NIGERIAN JOURNAL OF GENETICS

VOLUME 32, 2018

Published by The Genetics Society of Nigeria

ISSN 0189-9686

PRINTED IN NIGERIA

BY

Printed by
Niyi Printing & Publishing Company,
No. 47 Emir Road, Ilorin Kwara State, Nigeria. +2347032984291

NATIONAL EXECUTIVE OF GENETICS SOCIETY OF NIGERIA

SUGGESTIONS FOR CONTRIBUTIONS TO NIGERIA JOURNAL OF GENETICS

The Nigerian Journal of Genetics will consider for publication, original articles in the broad discipline of basic genetics (plant, livestock, fishery and human) and applied breeding. Only specially invited review articles will be published.

1. **Style of Manual:** Each article must contain the title in capital letters. This should be followed by name and address of the author(s) is suggested. The following sub-headings are suggested – Abstract; Introduction; Materials and Methods; Results; Discussion (or Results and Discussion); Summary and Conclusion (optional): Acknowledgements (if any); and Literature cited. These sub-headings should be centered and in capital letters and each must start on a separate page. The tables should follow the literature cited and this should be followed by figures. Three lines should be left for spaces where the author intends the tables and figures to be inserted and written into such spaces in pencil, should be the appropriate table or figure such as table 1 or fig. 2. Legend of figures should be typed on separate page while each figure, i.e., Fig. 1, Fig. 2 should appear on the figures. Only black and white pictures will be accepted. Magnifications should be indicated by line scale. Type each table on to separate sheet. Number (in Arabic) of table and its title should be typed at the top of each table. Measurements used must be indicated. When necessary, additional explanations should be supplied in foot notes.
2. **Manuscript:** The manuscript should be typed preferably on an A4 line numbered paper. The original plus two copies must be sent to the Editor-In-Chief. Two copies of the revised version of the accepted manuscripts (along with the diskette preferably on Word Perfect or Microsoft Word) shall be forwarded to the Editor-In-Chief. Running title, which should be an abbreviation of the heading, should be supplied. The Editor-In-Chief reserves the right to modify the running title.
3. **Footnotes:** Letters should be used as footnotes. For statistical significance: *, ** should be used to show 5% and 1% levels of significance, respectively.
4. **Handling fee:** An ₦2000.00K processing fee is charged per manuscript and this should accompany the manuscript in bank draft (cheque is not acceptable). Manuscripts not accompanied by the mandatory handling fee shall no longer be processed.
5. **Page Charges:** The page charge is ₦6000.00K per printed page while each figure and plate attracts ₦1000.00K and ₦1500.00K respectively. However, the minimum page charge per article is ₦20000.00K. This must be paid in full preferably in Bank draft, made payable to the Genetics Society of Nigeria, before accepted papers will be published. Publication charges may change from time to time, depending on the cost of materials. Authors will be informed accordingly.
6. **References**: A list of references should be presented in alphabetical order of the author's names. Names of authors should not be in capital letters. The author's names are followed by his initials and year. Example 1: Fatokun, C.A. (1976). Example 2: Kalia, H.R. and D.S. Padda (1962). Example 3: Ojo, O.O., D.F. Dada and A.A. Joshua (1962). For journal articles the title, volume and pages should be cited. For example, Rasmussen, D.C. and J.W. Lambert (1961). Variety x environment interactions in barley variety tests. *Crop Science,* 1: 261-262. Book titles should be underlined or italicized, and the number of pages specified.

Submit articles either online or by post.

Online

The Editor-In-Chief, Nigerian Journal of Genetics, genetics_nigeria@yahoo.co.uk and copy: (1) Prof. T.R. Fayeye: and (2) Dr. Kennedy Omokhafe

By Post

The Editor-In-Chief, Nigerian Journal of Genetics,
Department of Animal Production,
University of Ilorin. Kwara State, Nigeria.

TABLE OF CONTENTS

HETEROSIS AND RECIPROCAL EFFECTS IN GROWTH TRAITS OF F_1 GENERATION CROSSES BETWEEN THE FULANI ECOTYPE CHICKENS AND HUBBARD BROILERS STRAIN

***Sola-Ojo, F. E., Ayorinde, K. L and Toye, A. A.**
Department of Animal Production, University of Ilorin.
*Email: mofesola1@yahoo.com

Abstract: This study was designed to contribute to the genetic upgrading of the Fulani Ecotype (FE) a Nigerian local chicken through crossing with an exotic meat strain (Hubbard Broiler chickens). The heterotic and reciprocal effects of the crossing were observed in growth traits of the progenies of the Hubbard Broiler male and Fulani Ecotype female (HBxFE), and the Fulani Ecotype male and Hubbard Broiler Female (FExHB). The results show variations in heterosis from week 1 to 8. Heterosis for Bodyweight in crossbred produced with Hubbard broiler male was higher than in crossbreds from the female line from week 2 to 8. Weak and negative heterosis was obtained for morphometric traits measured from week 1 to 8. The Reciprocal Effects (RE) of Body Weight and morphometric measurements were low throughout the experimental period. Percentage heterosis obtained for weight gain and feed intake were positive and high for HBxFE (86.35 and 82.41, respectively), but low heterotic value was obtained for feed efficiency and viability (8.92 and 16.00). FE x HB had positive heterosis for feed intake and viability (89.25 and 7.60), and negative heterosis for weight gain and feed efficiency (-12.95 and -3.79). This study showed that the Hubbard broiler male crossed with Fulani Ecotype female (i.e. HB x FE) is the superior cross for upgrading growth traits, weight gain and viability, while the FE x HB cross is superior for improving feed efficiency over the Fulani Ecotype.

Keywords: Chicken, Exotic, Growth traits, Heterosis, Local, Performance

INTRODUCTION

The performance of village fowl in Africa is primarily affected by the poor management systems within which they are produced, as well as their relatively poor history of systematic genetic improvement relative to exotic chickens. Nevertheless, much variation has developed and maintained as a result of random breeding within and sometimes between diverse populations (El Houadfi, 1990; Sonaiya *et al.,* 1992; Nimbkar *et al.,* 2008). According to the authors, such birds can be improved genetically through selective breeding or by crossing with exotic stock. The wide variation in performance of the local chicken indicates their potential for improvement through genetic selection, cross breeding or both.

Crossbreeding which is a tool for improvement and upgrading genetic potential of animals is the mating of two or more different breeds. It is mainly used for commercial production especially in countries where highly developed pure breeds are available and it is known to maintain heterosis which cannot be fixed by inbreeding techniques. The gradual replacement of local genes through crossbreeding and artificial selection has been the basis of initial genetic development in many countries (Omeje and Nwosu, 1984). In developing world, most genetic changes are taking place through change of breeds via cross breeding programme aimed at 'grading up' of indigenous breeds towards exotic from the developed world (Nimbkar *et al*., 2008).

Introduction of high producing species of farm stock to the tropic and subtropical conditions is usually accompanied by performance degenerate due to lack of adaptability and resistance to tropical diseases (Ayorinde, 1990; Ibe, 1990). Also, grading up of nondescript livestock in developing countries has failed beyond a certain level of pure-bred inheritance due to the same reasons. Under such circumstances, crossbreeding of local breeds with improved exotic breeds can bring enough capacity for the animal to withstand the direct and indirect effects of unfavourable conditions. The native breeds of many species of livestock have very low genetic potential for production but they possess

qualities like adaptation to hot climatic conditions, resistance to many diseases prevalent in the tropical regions and general thriftiness under inferior feeding and management conditions (Akinnokun and Dettmers, 1976).

Nwosu and Asuquo (1984) opined that the local chicken can be developed into a broiler or meat type chicken. Crossing of the local chicken with Arbor Acre broilers have shown that crossbreeding of the local chicken with a broiler strain can significantly improve the meat performance of local chicken. Nwagu *et al.* (2001) showed that using the Arbor Acre cock and the local chicken hen was the preferred cross if a broiler strain is to be developed from the Nigerian local chicken.

The developmental breeding programs which introduce exotic males into villages as well as systematic breeding procedures used to establish synthetic breeds that are adapted to a specific environmental condition – as in India (Rao, 1983, Khan and Roy, 2003), have a sound basis. This is especially the case if higher productivity is to be combined with special genetically founded characteristics of local population such as body size, feathering type or pigmentation of skin and meat. Another genetic contribution of local strains can be seen in the provision of tropically relevant gene complexes for alternative breeding strategies. Since the fowl belongs to a well investigated species with over 250 known loci (Somes, 1990) fitting genes can also be directly identified in indigenous birds. Such major genes from local stocks can be easily integrated into appropriate breeding schemes, particularly those focused on the rural sector in developing countries.

Therefore, the Fulani Ecotype chicken which is an indigenous chicken characterized by low body weight and length of body parts (Sola-Ojo and Ayorinde, 2009) can be improved genetically through crossing with exotic stocks since the exotic breed are characterized by high productivity. It is therefore expedient to evaluate performance heterosis and reciprocal effects in progenies obtained from crossing of the Hubbard Broiler parent stock strain and the Fulani Ecotype chicken so as to have information on the performance of the resulting F_1 compared to the pure parent and to determine whether there is a benefit (growth performance) to using crosses in commercial production relative to inferior parent.

MATERIALS AND METHODS

Origin and Management of Parental Genotype: One hundred and twenty five matured Hubbard broiler parent stock and ninety seven adult Fulani Ecotype chickens (produced from a flock at the Teaching and Research farm, University of Ilorin) were allowed to mate randomly at a ratio 8 females to 1 male in pure bred Hubbard group and 10 females to 1 male in other groups. Group A (HBxHB) consist of 10 Hubbard broiler males and 80 Hubbard broiler females; group B (HBxFE) 3 Fulani Ecotype chicken males and 30 Hubbard Broiler males, group C (FExHB) 5 Hubbard Broiler males and 50 Fulani Ecotype chicken females, and; group D (FExFE) 4 Fulani Ecotype chicken males and 40 Fulani Ecotype chicken females.

Incubation and Hatching: Six hundred eggs were collected over a seven day period from the flocks and eggs were identified by group and stored in an aerated and ventilated room, after which they were taken to a commercial hatchery (Nefraday farm, Lasoju, Kwara State) for incubation and hatching. A total of 120 chicks from A, 80 chicks from group B, 60 chicks from group C and 65 chicks from group D were randomly selected for data collection per group.

Management of Experimental birds and Data collection: The chicks in each group (i.e. each genotype) were wing banded, weighed and randomly assigned to 4 pens such that each pen contained chicks of one genotype only. They were fed the same commercial broiler diet from day old to eight weeks of age. Vaccination and medication were administered (MVM, 1986). Measurement of body parts and body weight were done as described by Sola-Ojo and Ayorinde (2009).

Statistical Analysis: Heterosis (Specific) among the crossbred (HBxFE, FExHB) were estimated as the differences between the average parental genotype means (pure breed) and means of crossbred offspring as outlined by Falconer, (1989). It was calculated using the parental genotype F_1

contemporaneously with the hybrid performance under the same environmental conditions.

H_{AB} (%) = [(PF_1 - (P_A+P_B)/2] x100] / (P_A+P_B)/2

Where:

H_{AB} (%) is the directional heterosis (in percentage)

PF_1 is the mean performances of F_1

P_A is the mean performance of parental genotype A,

P_B is the mean performance of parental genotype B.

Reciprocal Effects were calculated as the differences between reciprocal F_1 performances.

$R_E = P_{F1}$ (HBxFE)–P_{F1} (FExHB) while % RE= [P_{F1} (HBxFE) – P_{F1} (FExHB)] x 100/ [P_{F1} (HBxFE) + P_{F1} (FExHB)]

Where:

R_E = the reciprocal effect.

P_{F1} (HBxFE) = the mean performance of the F_1 from Hubbard male and FE hen

P_{F1} (FExHB) = the mean performance of the F_1 from FE male and Hubbard hen.

RESULTS AND DISCUSSION

Heterosis and Reciprocal Effects of Body weight, Body parts and Growth Performance in Crosses involving Hubbard Broiler and Fulani Ecotype Chicken

The percentage heterosis (Table 1) for body weight in HBxFE was higher than that of FExHB from week 2 to 8 while FExHB had higher heterotic value for body weight at week 1 only. Heterotic value in HBxFE was 80.89 percent higher compared to that of FExHB at week 5 and 0.8% higher at week 6. The percentage heterosis for body weight was highest in both genotypes at week 7 (Table 1), thus reflecting performance in the range between the mid parental value and superior parent at that age. The heterosis values showed that with respect to body weight performance, HBxFE crosses are superior to the reciprocal which was in turn superior to the inferior parent and the mid parent value at all weeks (Table 1). Indeed, with respect to body weight, the performances of both F_1 crosses were comparable to the mid-parent value and heterosis was moderate for HBxFE from weeks 3 to 8 and FExHB from weeks 6 to 7.

In 6 weeks out of the 8 weeks examined, both F1 crosses performed less well in body length (BL) than the mid-parent value as evident from their negative heterosis. In HBxFE, BL heterosis was negative at weeks 1, 2, 6, 7 and 8, while that of FEXHB was negative at weeks 1, 2, 3, 6, 7 and 8 (Table 1). For body girth, the percentage heterosis for HBxFE and FExHB were negative at weeks 1 and 2, and also negative at week 8 in HBxFE, (Table 1), other values obtained were positively low.

Heterosis for shank length (SL) ranged from -3.88 to 15.67 for the HBxFE and -22.97 to 15.45 for the FExHB from weeks 1 to week 8. Both F1 genotypes had the highest SL at week 3 and identical heterosis at week 5 (Table 2). The shank diameter (SD) heterotic values ranged from 2.91 to 29.32 in HBxFE and -6.67 to 15.22 in FExHB from week 1 to week 8. Both F1 genotypes had identical SD heterosis at weeks 5 and 8. Positive heterotic values were obtained for the drumstick length (DL) in HBxFE from week 1 to 8, while FExHB had negative heterosis at week 1 (-8.03) with the highest positive value of 21.26 at week 3 (Table 2). DL heterosis was identical in both F1 genotypes at week 3.

The heterotic values obtained for wing length of HBxFE ranged from -13.78 to 17.86 from week 1 to week 8, while the percentage heterosis for the FExHB ranged from -12.93 to 12.84 (Table 3). The heterosis value obtained for Thigh length (TL) were positive for both HBxFE and FExHB throughout the 8 weeks period, and it ranged from 3.99 to 21.18 in HBxFE, and 3.38 to 19.70. The keel length (KL) heterotic values were positive for HBxFE, ranging from 0.94 to 18.72 whereas, in FExHB negative heterotic values were obtained at week 1, 2 and 5, and positive values ranging from 2.80 to 16.12 at weeks 3,4, 6, 7 and 8 (Table 3).

The percentage heterosis obtained for weight gain and feed intake were positive and high (86.35 and 82.41) for HBxFE while those of feed efficiency and viability were low (Table 4). High and positive percentage heterosis (89.25) was obtained for feed intake of FExHB while low or negative heterosis (7.6, -12.95 and -3.79) values were obtained for viability, weight gain and feed efficiency, respectively in the same cross.

Across the nine traits examined at weeks 1 to 8, reciprocal effect (RE) values ranged from 0 to

15.12 percent. The RE value was zero for BG at week 6, SL at week 5, SD at week 1, 5, 6 and 8, DL at week 2 and 3, KL at week 3, and TL at week 6 (Table 5). Reciprocal effect of 44.19 percent was obtained for weight gain, 5.81 percent for feed efficiency while feed intake and viability had values of 65.94 and 0.09 percent respectively as shown in Table 5. The reciprocal effects showed that HBxFE F1 genotype exhibited numerically superior performance over the reverse cross (FExHB) across all traits (Table 5).

These results indicate that the hybrids between Fulani Ecotype chicken and the Hubbard Broiler strain achieved an improved 8th week body weight over the inferior parent (Fulani Ecotype chicken) irrespective of the direction of crossing and indeed both F1 crosses performed better than the figure reported for another local breed (Nwagu, 1988). This should be relevant in the development of a meat line strain from the Nigeria local chicken.

Negative heterosis obtained for weight gain in FExHB is consistent with published literature showing that not all F1 crosses perform as well as the mid-parent (Verma and Chaudhary, 1980). This author observed body weight heterosis ranging from positive to negative values in 2, 3 and 4 way broiler crosses. The authors inferred that this is a usual pattern for body weight in broiler chickens and does not correspond to dominance or epistatic model. Positive heterosis obtained for HBxFE indicates that this genotype displayed better performance than the average of the parental types. The data showed that the HBxFE crosses were superior to the reverse (FExHB) for meat production. Positive heterosis in HBxFE cannot be explained by dominance or recessive action of autosomal genes, since they were F_1 hybrids, but may have been through sex chromosome-specific, mitochondrial origin specific or epi-genetic effects (Toye, 2013). Generally, the reciprocal effects of body weight and weight gain were poorer than the heterotic effects and this corresponds with the findings of Friars *et al*. (1992) that reciprocal effects are of low importance in crosses relative to meat strains. However, the low level of heterosis obtained for viability (7.6 and 16) is within the range of -9 to 24 % reported for heterosis for viability in poultry by Fairfull *et al*. (1987).

The result obtained agrees with the findings of Omeje and Nwosu (1984) where high heterosis of early body weight was observed for F1 offspring of cross of local chicken with an exotic GL strain of chickens. The results of better body weight performance when Hubbard cocks were mated to the Fulani hens correspond with the findings of Nwagu *et al.* (2001), where exotic (Arbor Acre) cock vs. Local chicken hen was the preferred cross in the development of an indigenous broiler strain. The result of the heterosis of the F_1 in this study indicates that there is a cross direction-specific dominance effect of high growth determinant genes, specifically in the HBxFE group from week 2 to 8 while it was experienced in FExHB only at week 1. The dominance effects obtained in this study reflects that the phenotypic values of body weight in progenies at those ages were higher than those of mid parental genotype values and is likely to be from epistatic effect of sex chromosomes or mitochondrial on autosomes as reported by Toye (2013).

The lack of concordance of heterosis between the reciprocal crosses indicates that cross breeding with Hubbard will lead to an improvement in the body weight of the indigenous chicken irrespective of direction of crossing, and this will be an additional benefit to choosing the superior cross direction i.e male line in HB crossed to female line in FE. Low heterotic values obtained for the feed efficiency suggest co-dominance of the genetic determinants contributed by the local chickens on one hand, and the HB on the other hand, also the absence of major sex chromosome, mitonchondrial and epi-genetic effect as stated by Toye (2013).

The superior performance of hybrid over both parents yielding positive heterosis for body weight points to haplo-complementation of allele contributed by each parent at some loci. The reverse cross from age 2-8 weeks while yielding superior weight gain heterosis, yielded mildly inferior feed efficiency heterosis than the reverse crosses (FExHB). This study also indicated that the lack of concordance in bodyweight heterosis between reciprocal crosses of parents is likely due to sex chromosome, mitochondrial genetic inheritance or epi-genetic factors and not autosomonal gene dominance. The HB male crossing with the FE female (HBxFE) should be encouraged because it

appears that body weight was more under paternal influence resulting in improved body weight over the FE indigenous chicken at all age points and yields superior BW compared to the reciprocal cross.

In conclusion, this study shows that reciprocal crosses revealed the extent; the direction of a cross will improve trait performance over that of the inferior parent, and the direction that will provide the better gain in performance over the inferior parent. In the present study, the body weight heterosis data favour HBxFE over FExHB, while feed efficiency heterosis data favour FExHB over HBxFE. The result of crossbreeding between Hubbard Broiler strain and the Fulani Ecotype chicken showed that the Hubbard Broiler male line and the Fulani Ecotype chicken female line are the preferred lines of F_1 crossing for an improved meat production.

Table 1. Percentage Heterosis of BW, BL and BG in Hubbard Broiler and Fulani Ecotype chicken crossbreds

Age	BW		BL		BG	
(weeks)	HBxFE	FExHB	HBxFE	FExHB	HBxFE	FExHB
1	4.18	4.88	-4.99	-13.99	-9.09	-12.29
2	9.50	8.82	-6.27	-14.50	-0.18	-1.74
3	40.20	38.60	4.68	-8.41	20.05	14.51
4	44.34	25.09	1.91	2.97	14.03	12.04
5	45.84	25.34	2.67	1.29	16.64	13.91
6	43.98	43.63	-4.25	-5.28	14.20	14.20
7	50.91	47.13	-5.07	-9.21	17.91	10.92
8	43.53	38.27	-3.24	-2.99	-9.01	29.96

HBxFE = Hubbard Broiler Male and Fulani Ecotype chicken Female
FExHB= Fulani Ecotype chicken Male and Hubbard Broiler Female
BW= Body weight; BL= Body length; BG= Body girth.

Table 2. Percentage Heterosis of SL, SD and DL in Hubbard Broiler and Fulani Ecotype chicken crossbreds

Age	SL		SD		DL	
(weeks)	HBxFE	FExHB	HBxFE	FExHB	HBxFE	FExHB
1	-3.88	-6.63	4.44	-6.67	14.70	-8.03
2	-10.59	-22.97	2.91	0.97	15.14	15.14
3	15.67	15.45	29.32	-0.75	21.26	21.26
4	13.81	12.36	12.82	5.13	15.56	14.52
5	13.89	13.89	15.22	15.22	18.49	14.08
6	1.30	-0.16	6.53	6.53	16.66	16.44
7	12.90	11.68	7.62	5.71	20.22	17.18
8	13.88	6.87	13.91	13.91	15.09	14.99

HBxFE = Hubbard Broiler Male and Fulani Ecotype Chicken Female
FExHB= Fulani Ecotype chicken Male and Hubbard Broiler Female
SL =Shank length; SD= Shank diameter; DL = Drumstick length.

Table 3: Percentage Heterosis of WL, TL and KL in Hubbard Broiler and Fulani Ecotype chicken crossbreds

Age	WL		TL		KL	
(weeks)	HBxFE	FExHB	HBxFE	FExHB	HBxFE	FExHB
1	-13.78	-12.93	10.41	8.60	8.70	-3.84
2	-12.78	-7.24	5.56	5.28	0.94	-1.65
3	-5.58	12.84	21.18	19.70	16.12	16.12
4	17.86	5.48	3.99	3.38	2.98	2.80
5	11.00	7.69	14.73	14.54	1.99	-0.95
6	3.88	3.72	14.29	14.29	16.90	15.65
7	6.38	-2.08	16.44	12.30	18.72	14.61
8	11.47	5.11	11.83	11.57	13.82	13.70

HBxFE = Hubbard Broiler Male and Fulani Ecotype chicken Female
FExHB= Fulani Ecotype chicken Male and Hubbard Broiler Female
WL= Wing length; TL = Thigh length; KL = Keel length.

Table 4: Heterosis and Reciprocal Effect of Growth and Performance Traits in the Hubbard Broiler and Fulani Ecotype crossbreds

	Heterosis		
Parameters	HBxFE	FExHB	RE
Weight gained	86.35	-12.95	44.19
Feed Intake	82.41	89.25	65.94
Feed Efficiency	8.92	-3.79	5.81
Viability	16.00	7.60	0.09

HBXFE = Hubbard Broiler Male and Fulani Ecotype chicken Female
FEXHB =Fulani Ecotype Male and Hubbard Broiler Female
RE = Reciprocal Effect.

Table 5: Reciprocal Effect of Crossing Hubbard Broiler and the Fulani Ecotype chicken

Age	Traits								
weeks	BW	BL	BG	SL	SD	DL	WL	KL	TL
1	0.68	0.27	3.58	7.98	0.00	22.0	0.18	12.23	1.65
2	0.62	2.31	1.57	14.88	1.90	0.00	1.77	2.60	0.27
3	1.15	1.64	4.73	0.19	26.32	0.00	4.11	0.00	1.23
4	14.28	0.61	1.77	1.29	7.06	0.91	2.23	0.18	0.59
5	15.12	1.44	2.31	0.00	0.00	3.79	3.03	2.93	0.17
6	0.24	1.09	0.00	1.45	0.00	0.19	0.15	1.08	0.00
7	2.54	4.46	6.11	12.57	1.79	2.56	8.28	3.53	3.63
8	3.73	0.26	13.0	0.00	0.00	0.08	5.87	0.11	0.24

BW = Body weight, BL=Body length, BG= Body girth, WL= Wing length, DL= Drumstick length, KL= Keel length, TL =Thigh length SL= Shank length SD= Shank diameter.

REFERENCES

Akinnokun, O. and Dettmers, A. (1976). Genotype and Environment Interactions in an exotic commercial egg strain and the local chicken of Nigeria. *Ife Journal of Agric*., 1: 57-62.

Ayorinde, K.L. (1990). Problems and prospects of guinea fowl production in the rural of Nigeria. *In Rural Poultry in Africa Sonaiya E.B, Ed :106-115*.

El Houadfi, M. (1990). *Rapport sur la production Avicole et probleme liels aux Elevages Traditionnels au Maroc.* Proc. CTA International Seminar on small holder Rural Poultry Production. Thessaloniki, Greece, Oct. 9- 13. 2: 161- 172.

Fairfull, R.W., Gowe, R.S. and Nagal, J. (1987). Dominance and epistasis in heterosis of White Leghorn strain crosses. *Canadian Journal of Animal Sci*., 67: 663-680.

Falconer, D.S. (1989). Introduction to Quantitative Genetics, 3rd edition Longman Scientific and Technical, London.

Frairs, G.W., Boren B.B. and McKean, H.E. (1992). Time trends in estimates of genetic parameters in a population of Chicken subjected to multiple objective selections. *Poultry Science*, 41: 1773- 1784.

Ibe, S.N. (1990). Increasing rural poultry production by improving the genetic endowment of rural poultry. *In workshop in Rural Poultry Development in Africa. OAU. Ile-Ife. Nigeria, pp 78-81.*

Khan, M.R and Roy , P.C. (2003). Credit policy, disbursement and its impact on poultry industry in Bangladesh, Proc. 3rd International Poultry Show and Seminar Dhaka, Bangladesh. Worlds Poultry Science Association, Bangladesh Branch: 43 -51.

MVM, (1986). The Merck Veterinary Manual. 6 Edn. York. Merck and Co., INC. Rahway, N.J., USA: 1-19.

Nimbkar, C., Gibson, J., Okeyo, M., Boettene, P. and Soelknor, J. (2008). Sustainable use and genetic Improvement. *Animal Genetic Resources Information*, 42: 49 – 69.

Nwagu, B.I. (1988). Heterosis of Biometrical characteristics in crosses between Arbor Acre Broilers and local chickens of Nigeria. *MSc. Thesis, University of Nigeria, Nsukka, Nigeria.*

Nwagu, B.I., Nwosu, C.C., Abubakar, B.Y., Oni O.O. and Adeyinka, I.A. (2001). Productive Performance of F_1 Crosses between Arbor Acre Broilers and Local Chickens. *Nigeria Journal of Animal Production*, 17 (1 & 2): 66-77.

Nwosu, C. C. and Asuquo, B.O. (1984). Heritability estimates of body weight in the local chicken. *In proceedings of the 9th Annual Conf. of NSAP, Nsukka, Nigeria: 41-49.*

Omeje, S.S.I. and Nwosu, C.C. (1984). Heterosis and superiority in body weight and feed efficiency evaluation of exotic parent stock by local chicken F_1 Crossbreds. *Nig. Journal of Genetics*, 5: 11- 26.

Rao, G.V. (1983). Improved dessi birds for poultry farming in tribal areas. *Indian Farming*, 33: 36-38.

Sola-Ojo, F.E. and Ayorinde, K.L. (2009). The Fulani Ecotype Chicken: Growth and Feed Utilization Potentials. *World Journal of Applied Science and Technology*, 1: 37-44.

Somes, R. G. (1990). Mutations and major variants of plumage and skin in chickens. (Crawford R.D., Ed.). *Poultry Breeding and Genetics*: 169-208.

Sonaiya, E. B, Laogun, E.A., Matanmi, O., Daniyan, O. C., Akande, B.E., Oguntade, E. A., Omisebi, R. O. and Olori, V. E. (1992). Health and husbandry aspects of Village extensive poultry production in south western Nigeria. *In Proc. of an International Workshop on village Poultry in Africa (Pandey, W.S and Demey F., Eds). Rabat, Morroco: 34- 41.*

Toye, A.A. (2013). Systematic Elucidation of the role Nuclear genes in mitochondrial health and disease. Published by Omega Initiative, Causal Genetics LTD. UK.

Verma, S.K. and Chaudhary, R.P. (1980). Note on the influence of two-three-and four-way crosses on growth for broiler production. *Indian Journal of Animal Science*, 50: 213-214.

MULTIVARIATE ANALYSIS OF SEXUAL DIMORPHISM IN THE MORPHOMETRIC TRAITS OF MUTURU CATTLE IN NORTH CENTRAL NIGERIA

A. Yakubu[1]*, A. V. Hingir[1] and A. R. Abdullah[2]

[1]Department of Animal Science, Faculty of Agriculture,
Nasarawa State University, Keffi, Shabu-Lafia Campus, P.M.B. 135, Lafia, Nasarawa State, Nigeria.
[2]Department of Animal Science, School of Agriculture and Industrial Technology,
Babcock University, Ilishan Remo, P.M.B. 21244, Lagos State, Nigeria.
*Correspondence: abdulmojyak@gmail.com

Abstract: The study aimed at evaluating sexual dimorphism in Muturu cattle using multivariate Principal Component (PC) and discriminant analyses. Eleven morphometric traits namely, Withers Height (WH), Body Length (BL), Horn Length (HL), Ear Length (EL), Face Length (FL), Rump Length (RL), Neck Length (NL), Neck Circumference (NC), Chest Girth (CG), Shoulder Width (SW) and Rump Width (RW) were measured in a random sample of 105 Muturu cattle of both sexes (60 females and 45 males). The animals were within the 8-tooth age (greater than 48 months old) and were reared through the extensive system of management in North Central Nigeria. With the exception of HL and FL, the univariate analysis indicated sex-associated differences ($P<0.05$) in all the body parameters, with higher values recorded for males. Although the estimates of phenotypic correlations were positive and significant ($P<0.01$) in both sexes, varying coefficients (0.37-0.97 and 0.61-0.94 for males and females, respectively) were obtained. In the PC analysis of morphometric traits, three components (PC1, PC2 and PC3) were extracted in bulls, each accounting for 75.02%, 6.91% and 6.16% of the total variance, respectively. However, two components (PC1 and PC2) were extracted in cows, which explained 82.55% and 5.55%, respectively of the generalized variance. The most discriminant variables to separate the two sexes were NL, HL, FL, BL and WH. The discriminant model was able to allocate correctly 100.0% of Muturu cows and bulls to their a priori sexes. The present information may be exploited in management, ecological and conservation studies of Muturu cattle populations.

Key words: Biometric traits, cattle, multivariate analysis, Nigeria, sexual dimorphism

INTRODUCTION

Maintenance of biodiversity is one of the most important current concerns of human kind, as wild species and domestic breeds and strains are disappearing at an alarming rate (Corrales *et al*. 2010). Muturu, meaning humpless, is the Hausa name for the West African Shorthorn in Anglophone West Africa. Most of the Muturu cattle of Nigeria belong to the Savanna type, spread over the Benue plateau, and smaller numbers are found further to the Southwest (ILRI, 2011). The survival of the Muturu cattle breed in Nigeria stems from the fact that the animal is still sacred in so many communities and its milk is widely used for medicinal purposes (Adebambo, 2001). They constitute 8.3 percent of the total cattle population of Nigeria (RIM, 1992). The issue, however, is how to design sustainable breeding schemes for indigenous breeds under inherent tropical conditions (Rege *et al*., 2011). Appropriate design of breeding programmes is impossible for breeds that have not been adequately characterized either phenotypically and/or genetically (Mwacharo *et al*., 2006). The first step of the characterisation of local genetic resources is to assess variation of morphological traits (Delgado *et al*., 2001).

Morphological variation within a species is of great biological interest, both as a phenomenon and as a descriptive and an analytical tool. Sexual differences in external morphology are of interest in studies of reproductive biology and to analyse population composition. This can be used to detect the amount and distribution of genetic variation within Muturu cattle population. Basic information for selection and breeding programmes of the cattle populations could also be obtained through sex differences (Chebo *et al*., 2014). Sexual differences are often dramatic and widespread across taxa (Wyman *et al*., 2013). The evolution of sex-specific genetic variation allows each sex to approach, or possibly achieve, its

optimum phenotype, thereby generating sexual dimorphism (Ingleby *et al*., 2014; Polak and Frynta, 2009). In most organisms, it goes beyond the fundamental differentiation of reproduction organs to include dimorphisms for body size, shape, colour, as well as the presence of specific morphological structures in one sex (Fairbairn and Roff, 2006). Recently, physiologists, geneticists and developmental biologists have sought to integrate this ultimate perspective with an understanding of the proximate mechanisms that facilitate the expression of dimorphic phenotypes from a genome that is largely shared between the sexes (Rhen, 2007). Additionally, theory has long suggested that the evolution of sexual dimorphism is facilitated by sex chromosomes, as these are the only portions of the genome that differ between males and females (Mank, 2009).

Analysis of variance and correlations are used to obtain relationships among different body measurements. However, PC can explain relationships in a better way when the recorded traits are correlated. This permits the analysis of a large number of traits that are strongly interrelated (Hair *et al*., 2009; Parés-Casanova and Villalba, 2013) without presenting any problem associated with multicollinearity. The correlation between the factors and original traits is represented by its weight where traits with a higher weight are more representative of that factor (Kern *et al*., 2014). Although in practice there is no limit in the number of traits to be evaluated in a genetic programme or in an experiment, it is relevant to use adequate statistical analysis to identify important and informative variables among a great number of traits (Yamaki *et al*., 2006; Nafti *et al*., 2014). For breeding purpose, it is fundamental to better morphologic evaluation through the use of multitrait information (Pinto *et al*., 2008) as discriminant functions can be useful for the selection of breeding stock and to avoid registration of animals not meeting the phenotypic standards of the breed association.

In North Central Nigeria, information on the morphometric traits of Muturu cattle appears virtually non-existent. The present investigation therefore, aimed at providing baseline information on sexual dimorphism in Muturu cattle using multivariate principal component and discriminant analyses. The information so obtained will ensure better characterization which could aid in the ecological studies and conservation of the Nigerian indigenous cattle breed.

MATERIALS AND METHODS

Study Site and Experimental Animals: The experiment made use of a random sample of 105 Muturu cattle of both sexes (60 females and 45 males). The animals were selected in certain villages located in Gboko, Benue State, Nigeria. The choice of the sampling site was informed by the concentration of the Muturu cattle in the area. Animals were selected to be as unrelated as possible to ensure that the full range of genetic diversity present within the breed was included in the study. This was done by asking the farmers the sources of their breeding stock, the mating and grazing patterns of the animals. Gboko is located within a sub-humid tropical region between Latitudes 07º 08′ 16″ and 07º 31′ 58″, and Longitudes 08º 37′ 46″ and 09º 10′ 31″. It has mean annual temperature ranging from 23ºC to 34ºC, and is characterized by two distinct seasons: the dry season and rainy season. The mean annua1 precipitation is about 1,370mm, with an average wind speed of 1.50 m/s (NMA, 2012). All the animals sampled were within the 8-tooth age (greater than 48 months old). The age of the animals was determined using permanent dentition. The animals were extensively managed with little or no provision for shelter in the night. The animals grazed during the day on natural pasture while this was supplemented occasionally with local concentrates.

Body traits measured: Eleven morphometric traits were measured on each animal. The body traits were Withers Height (WH), Body Length (BL), Horn Length (HL), Ear Length (EL), Face Length (FL), Rump Length (RL), Neck Length (NL), Neck Circumference (NC), Chest Girth (CG), Shoulder Width (SW) and Rump Width (RW). Anatomical reference points were as earlier described (Yakubu *et al*., 2009). The height measurement (centimetres) was done using a graduated measuring stick. The length and circumference measurements (centimetres) were effected using a tape rule while the width measurements (centimetres) were taken using a calibrated wooden calliper. Measurements were done

in the morning before the animals were released for grazing. All measurements were carried out by the same person in order to avoid between-individual variations.

Statistical analysis

Means (±S.E.), standard deviations and coefficients of variation of the morphometric characteristics of both male and female Muturu cattle were computed using the MEANS procedure. The linear additive model was adopted to test the effect of sex on the morphometric measurements. Means were separated using the two-tailed, two-sample t-test of the same statistical package. Pearson's coefficients of correlation (r) among body weight and the various morphometric traits were estimated. From the correlation matrix, data were generated for the principal component (PC) factor analysis. PC analyses relationships among several quantitative variables measured on a single object, reducing the number of variables under analysis to a small number of indices (called the principal components) that are linear combinations of the original variables (Mavule *et al*., 2013). In the PC analysis of the present study done separately for each sex, cumulative proportion of variance criterion was employed in determining the number of factors to extract. The varimax criterion of the orthogonal rotation method was employed in the rotation of the factor matrix to enhance the interpretability of the factor analysis. The overall reliability of the PC analysis was tested using Kaiser-Meyer- Olkin measures of sampling adequacy, Bartlett's Test of Sphericity and Chronbach's Alpha. Canonical discriminant analysis, a multivariate technique was used to identify the combination of variables that best separate the two sexes. In the present study, the 11 morphometric variables were stepwise introduced as predictor variables into the discriminant analysis. The relative importance of the morphometric variables in discriminating the two sexes was assessed using the F-to-remove statistic. For sex identification, the unstandardized discriminant function procedure of the canonical discriminant analysis was employed (this function has the form of a multiple regression equation). The ability of this function to identify each Muturu sex was indicated as the percentage of individuals correctly classified from the sample that generated the function. Accuracy of the classification was evaluated using split-sample validation (cross-validation). All analyses were done using SPSS (2001) statistical package.

RESULTS

Table 1: Descriptive statistics for the morphometric characters of Muturu cattle

Traits	Bulls		Cows	
	Mean±SE	CV (%)	Mean±SE	CV (%)
Withers height	111.73±2.00[a]	12.04	100.73±1.84[b]	14.16
Body length	175.54±4.35[a]	16.62	156.33±3.59[b]	17.79
Horn length	22.68±0.61[a]	18.03	22.02±0.52[a]	18.48
Ear length	21.80±0.28[a]	8.53	20.59±0.36[b]	13.65
Face length	40.99±1.41[a]	23.08	38.73±1.21[a]	24.40
Neck length	39.08±0.64[a]	10.95	31.02±0.54[b]	13.51
Rump length	37.47±0.75[a]	13.48	31.60±0.65[b]	15.92
Chest girth	135.48±1.83[a]	9.07	124.80±1.60[b]	9.95
Shoulder width	36.57±1.74[a]	31.94	30.37±1.02[b]	26.01
Rump width	42.11±1.48[a]	23.56	36.13±0.80[b]	17.19
Neck circumference	56.40±1.33[a]	15.78	48.53±1.22[b]	19.51

SE= standard error

CV=coefficient of variation

[a,b] Means in the same row with different superscripts are significantly different (P<0.05)

Table 2: Phenotypic correlations of the morphometric traits of the sexes of Muturu cattle*

Traits	WH	BL	HL	EL	FL	NL	RL	CG	SW	RW	NC
WH		0.96	0.71	0.57	0.69	0.76	0.73	0.95	0.88	0.79	0.76
BL	0.96		0.69	0.58	0.65	0.82	0.72	0.92	0.86	0.76	0.77
HL	0.71	0.84		0.56	0.51	0.76	0.37	0.80	0.84	0.84	0.56
EL	0.85	0.80	0.81		0.67	0.72	0.54	0.68	0.62	0.64	0.68
FL	0.68	0.62	0.74	0.63		0.77	0.48	0.77	0.69	0.69	0.58
NL	0.76	0.86	0.84	0.74	0.84		0.48	0.86	0.82	0.80	0.69
RL	0.88	0.77	0.62	0.78	0.70	0.74		0.75	0.65	0.62	0.57
CG	0.94	0.89	0.87	0.84	0.81	0.88	0.89		0.94	0.90	0.73
SW	0.94	0.89	0.81	0.84	0.75	0.83	0.87	0.94		0.97	0.72
RW	0.87	0.86	0.86	0.85	0.83	0.85	0.81	0.95	0.94		0.70
NC	0.80	0.75	0.66	0.89	0.61	0.67	0.73	0.73	0.73	0.74	

*Significant at P<0.01 for all correlation coefficients

Upper matrix: Male cattle

Lower matrix: Female cattle

Withers Height (WH), Body Length (BL), Horn Length (HL), Ear Length (EL), Face Length (FL), Neck Length (NL), Rump Length (RL), Chest Girth (CG), Shoulder Width (SW), Rump Width (RW) and Neck Circumference (NC)

Table 3: Eigenvalues and share of total variance along with factor loadings after varimax rotation and communalities for comparing the morphometric traits of the sexes of Muturu Cattle

Traits	Bulls				Cows		
	PC1	**PC2**	**PC3**	**Communality**	**PC1**	**PC2**	**Communality**
WH	0.592	0.687	0.323	0.927	0.804	0.530	0.928
BL	0.578	0.678	0.334	0.906	0.712	0.589	0.854
HL	0.910	0.143	0.264	0.918	0.506	0.751	0.820
EL	0.241	0.263	0.860	0.866	0.848	0.420	0.896
FL	0.372	0.277	0.744	0.769	0.291	0.881	0.860
NL	0.659	0.255	0.617	0.880	0.452	0.829	0.892
RL	0.149	0.904	0.251	0.903	0.739	0.503	0.798
CG	0.664	0.589	0.429	0.971	0.652	0.732	0.961
SW	0.771	0.481	0.353	0.949	0.697	0.654	0.914
RW	0.763	0.398	0.391	0.893	0.622	0.737	0.930
NC	0.370	0.516	0.553	0.708	0.867	0.295	0.839
Eigenvalue	8.252	0.760	0.677		9.081	0.611	
Percentage variance	75.02	6.91	6.16		82.55	5.55	

Table 4: Morphometric traits selected by stepwise discriminant analysis to separate Muturu bulls and cows

Traits	Wilk's Lambda	F-remove	P-level
NL	0.535	89.401	0.001
HL	0.320	108.446	0.001
FAL	0.231	111.942	0.001
BL	0.208	95.391	0.001
WH	0.136	125.389	0.001

Table 5: Classification results for the discriminant analysis of the two sexes of Muturu cattle

		Predicted Group Membership		Total
	Sex	Bull	Cow	
Original count	Bull	45	0	45
	Cow	0	60	60
%	Bull	100.0	0.0	100.0
	Cow	0.0	100.0	100.0
Cross-validated count	Bull	45	0	45
	Cow	0	60	60
%	Bull	100.0	0.0	100.0
	Cow	0.0	100.0	100.0

Means, standard errors and coefficients of variation and significance of sex effect on each morphological trait measured in mature Muturu cattle are presented in Table 1. Male animals had significantly higher WH, BL, RL, NL), NC, CG, SW and RW. However, the sexes were not different (P>0.05) in HL, EL, FAL.

The phenotypic correlations among the morphometric traits were positive and significant (P<0.01) in both male and female animals (Table 2). However, the degree of the correlation coefficients between the traits varied in both sexes (0.37-0.97 and 0.61-0.94 for males and females, respectively).

The Kaiser–Meyer–Olkin measure of sampling adequacy (0.67 and 0.63 for bulls and cows, respectively), Bartlett's Test of Sphericity (chi-square=3808.83 versus 1303.00; P<0.001 for bulls and cows, respectively) and Chronbach's Alpha (0.896 and 0.786, respectively for bulls and cows) revealed the appropriateness and reliability of PC analysis (Table 3). Three components were extracted from the morphometric traits of bulls. The first component which explained 75.02% of the generalized variance had its loadings for HL, SW, RW, CG and NL. The second component, which accounted for 6.91% of the total variability assigned positive weights to RL, WH and BL. The traits that were more associated with the third component were EL and FL. In cows however, two components, each contributing 82.55% and 5.55% to the total variance were extracted. The first component was characterized by relatively high positive loadings for NL, WH, EL, BL, RL and SW while the second component was influenced by FL, NL, HL, RW and CG. The communalities ranged from 0.708-0.971 and 0.798-0.961, respectively for bulls and cows.

Results of the stepwise discriminant analysis showing Wilk's Lambda values, F-values, and probability are presented in Table 4. The discriminant analysis based on significant F-values indicated NL, HL, FL, BL and WH as the linear measures permitting discrimination between bulls and cows. The unstandardized stepwise discriminant function was used to classify individual cattle. The five discriminating variables earlier extracted were the variables included in the discriminant (D) equation as follows:

$D = 13.779 + 0.096BL - 0.722NL - 0.156WH + 0.165FL + 0.240HL$

The discriminant function was able to correctly classify 100.0% of the 45 bulls and 60 cows investigated (Table 5). Cross-validation with the split-sample method also indicated a 100.0% success rate.

DISCUSSION

The higher morphometric values recorded for males in the present study may be attributed to sexual dimorphism. Significant differences in different body measurement/biometric traits due to sex have been reported by earlier workers in cattle (Gilbert *et al*., 1993; Singh *et al*., 2008). Li *et al*. (2014) reported that there was no significant difference in the growth rate (withers height, body length, chest girth and shin circumference) of F1 Angus × Chinese Xiangxi yellow cattle, although higher values were recorded for males compared to their female counterparts. These authors focused on heifers and steers and not mature bulls and cows of the present study. In his own findings, Isaac (2005) reported that sexual dimorphism in body size is clearly widespread among mammalian taxa, with male-biased dimorphism being the more common, but certainly not exclusive pattern. The implication of the present result is that degree of dimorphism in the morphometric traits may be driven by both growth

constraints associated with different functional regions of the animals and by the independent action of natural selection on the morphology of those regions. Males and females share most of the same genomic architecture for growth and body size, but that these shared genes are differentially regulated by sex-specific modifiers (Badyaev, 2002). In earlier findings, the influence of sex factor on growth was attributed to physiological characteristics and the endocrinal system, type and measure of hormone secretion, especially sexual hormones (Jafari and Hashemi, 2014). Sex steroids (i.e. androgens, estrogens and progestins) are excellent candidates for the regulation of sex differences in growth and body size because they are produced and secreted in sex-specific fashion by the gonads (Cox *et al*., 2009).

Considering the fact that many additive genes of common action are responsible for the expression of morphometric traits, the formation of one body part is related with the formation of the other part (Lener and Donald, 1996). The varying phenotypic correlation coefficients in the two sexes suggest sexual differences in the genetic architecture of the Muturu cattle. The present association may be useful as selection criterion, since positive correlations of traits suggest that the traits are under the same gene action (pleiotropy). It has also been established that high positive phenotypic relationship could be an indicator of genetic correlations between body measurements (Maiwashe *et al*., 2002; Sole *et al*., 2014). The phenotypic and genetic relation between conformation and functional performance traits has also been reported (Alberti *et al*., 2008). Therefore, where data and resources are not available for genetic evaluation as is the case in most smallholder farms, selection could be carried out on the basis of body measurements of Muturu cattle.

The factor loadings of the extracted PCs for each sex in the present study give credence to the widely reported sexual dimorphism between male and female cattle. Similar characterization using principal component analysis of morphological traits has been reported for various livestock species (Yakubu *et al*., 2009; Yakubu *et al*., 2011a and b; Pundir *et al*. 2011; Parés-Casanova and Mwaanga, 2013; Oguntunji and Ayorinde, 2014). The classification function obtained in this study could directly be used to separate the two sexes, since positive D scores indicate male animals and negative D scores indicate female animals. The reduction in the number of measurements saves time and energy required to distinguish between the sexes of Muturu cattle, and could aid in the ecology, conservation, selection and breeding process. The use of morphometric traits as a discriminant index was accentuated in a related study in cattle where body size measurements were more useful to discriminate among cattle breeds, than either live weight or daily gain (Alberti *et al*., 2008). In related studies, Yakubu and Akinyemi (2010) was able to allocate correctly Uda ewes and rams to their a priori sexes using discriminant analysis; while Pinto *et al*. (2008) was able to separate stallions from mares.

CONCLUSION

The study revealed sexual dimorphism in Muturu cattle as male animals have higher WH, BL, EL, RL, NL, NC, CG, SW and RW compared to their female counterparts. The phenotypic relationships also showed varying correlation coefficients between the morphometric traits of bulls and cows. While three PCs were extracted in bulls, two PCs were sufficient to explain the generalized variance in the body parameters of cows. However, NL, HL, FL, BL and WH were the linear measures permitting discrimination between bulls and cows in the stepwise discriminant analysis. The use of multitrait information in separating the sexes of Muturu cattle appears promising since objective evaluation of populations is a major point for ecological studies, conservation and rational management of breeds.

REFERENCES

Adebambo, O. A. 2001. The Muturu: A rare sacred breed of cattle in Nigeria. *AGRI*, 31: 27-36.

Albertí, P., Panea, B., Sañudo, C., Olleta, J.L., Ripoll, G., Ertbjerg, P., Christensen, M., Gigli, S., Failla, S., Concetti, S., Hocquette, J.F., Jailler, R., Rudel, S., Renand, G., Nute, G.R., Richardson, R.I. & Williams, J.L. 2008. Live weight, body size and carcass characteristics of young bulls of fifteen European breeds. *Livest. Sci*., 114: 19–30.

Badyaev, A.V. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends Ecol. Evol*., 17: 369–378.

Chebo, C., Ayalew, W. and Wuletaw, Z. 2014. Traditional breeding practices and trait preferences of cattle farmers in Gamo Goffa Zone, Southern Ethiopia. *Animal Genetic Resources,* 55: 19–27.

Corrales, J.A., Cerón-Muñoz, M., Cañas, J.A., Herrera, C. R. & Calvo, S.C. 2011. Relationship between type traits and

milk production in Holstein cows from Antioquia, Colombia. *Rev. MVZ Córdoba*, 16: 2507-2513.

Corrales, R., Nasholm, A., Malmfors, B. & Philipsson, J. 2010. Population structure of Reyna Creole cattle in Nicaragua. *Trop. Anim. Health Prod*., 42: 1427–1434.

Cox, R. M., Stenquist, D.S. & Calsbeek, R., 2009. Testosterone, growth and the evolution of sexual size dimorphism. *J. Evol. Biol*., 22: 1586–1598.

Delgado, J. V., Barba, C., Camacho, M.E., Sereno, F. T. P. S., Martinez, A. & Vega-Pla, J.L. 2001. Livestock characterisation in Spain. *AGR*I, 29: 7-18.

Fairbairn, D. J. & Roff, D.A. 2006. The quantitative genetics of sexual dimorphism: Assessing the importance of sex linkage. *Heredity,* 97: 319-328.

Gilbert, R.P., Bailey, D. R. C. & Shannon, N. H. 1993. Linear body measurements of cattle before and after 20 years of selection for post-weaning gain when fed two different diets. *J. Anim. Sci*., 71: 1712-1720.

Hair, J. F., Black, W.C., Babin, B. J. & Anderson, R. E. 2009. Multivariate Data Analysis, 7th Edition, Prentice Hall.

ILRI. 2011. Cattle Breeds: Muturu. International Livestock Research Institute (ILRI) Animal Genetics Training Resource.

Ingleby, F. C, Innocenti, P., Rundle, H.D., & Morrow, E.H. 2014. Between-sex genetic covariance constrains the evolution of sexual dimorphism in Drosophila melanogaster. *J. Evol. Biol*., 27: 1721-1732.

Isaac, J. L. 2005. Potential causes and life-history consequences of sexual size dimorphism in mammals. *Mammal Review*, 35: 101-115.

Jafari, S. & Hashemi, A. 2014. Estimation of genetic parameters for body measurements and their association with yearling live weight in the Makuie sheep breed. *South Afr. J. Anim. Sci*., 44: 140-147.

Kern, E. L., Cobuci, J. A., Costa, C. N. & Pimentel, C. M. M. 2014. Factor analysis of linear type traits and their relation with longevity in Brazilian Holstein cattle. *Asian* Australas. *J. Anim. Sci.,* 27: 784-790.

Lener, L. M. & Donald, H. P. 1996. Modern development in animal breeding. Academic Press, London. 295 pp.

Li, L., Zhu, Y., Wang, X., He, Y. & Cao, B. 2014. Effects of different dietary energy and protein levels and sex on growth performance, carcass characteristics and meat quality of F1 Angus × Chinese Xiangxi yellow cattle. *J. Anim. Sci. Biotech*., 5: 21. doi:10.1186/2049-1891-5-21.

Maiwashe, A. N., Bradfield M. J., Theron, H.E. & van Wyk, J.B. 2002. Genetic parameter estimates for body measurements and growth traits in South African Bonsmara cattle. *Livest. Sci*., 75: 293-300.

Mank, J.E. 2009. Sex chromosomes and the evolution of sexual dimorphism: lessons from the genome. *Am. Nat*., 173:141-150.

Mavule, B.S., Muchenje, V., Bezuidenhout, C.C. & Kunene, N.W. 2013. Morphological structure of Zulu sheep based on principal component analysis of body measurements. *Small Rumin. Res*., 111: 23– 30.

Mwacharo, J.M., Okeyo, A.M., Kamande, G.K. & Rege, J.E.O. 2006. The small East African shorthorn zebu cows

Yakubu, A. & Ibrahim I. A. 2011. Multivariate analysis of morphostructural characteristics in Nigerian indigenous sheep. *Ital. J. Anim. Sci*., 10: 83-86.

in Kenya. 1: Linear body measurements. *Trop. Anim. Health Prod*., 38: 65-76.

Nafti, M., Khaldi, Z. & Haddad, B. 2014. Multivariate characterization of morphological traits in local Tunisian oases goats. *Animal Genetic Resources,* 55: 29–38.

NMA. 2012. Nigeria Meteorological Agency (NMA) Weather Data for Benue State. MIMET, Makurdi Office.

Oguntunji, A.O. & Ayorinde, K.L. 2014. Sexual size dimorphism and sex determination by morphometric measurements in locally adapted Muscovy duck (*Cairina moschata*) in Nigeria. *Acta Agric. Slovenica,* 104: 15–24.

Parés-Casanova, P.M. & Villalba, S.D. 2013. Application of varimax rotated principal component analysis in quantifying some zoometrical traits of a relict cow. *Korean J. Vet. Res*., 53: 7-10.

Parés-Casanova, P.M. & Mwaanga, E.S. 2013. Factor analysis of biometric traits of Tonga cattle for body conformation characterization. *Glob. J. Mul. App. Sci*., 1: 41-46.

Pinto, L.F.B., Almeida, F.Q., Quirino, C.R., Azevedo, P.C.N., Cabral, G.C., Santos, E.M. & Corassa, A. 2008. Evaluation of sexual size dimorphism in Mangalarga Marchador horses using discriminant analysis. *Livest. Sci*., 119: 161-166.

Polak J. & Frynta D. 2009. Sexual size dimorphism in domestic goats, sheep and their wild relatives. *Biol. J. Linn Soc.* 98: 872–883

Pundir, R. K., P. K. Singh, K. P. Singh &. Dangi, P.S. 2011. Factor Analysis of Biometric Traits of Kankrej Cows to Explain Body Conformation. *Asian Australas. J. Anim. Sci*. 24: 449-456.

Rege, J. E. O., Marshall, K. Notenbaert, A., Ojango, J. & Mwai, A.O. 2011. Pro-poor animal improvement and breeding—What can science do? *Livest. Sci*. 136: 15–28.

Rhen, T. 2007. Sex differences: genetic, physiological, and ecological mechanisms. In: Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism (D.J. Fairbairn, W.U. Blanckenhorn & T. Szekely, eds), Oxford University Press, Oxford. Pp. 167–175.

RIM. 1992. Nigerian Livestock Resources Vol. II. National Synthesis. Report by Resource Inventory and Management Limited (RIM) to Fed. Dept. of Livestock and Pest Control Services, Abuja, Nigeria.

Singh, P. K., Pundir, R.K., Alhawat, S.P.S., Kumar, N.S., Govindaiah, M.G., Asija, K. 2008. Phenotypic characterization and performance evaluation of Hallikar cattle in its native tract. *Ind. J. Anim. Sci.,* 78: 211-214.

Sole, M., Cervantes, I., Gutierrez, J.P., Gomez, M.D. and Valera, M. 2014. Estimation of genetic parameters for morphological and functional traits in a Menorca horse population. *Span. J. Agric. Res*., 12: 125-132.

SPSS, 2001. Statistical Package for the Social Sciences. SPSS Inc., New York.

Wyman, M. J. Stinchcombe, J. R. & Rowe, L. 2013. A multivariate view of the evolution of sexual dimorphism. *J. Evol. Biol*., 26: 2070-2080.

Yakubu, A., Salako, A.E., & Imumorin, I. G. 2011a. Comparative multivariate analysis of biometric traits of West African Dwarf and Red Sokoto goats. *Trop. Anim. Health Prod*., 43: 561-566.

Yakubu, A., Salako, A.E. & Abdullah A-R. 2011b. Varimax rotated principal component analysis of the zoometrical traits of Uda sheep. *Archiv. de Zootec*., 60: 813-816.

Yakubu, A. & Akinyemi, M. O. 2010. An evaluation of sexual size dimorphism in Uda sheep using multifactorial discriminant analysis. *Acta Agriculturae Scand. Section A,* 60: 74-78.

Yakubu, A., Ogah, D. M. & Idahor, K. O. 2009. Principal component of the morphostructural indices of White Fulani cattle. *Trakia J. Sci*., 7: 67-73.

Yamaki, M., Correa, G. S. S., Silva, M.A., Barbosa, L. & Torres, R.A. 2006. Evaluation of quail carcass traits using principal component analysis. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, August 13-18, Belo Horizonte, MG, Brazil, 2006.

REPEATABILITY ESTIMATES FOR SOME GROWTH AND REPRODUCTIVE TRAITS IN Napri-X BROILER FOUNDATION STOCK

[1]Adejoh-Ubani, E. O,* [1]Nwagu, B. I. [1]Adeyinka, I. A.[2] Orunmuyi, M. [3]Akinsola, O. M. [1]Olutunmogun, A. K., and [1]Umar, A. U.,

[1]National Animal Production Research Institute (NAPRI), Shika, Zaria, Kaduna State, Nigeria.
[2]Department of Animal Science, Federal University Oye-Ekiti, Ekiti State, Nigeria
[3]Department of Animal Science, University of Jos, Jos, Plateau State, Nigeria
Corresponding Author: lizzyubani@yahoo.com

Abstract: The objective of this study was to estimate repeatability of some growth and reproductive traits by generation and line in Napri -X broiler foundation stock under selection for three generations. A total of 6747 birds were used for the work. Traits considered were body weights at day old, 2,4,6 and 8 weeks of age, Neck length (NL), Back length (BL), Shank length (SL) and keel length (KL), Breast length (BRL) and Thigh length(TL). Reproductive traits include: {egg set, number fertile (NFERT), number hatch (NHAT), number dead in shell (NDD), percent fertility (PFERT), percent hatch (PHAT) and percent hatchability (PHATY)}. The lines (Sire line and Dam line) were developed respectively through selective breeding for body weight at eight weeks (BWT8) in a pedigreed foundation stock of broilers. Mating ratio was 1 cock: 6 hens within families and across lines. Selection was carried out from base to the third generation. Eggs for the study were appropriately marked and hatched in individual hatching compartments. Body weights were taken at hatch, 2, 4, 6 and 8 weeks of age. Morphometric traits were measured using a tape rule. Data on reproductive traits were recorded for seven hatches for each line. Statistical analyses were done using SAS.9.2 software for basic descriptive statistics. Fertility and hatchability percentages were transformed to arcsin ! % and analyzed according to Student's t-test and the trend of the results represented in charts. All measured variables (egg set, number fertile, number hatched and number dead in shell) and reproductive indices (percent fertility, percent hatchability and percent fertile) were significantly ($P<0.01$) different between the two lines. Sire line recorded a decreasing trend with an estimate of 15.57(egg set), 14.46 (number fertile), 11.46 (number hatch) and 2.86 (number dead in shell). Egg set (10.45, 11.85, 11.69 and 7.14), number fertile (9.47, 10.46, 9.65 and 5.25), number hatch (7.34, 5.66, 6.81 and 3.25) and number dead in shell (4.86, 5.23, 7.14 and 7.38) were the averages for sire and dam selected and control lines. Sire and dam lines selected and control recorded values of 89.16, 89.46, 83.06 and 76.58% (percent fertility), percent hatch (70.88, 53.52, 59.24 and 50.39%) and percent hatchability (80.27, 59.08, 71.32 and 64.12%), respectively. This implies that traits under selection were highly variable Generation 3 had the highest range of body weight from day old to eight weeks of age (39.91-2162.41g) compared to other generations (0-2). Repeatability estimates of reproductive measurable and computed indices by generation and line shows that line effect had higher estimates (0.125, 0.001, 0.045 and 0.023) compared to generation (0.018, 0.000, 0.041 and 0.020) with the least estimates for eggset, pfert, perhat and phaty. Conversely, pdd (0.009) for generation effect was numerically higher compared to the line effect (0.001). The implication of this is that the ability of these birds to repeat their present performance in the future is low and that high number of records is required to estimate the potentials of these birds across lines and generations.

INTRODUCTION

Repeatability (*R*) is the proportion of the phenotypic variance that is due to permanent effects (genetic effects and permanent environmental effects). It is a measure of the tendency for an animal to repeat the present performance in future (Fayeye, 2014). It is the average proportion of differences in the present records that is likely to be repeated in later records. It is important in prediction of breeding values from multiple records on the same animals as well as being very important in making culling decisions: When R is high we can cull animals of poor performance on the basis of the first record When R is low one should wait for more records before making a culling decision on the animal.

The magnitude of a repeatability estimate gives an indication of the extent to which selection applied at any stage will affect subsequent flock performance (Ibe, 1995). This research was conducted to estimate the repeatability of body- weight, morphometric traits and reproductive traits by generation and line in Napri-X broiler foundation stock.

MATERIALS AND METHODS

Location of the Study

The research was conducted at the Poultry Breeding Unit of Poultry Research Programme of the National Animal Production Research Institute (NAPRI) Shika, Zaria. Shika is located in the semi-arid, Northern Guinea savanna zone of Nigeria within latitude 11o8'N and 07o4'E with an elevation of 2178 feet (663.77 metres) above sea level. The average annual precipitation is 1,100mm, which spreads from late April or early May to mid- October, with a peak between June and September (wet season). The wet season is usually followed by "Harmattan", a period of cool, dry weather which lasts from mid-October to February (post rains). The mean maximum temperature varies from 27oC to 35oC depending on the season; and the mean relative humidity during Harmattan and wet season are 21 and 72 %, respectively (Ovimaps, 2014).

Experimental Birds

A total of 6747 birds were used for the work. Chicks were wing-banded at day old, brooded and reared to point of lay. Chicks were managed on deep litter system in pens. The pens were cleaned, disinfected and littered with wood shavings, before the arrival of the chicks. Adequate temperature was maintained in the brooding and rearing houses, clean drinking water and broiler starter mash/finisher were provided *ad lib.* The starter mash contained 19.44%CP, while the finisher had 18.60%CP. Routine vaccinations were appropriately ensured as at when due.

Data collection

Reproductive Traits

Data on fertility and hatchability were recorded for seven hatches for each line.

Percent fertility, percent hatch and percent hatchability were calculated as follows.

$$\text{Percent Fertile} = \frac{Total\ number\ of\ fertile\ eggs}{Total\ number\ of\ eggs\ set} \times 100$$

$$\text{Percent Hatch} = \frac{Total\ number\ of\ chicks\ hatched}{Total\ number\ of\ eggs\ set} \times 100$$

$$\text{Percent Hatchability} = \frac{Total\ number\ of\ chicks\ hatched}{Total\ number\ of\ fertile\ eggs} \times 100$$

Fertility and hatchability percentages were transformed to arcsin ! % and analysed according to Student's t-test (Snedecor and Cochran, 1967).

Data Analysis

Data was subjected to analysis of variance (ANOVA) using the SAS system (1998) and means were compared by Duncan's Multiple Range Test (Duncan, 1995).

Repeatability (R) was estimated using the standard expression giving by Falconer (1989), expressed as:

$$\mathbf{R} = \frac{\hat{\sigma}^2_p}{\hat{\sigma}^2_p + \hat{\sigma}^2_e}$$

Where;

$\hat{\sigma}^2_p$ is the individual variance component

$\hat{\sigma}^2_e$ is the variance due to error

$\hat{\sigma}^2_p + \sigma^2_e$ is the total phenotypic variance.

RESULTS AND DISCUSSION

Table 1: Least Squares Means for body weight and morphometric traits for pooled data of the two lines at different generations

Traits	GEN 0 (N=1401)	GEN 1 (N=1949)	GEN 2 (N=2070)	GEN 3 (N=1327)
BWT0	38.37	38.92	38.93	39.88
BWT2	214.24^{ab}	147.19^{b}	222.69^{a}	224.84^{a}
BWT4	601.84^{b}	438.15^{c}	616.12^{a}	613.78^{a}
BWT6	1145.04^{a}	1072.60^{c}	1140.08^{a}	1100.89^{b}
BWT8	1805.91^{d}	1936.21^{c}	2085.88^{b}	2162.41^{a}
NL	11.45	9.64	9.95	8.48
BL	22.86	21.90	21.51	19.75
KL	10.68	9.30	9.68	8.67
BRL	10.95	9.49	9.70	9.12
TL	9.36	8.50	9.53	7.97
SL	9.61	7.54	7.29	7.56

[abcd]Means with different superscripts on the same row are significantly different (P<0.05). BWT0- BWT8 = Body weight from 0-8 weeks of age, NL-Neck length, BL-Backlength, KL-Keel length, BRL-Breast length, TL-Thigh length, SL- Shank length. Gen 0- Gen 3=Base generation – Generation 3, N=Total number.

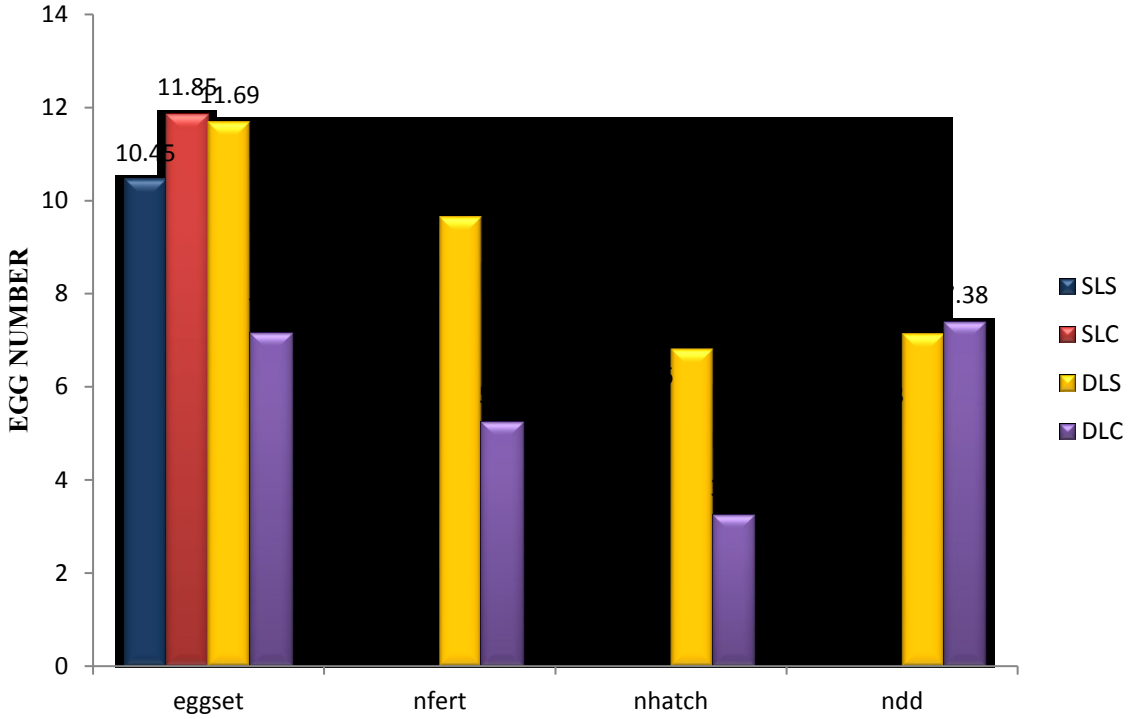


Figure 1: Pooled analysis of reproductive measured variables of sire and dam lines

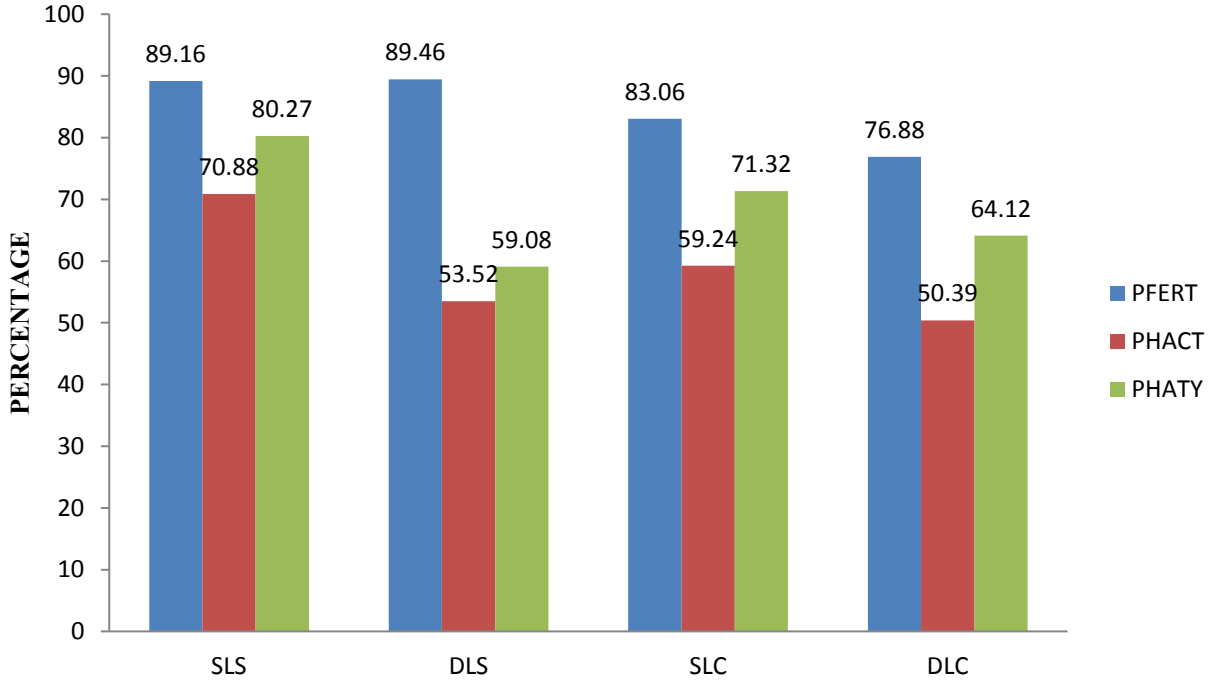


Figure 2: Pooled effects of reproductive indices of sire and Dam lines

Table 2: Repeatability estimates of body weight and body measurement traits

	Generation	Line
BWT0	0.016	0.007
BWT2	0.36	0.023
BWT4	0.213	0.113
BWT6	0	0.252
BWT8	0.086	0.28
NL	0.348	0
BL	0.103	0.008
KL	0.232	0

BRL	0.138	0
TL	0.114	0
SL	0.281	0

BWT0- BWT8= Body Weight at 0- 8weeks of age; NL=Neck length, BL= Body length, KL=Keel length, BRL= Breast length, TL=thigh length SL= Shank length

Table 3: Repeatability estimates of reproductive measurable and computed indices Traits

TRAITS	Repeatability by generation	Repeatability by line
EGGSET	0.018	0.125
PFERT	0.000	0.001
PERHAT	0.041	0.045
PHATY	0.020	0.023
NDD	0.009	0.001

Pfert-percent fertility, pdd-percent number dead in shell, phaty-percent hatchability

Least squares means for body weight and morphometric traits when data were pooled across generations are shown in Table 1. Except for BWT0 and morphometric traits that did not differ significantly ($p>0.05$), all other traits showed highly significant ($p<0.01$) differences across generations. Generation 3 recorded higher values for body weight traits and differed statistically ($p<0.01$) compared to other generations except generation 2 which had statistical similarity with generation 3. Generation 1 had higher values for all the morphometric traits except for TL which was highest in generation 2. The results of the mean body weight and morphometric traits showed an increasing trend across ages in generations for all lines as expected, which shows a positive relationship between body weight and age of the flock. This trend is supported by the works of Adejoh- Ubani (2006) and Adeyinka *et al.* (2006) on naked neck chicken flocks. The results suggest that age is a major determinant of growth and physiological development of animals. Body weights ranged from 38.37 to 2162.41g in the three generations in this study. These estimates exceeded some of the estimates reported in literature at similar ages by several researchers (Adeyinka *et al*., 2006; Faruque *et al*., 2007). Body weights of broiler naked neck chickens at day old, 2, 4, 6 and 8 weeks were reported by Adeyinka *et al*. (2006) as 37.22 ± 0.32g, 210.46 ± 1.97g, 744.33 ± 4.31g 1351.3 ± 7.91g and 2428.1 ± 14.61g, respectively. Faruque *et al*. (2007) reported body weight at hatch and 8 weeks of age for Non-descript, Hilly and Naked neck genotypes as 31.2, 30.5, 31.7 g and 481.9, 449.0 and 476.0 g, respectively. The estimate obtained in this study was higher than the range (374-1981 g) reported by Ojedapo (2013) from 2 weeks through 8 weeks of study in Marshall Broiler birds. The range obtained in this study was lower compared to range (40.90 – 2576 g) reported by Yalcin *et al*. (1997) from day old to 7 weeks of age in a study conducted in Israel for selected commercial broiler lines. The observed differences in reported estimates could be due to genetic and environmental differences. Genetic differences were found to have a significant and non-significant effect on body weights of chicks at day old through 8 weeks of age. Earlier investigators including Taha, (2010), Nadia *et al*. (2009) and Enaiat *et al*. (2010) also obtained significant differences using different strains and breeds. In Iraq, Ali (2006) reported a non-significant difference between three broiler hybrids (Lohman, Ross and Hubbard) in their body weights at 1, 14, 28, 42, and 56 days of age. The implication of significant and non-significant effect of genetic lines on body weight is that body weight traits are being governed by additive and non-additive gene action in this study. Differences in least squares means for BWT0 through BWT8 observed among the lines within the generations could be due to changes in the genetic constitution of the lines after selection and the influence of environmental factors such as the different climatic conditions in which each generation was raised. This observation is supported by the report of Aboul-Seoud, (2008) who concluded that observed differences between the various estimates reported in literature for body weight of poultry birds (Chickens, quails, ducks etc) recorded at a particular age may be possibly due to one or more of the different reasons such as: differences in the climatic and managerial conditions under which different flocks were reared, to the possible differences in genetic makeup of the different flocks, or differences in the statistical manipulation of the data used to obtain the estimates. Estimates of morphometric traits obtained in this study were higher than some of the numerical values obtained for broiler lines in tropical regions by several researchers; Kabir *et al.* (2008), Ubani and Adeyinka (2008), Ubani *et al*., 2011, Ojedapo 2013) but lower than some of the ranges obtained in the temperate regions by several researchers {(Cahaner *et al*. (1992)}. Ubani *et al*. (2011) reported least square means of naked neck broiler chickens as 9.43 ± 0.03cm at 8

weeks of age for thigh length. Adeyinka *et al*. (2006) obtained back length of 15.99 ± 0.05 cm and values of 5.63 ± 0.04 for keel length in a flock of naked neck broiler chickens. Ubani *et al.* (2011) reported keel length value of 10.53 ± 0.10cm at 8 weeks in broiler chickens. Ojedapo (2013) obtained thigh length of 6.10cm in ecotype breeds of chickens. Cahaner *et al*. (1992) recorded keel length value of 12.22cm in a naked neck broiler stock, which was higher than the range (8.40 – 10.71 cm) obtained in this study.

Pooled Analysis for Measured Egg Traits and Reproductive Indices

Pooled measured reproductive variables and computed indices in the sire and dam broiler lines for all generations are shown in Figures 1 and 2, respectively. The measured variables {egg set, number fertile (NFERT), number hatch(NHAT) and number dead in shell (NDD)} and reproductive indices {percent fertility (PFERT), percent hatch (PHAT) and percent hatchability (PHATY)} were statistically significant ($P<0.01$) between the sire and dam lines selected and control. Egg set (10.45, 11.85, 11.69 and 7.14), number fertile (9.47, 10.46, 9.65 and 5.25), number hatch (7.34, 5.66, 6.81 and 3.25) and number dead in shell (4.86, 5.23, 7.14 and 7.38) presented the above averages for sire and dam selected and control lines. Sire and dam lines selected and control recorded values of 89.16, 89.46, 83.06 and 76.58% (percent fertility), percent hatch (70.88, 53.52, 59.24 and 50.39%) and percent hatchability (80.27, 59.08, 71.32 and 64.12%), respectively. The significant difference observed between the lines across different generations in egg set, number fertile, number hatch and number dead in shell agrees with the report of Chao and Lee (2001) in Taiwan County chickens. Estimated values for all the measurable traits were within the range reported by Szwaczkowski *et al*. (2003) and could be attributed to sex-linked effects, but not consistent with estimates of some authors (Baffour-Awuah *et al*., 2000; Saatci *et al*., 2006).

Repeatability Estimates for all the Body weight and Morphometric Traits

Table 2 shows the repeatability estimates for all the body weight and body measurements traits under consideration. Estimates were low to moderate for pooled analysis for generation and line traits. Low estimates of repeatability had the following distributions (0.016, 0, 0.103, 0.138 and 0.114) for BWT0, BWT6, BWT8, BRL and TL respectively. BWT2, BWT4, NL, KL and SL showed respective moderate estimates of 0.36, 0.213, 0.348, 0.232 and 0.281 for generation effect. The frequencies of low distributions were higher in pooled line effect as compared to the generation effect among the growth traits under study. The distribution of low estimates of repeatability was as follows: 0.007, 0.023, 0.113, 0, 0.008, 0, 0, 0 and 0 (BWT0, BWT2, BWT4, NL, BL, KL, BRL, TL and SL) while moderate estimates were observed between BWT6 and BWT8 (0.252 and 0.28).

The low to moderate repeatability estimates for all groups for both body weight and morphometric traits across all ages, lines and generations observed in this study could be due to the effects of non-additive genes or high environmental effects. The implication of this is that the ability of these birds to repeat their present performance in the future is low. The estimates obtained in this study (0.018, 0.35, 0.10, 0.23, 0.13, 0.11, 0.28 for BWT8, NL, BL, KL, BRL, TL and SL respectively in generation and 0.28, 0, 0.008, 0, 0, 0, 0 for line) disagree with the results of Ubani *et al*. (2011), who reported high repeatability estimates of 0.99, 0.95, 0.96, 0.92 and 0.94 for BWT8, NL, BL, SL and KL respectively in naked neck broiler chickens. Differences obtained in this study could be attributed to breed/ line differences and environmental effect.

Repeatability estimates of reproductive measurable and computed indices by generation and line in broiler foundation stock

Repeatability estimates of reproductive measurable and computed indices by generation and line in broiler foundation stock are shown in Table 3 Line effect had higher estimates (0.125, 0.001, 0.045 and 0.023) compared to generation (0.018, 0.000, 0.041 and 0.020) with the least estimates for eggset, pfert, perhat and phaty. Conversely, pdd (0.009) for generation effect was numerically higher compared to the line effect (0.001). Causes of low estimates could be due to relatively small numerator, which may have occurred due similarity of individuals which is attributable to either genetic or environmental effects. A second cause of low repeatability could be attributed to environmental influences which could have arisen as a

result of insufficiently controlled conditions such as temperature,

CONCLUSION

Low repeatability values were generally obtained for all traits in this study, which implies that prediction of the next record has low accuracy. It therefore means that more number of records will be required to adequately characterize individuals for their transmitting ability for growth and reproductive traits in this flock of birds between lines and across generations.

REFERENCES

Aboul-Seoud, D.I.M. (2008). Divergent selection for growth and egg production traits in Japanese quail. A Ph.D Thesis, Department of Animal Production, Faculty of Agriculture Al-Azhar University, Egypt.Pp 145.

Adeyinka, I.A., Oni, O.O., Nwagu, B.I. and Adeyinka, F.D. (2006). Genetic parameter estimates of body weight of naked neck broiler chicken. *International Journal of Poultry Science*, 5(6): 509-592.

Ali, E.A. (2006). Effect of dietary supplementation with organic chromium on productive, physiological and immunological traits of three hybrids of broiler chicken. M.Sc. Thesis, College of Agriculture, Baghdad University, Iraq, Pp 71-80.

Baffour-Awuah, O., Ampofo E. and Dodoo, R. (2000). Predicting the live weight of sheep by using linear body measurement. *Ghana Journal of Agricultural Science*. **33:** 207-212.

Cahaner, A., Deeb, N. and Gutrnan, M. (1992). Improving broilers growth at high temperature by the naked-neck gene; *In Proceedings of the 19th World Poultry Congress,* Amsterdam. Netherlands. 57-60.

Chao, C.H. and Lee, Y.P. (2001). Relationship between reproductive performance and immunity in Taiwan country chickens. *Poultry Science*, 80: 535-540.

Enaiat, M. M., Amina, A. S. and Eman, M. A. (2010). A comparative study of productive and physiological performance between two local strains of chickens. *Egyptian Poultry Science,* 30 (1): 297-316.

Falconer, D. S. (1989). Introduction to quantitative genetics. 3rd edition. Longman group, Essex, England. Pp 388.

Faruque, S., Sarker, N. R.; Islam, M. N.; Sarker, M. S. K.(2007). Performance of native chicken under intensive system. *Journal of the Bangladesh Agricultural University*, 5 (2) 283-288.

Fayeye, T.R. (2014). Genetic principles and animal breeding. Happy Printing Enterprises, Ilorin. Kwara State. Pp 256.

Ibe, S.N. (1995). Repeatability of growth trait in Nigerian local chickens using early records. *Nigerian Journal of Animal Production*, 23(2): 103-106.

Kabir, M., Yakubu, H., Akpa, G.N., Jokthan, G.E, Abdu, S.B., Abdulrashid, M. and Adamu H.Y. (2008). Estimates of repeatability of body weight and body conformation traits in Anak 2000 strain of broiler chickens. *Proceedings 13th Annual Conference, Animal Science Association of Nigeria, Ahmadu Bello University, Zaria*.Pp 18-21.

Nadia, R.L., R.A. Hassan, E.M. Qota and H.M. Fayek, (2009). Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. *International Journal of Poultry Science*. 7: 134-150.

Ojedapo, L.O (2013) Evaluation of body weight and other linear parameters of Marshall broiler for repeatability estimates. *International Journal of Applied Agricultural and Apicultural Research,* 9(1&2): 175-181.

Okon, B., Ogar, I.B. and Mgbere, O.O. (1996). Interrelationship of live body measurements of broiler chickens in a humid tropical environment. *Nigerian Journal of Animal Production,* 24(1): 7-12.

Ovimaps, (2014). Ovi location map; Ovi earth imagery date; July 3rd, 2014.

Saatci, M., Omed, H. and Dewi, I.A. (2006). Genetic parameters from univariate and bivariate analysis of egg and weight traits in Japanese quail. *Poultry Science,* 85: 185-190.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods, 8th Edition, Iowa State University Press. ISBN 978-0-8138-1561-9.Pp 255.

Szwaczkowski, T., Cywa-Benko, K. and Wezyk, S. (2003). A note on inbreeding effect on productive and reproductive traits in laying hens. *Animal Science Papers and Reports*, Page 21.

Taha, A.E. (2010). Laying performance of Japanese quails divergently selected for body weight under different rearing and lighting systems. Ph.D. Thesis. Faculty of Veterinary Medicine Alexandra University Egypt. Pp 78.

Ubani, E.O.A., Adeyinka, I.A., Nwagu, B.I., Abeke, F.O., Sekoni, A.A., Otu, M.O. and Iyiola–Tunji, A.O. (2011). Estimates of repeatability for some growth traits in naked neck broiler chickens. In: *Proceedings of the 36th Conference of Nigerian Society for Animal Production* (NSAP) 48–50.

Yalcin, S., Settar, P., Ozkan, S. and Cahaner, A. (1997). Comparative evaluation of three commercial broiler stocks in hot versus temperate climates. *Poultry Science*, 76: 921-929

.

HERITABILITY AND REPEATABILITY OF SOME EGG PRODUCTION TRAITS IN SELECTED JAPANESE QUAILS (*Cortunix cortunix japonica*)

[1]Attah, E. O., [2]Nwagu, B. I., [1]Kabir, M. and [3]Abbaya, H. Y

[1]Department of Animal Science, Ahmadu Bello University, Zaria, PMB 1044, Zaria, Nigeria.

[2]National Animal Production Research Institute (NAPRI), Shika, Zaria, Nigeria.

[3]Department of Animal Production, Adamawa State University Mubi,
P. M. B 025, Mubi, Adamawa State, Nigeria.

Abstract: This experiment was conducted to estimate heritability and repeatability parameters of some egg production traits in three generations of Japanese quails under selective breeding. A total of 600 healthy chicks were sexed at day 21 and randomly shared into 2 groups consisting of 450 birds (300 females and 150 males) in the selected line and 150 birds (100 females and 50 males) in the control bred line. The birds were mated at 7 weeks of age and in ratio of 1 male: 2 females throughout the generations of selection. Selection index was constructed for all the traits of interest (age at sexual maturity, egg number and egg weight). Multi-trait animal models were used to estimate the (co)variance components based on average information restricted maximum likelihood method (AIREML) using R software. Heritability estimates for egg production traits across the various generations ranged from low to high (0.11- 0.46) while high values were recorded for repeatability (0.48 - 0.80). Moderate to high heritability estimates of age at sexual maturity, body weight at first egg, egg number and egg weight indicate that the use of selection index could lead to genetic improvement. Therefore, the study recommends that selection based on an index should be applied in breeding programs for the improvement of egg production traits in Japanese quails.

INTRODUCTION

Quail farming serves as a form of alternate poultry production in many countries and is gaining attention from the entrepreneurs, farmers and researchers. According to Muthukumar and Dev-Roy (2005), quails are efficient in converting feed into animal protein of high biological value and hence are the cheapest source of protein for human diet.

Japanese quails are characterized by many favorable traits such as fast growth rate, quick sexual maturity, short generation interval, small body size and significant egg production ratio compared to other farm birds (Narinc *et al*., 2014; Molino *et al*., 2015). Quail meat is popular for increasing the sexual instinct in human beings (Jadhav and Siddiqui, 2007), can adopt excellent market potential with higher proteins (26%) and less fat (3%). It is further claimed that the quails, being delicate, sensitive and friendly birds, occupying small space, consuming less feed and maturing within short time, are the most appropriate avian specie to be used for all types of poultry research work (Minvielle, 2004). Days needed to produce a certain number of eggs is an important economic trait in poultry as any decrease in days needed will consequently decrease production cost (Khadiga *et al*., 2016). Mahmoud *et al*. (2015) indicated that when days needed to produce the first ten eggs is included as a selection criterion, the total merit of egg production and growth traits was improved and showed favorable genetic correlation with production traits in Japanese quails, increasing the profitability of such selection program.

In order to have success through selection for a particular trait or a set of traits, proper knowledge of genetic parameter is the most important. Genetic parameters describe genetic and environmental variation and might vary among populations and environments and should thus be estimated in different populations and environments (Khaldari *et al*., 2010).

Genetic studies on quails will help breeders to design suitable programs for genetic improvement of this specie using reliable estimates of genetic parameters to evaluate direct and indirect responses to selection.

Adequate information on genetic parameter estimates (heritability and repeatability) of egg production traits in quail population is necessary for designing appropriate breeding plans for genetic improvement. Since egg production traits are inter-related, consideration of such relationships is relevant to choosing appropriate selection methods.

This research is therefore, geared towards estimating genetic parameters based on individual selection for

body weight, egg weight, body weight at first egg, egg number and age at first egg over three generations.

MATERIALS AND METHODS

The Study Site: This study was conducted at the Poultry Unit of the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria. Zaria is located in the Northern guinea Savannah ecological zone of Nigeria. The area lies between latitude $11^0 11'$ N and longitude $7^0 38'$E, at an altitude of 686m above sea level. The climate is relatively dry, with a mean annual rainfall of 700-1400mm, occurring between the months of April and September as reported by (Akpa *et al*., 2002; Ovimaps, 2015). The dry season begins around the middle of October, with dry cold weather that ends in February. This is followed by relatively hot, dry weather from March to sometimes in April, when the rain begins. The mean minimum and maximum daily temperature range from 14^0C to 24^0C during the cool season and from 19^0C to 36^0C during the hot season. The relative humidity varies between 19% and 35% in dry season and between 63% and 80% in the wet season (IAR, 2013).

The Foundation Stock: The foundation stock refers to the reference population. A total of 700 adult Japanese quails (*Coturnix coturnix japonica*) were obtained from National Veterinary Research Institute (NVRI) Vom, Plateau state at 3 weeks of age. Six hundred (600) healthy quails comprising of 400 female quails and 200 male quails were selected and reared together on deep litter to generate a broad-based population. For effective fertilization of eggs, mating ratio of 1 male: 2 females were used.

The birds were mated at 7 weeks of age, collection of eggs for incubation commenced when the birds were at least 9 weeks of age as this allowed for effective fertilization of the eggs. Eggs for incubation were collected twice a day for a period of 5 days. The frequency of collection was to avoid damage of the eggs. On the fifth day, wholesome and clean eggs were fumigated and placed in the incubator. Seven batches of eggs were incubated at an interval of five days. On the 15^{th} day of incubation, the eggs were candled. The number of infertile eggs were taken note of and removed, while fertile eggs were transferred to the hatchery. Immediately the chicks were hatched, they were individually weighed on an electronic sensitive scale to obtain hatch weight and each chick was identified by numbered leg band. Hatched chicks in each week were brooded and reared together before selection. These birds formed the foundation stock which was divided into two, namely: the random bred control and the selected line.

Management of Experimental Birds: Two weeks before the hatching of the quail chicks, preparation for brooding commenced. On arrival, the quail chicks were rapidly unboxed and inspected before placing them in the brooding pen. The respective six hatches were brooded separately on floor pens. The brooding period lasted for a period of 2 weeks, after which the chicks were transferred to rearing pen. Birds were fed measured quantity of feed containing 24% CP, 2904 ME Kcal/kg throughout the starter phase (from hatch to four weeks of age), after which they were fed a breeder diet containing 21% CP and 2800 ME Kcal/Kg (Kaye 2014) from the 5^{th} week of age to the end of the experiment in all the three generations. Feed intake and body weight gain was observed on a weekly basis. The feed composition fed to the birds is shown below.

Table 1: Starter and breeder diet used for the experiment

Ingredient	Starter diet (%)	Breeder diet (%)
Maize	53	55.25
Groundnut cake	25	24
Soya bean cake	18.3	12
Bone meal	3	3
Limestone	-	5
Salt	0.2	0.25
Methionine	0.25	0.2
Lysine	0.10	0.1
Vitamin premix	0.25	0.2
Total	100	100

Breeding Programme: In other to generate enough birds for the experiments, mating was carried out within the base population in a mating ratio of 1: 2 male to female ratio for better fertility (El-Fiky, 2002; Dogan *et al*., 2013). Eggs were collected twice a day one week after the beginning of mating to ensure that all eggs were fertile and pedigreed (identified according to dam). The eggs were weighed on an electronic sensitive scale (Electronic pocket scale, EHA 251, 500g capacity). Whole labeled eggs were taken to Umar hatchery at Galma along Jos road, Zaria where they were fumigated and placed in an incubator (37.8^0 C; 65-75% humidity). On the 15^{th} day of incubation, the eggs were candled, infertile eggs were removed and

recorded, recorded the eggs were removed and recorded, while fertile eggs were transferred for the last three days of incubation to the hatcher (37.8^0 C; 65-90% humidity) in marked compartment according to dam number. The chicks were wing tagged and brooded together on deep litter system to obtain the G_1 generation. Adequate heat was supplied during the brooding period using electric bulbs and kerosene stove.

At 4 weeks of age, birds were sexed and a total number of 600 chicks gotten from the foundation were randomly selected and divided into two, namely; the random bred control and the selected line. 300 female quails and 150 male quails were used as selected line while 100 females and 50 males were used as random bred control lines in the experiment. The random bred control was maintained as non-selected pedigreed population to correct for environmental trends and/or fluctuations brought about by random genetic drift (Havenstein *et al*., 1988). In each contemporary selected line, male quails were separated from the female quails to prevent uncontrolled mating. Each of the experimental birds were placed in individual wooden wire mesh cages measuring 35 by 30cm and monitored for onset of sexual maturity, weekly body weight, weekly feed intake and short term (60 days) egg production. Known quantity of feed was offered. All possible preventive measures were taken to avoid feed spillage. Clean drinking water was provided *ad libitum*. Eggs collected from each selected female quail was labeled, weighed on an electronic sensitive scale (EHA 251, 500G capacity) and recorded on a daily basis.

Selection in the different Generations: At the end of 30 days, the female quails were subjected to selection. For this purpose, top 60% of the female quails from each selected line were selected based on a selection index incorporating body weight at first egg (BWFE), egg weight (EW) and egg number (EN). Male quails in each selected line were selected based on their breeding value which were predicted based on the means of their half and full-sisters. The male quails were more intensely selected than the female quails because fewer male quails are needed to maintain a mating ratio of 1 male: 2 Females. These selected male and female quails were then used as parents of the next generation of birds. Mating was controlled by allowing a male to stay with allocated female for one day. Special care was taken to avoid inbreeding among parental birds used for mating. The selective breeding of the Japanese quail was carried out in 3 successive generations. Top 60% of female quails and top 40% of male quails selected from G_1 population became the parents of G_2 population, which in turn, yielded the parents of G_3 generation

Data Collection: Data on the following growth and performance traits were collected:

1. Body weight at first egg (g): The female quails were monitored closely. Once any female quail drops her first egg, the body weight of the quail was recorded using an electronic sensitive scale (Electronic pocket scale, EHA 251, 500g capacity) and recorded in grams.
2. Egg weight (g): All eggs laid by the female quails during the short-term egg production were weighed individually and recorded on a daily basis using an electronic sensitive scale calibrated in grams (Electronic pocket scale, EHA 251, 500g capacity).
3. Age at sexual maturity (days): Male quails were inspected daily from 25^{th} day of age to determine the onset of cloacal gland foam production. This day was recorded as the age of reaching sexual maturity of a male. Age at sexual maturity in female quails was expressed in days from hatching till the day of the first egg laid for each female quail.
4. Egg number: Eggs laid by each female quails was collected daily and the total over 60day period was recorded as the egg number.
5. Feed intake: Measured quantities of feed were served to each quail daily and the leftover measured the following morning. Adequate measures were taken to prevent feed spillage.
6. The formula below was used to determine the feed conversion ratio;

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed consumed}}{\text{body weight gain}}$$

Data Analysis: Model equation for the trial was Nested design with the following models:

$Y_{ijklm} = \mu + G_l + B_k + D_k + S_i + e_{ijklm}$

Where; Y_{ijklm} = observation of the m^{th} offspring on the j^{th} dam mated to the i^{th} sire with the k^{th} bodyweight in the 1^{st} generation

μ = the overall mean; G_l = effect of the 1^{st} generation (G= 0,1,2,3); B_k = effect of the k^{th} bodyweight on

productive traits; D_k = effect of the j^{th} dam mated to the i^{th} sire; S_i = effect of the i^{th} sire; e_{ijkl} = random error.

Genetic Parameter Estimates Heritability: Heritability estimates in generation 1,2 and 3 was estimated using sire variance and calculated according to Becker (1984). Here, individual families within lines were considered as groups, the group sum/trait was assigned to each family as individual observations. All variance components were generated using the nested procedure of SAS and this was used to evaluate heritability due to sire using the model shown below:

$Y_{ij} = \mu + S_i + e_{ij}$

Where Y_{ij} = Observation on the j^{th} offspring of the i^{th} sire in the trait being considered.

μ = Overall population mean for the trait; S_i= Random effect of the i^{th} sire; e_{ij} = Random error

The above model also assumes that the reference population is random bred with no inbreeding, experimental units share common environment, maternal effect is absent, non-additive genetic effects or variances are absent or zero and no sex linkage effect.

Heritability was estimated with the expression:

$$h^2s = \frac{4\sigma^2 s}{\sigma^2 s + \sigma^2 w}$$

Where h^2s = narrow sense heritability, using sire model; σ^2_S = sire variance component; σ^2w = error variance component

Standard error (S.E.) of the estimate given by Becker (1984) was used and is as follows:

$$S.E.(h^2) = \sqrt[4]{\frac{2(1-t)^2[1+(k-1)t]^2}{k(k-1)(s-1)}}$$

Where; t = interclass correlation $= \frac{\sigma^2 s}{\sigma^2 w + \sigma^2 s}$

K = number of offspring per sire; S = number of sires

Repeatability Estimates: Repeatability coefficient was estimated using the expression below;

$$R = \frac{{\sigma_1}^2}{{\sigma_1}^2 + {\sigma_e}^2}$$

Where; ${\sigma_1}^2$ = Variance component due to differences among individuals; ${\sigma_e}^2$ = Error variance component.

The formula below according to Becker (1984) was used to estimate standard error of the estimated repeatability coefficient.

$$S.E(R) = \frac{2(1-R)^2(1+(k-1)R)^2}{k(k-1)(n-1)}$$

Where; k = number of records taken on each individual; n = number of individuals; R = estimated repeatability of a trait.

RESULTS AND DISCUSSION

Table 2 shows the heritability estimates of egg production traits and feed conversion ratio in Japanese quail in Generation one, two and three. The heritability estimates of egg production trait across the three different generations ranged from moderate to high. Heritability values for egg number ranged from 0.11 in generation one to 0.23 and 0.26 in generation two and three respectively.

Heritability value of body weight at first egg was estimated as 0.21 to 0.0.34 with an increase in trend across the selected population from generation one to generation three. This implied that selection using index increased the inheritance of weight at first egg as generation of selection increased. Higher value (0.58) was reported by Ozsoy and Aktan (2011) while Peng *et al*. (2010) reported lower value (0.11) for the same trait in Japanese quails. Similar high value for heritability of body weight at first egg (0.56) in chickens has been reported by Oleforuh-Okoleh (2011).

Heritability values of body weight at first egg observed in this study vary from low to high. The estimate of heritability for body weight at first egg reported in the present study is however within the range (0.12-0.54) reported by some previous researchers (Lofti *et al*., 2012: Okenyi *et al*., 2013; Momoh *et al*., 2014) for body weight at first egg in Japanese quail.

Variations in heritability values of body weight at first egg could be attributed to differences in selection methods and population size. According to reports by Falconer and Mackay (1995), heritability for a particular trait may take different values according to the population, environmental conditions and method of calculation. The high estimates of heritability of body weight at first egg obtained in the present study clearly indicate the existence of substantial amount of additive genetic variance for the traits studied in this population.

The heritability of egg number in generation one, two and three was low (0.11, 0.21 and 0.26 respectively) with an increase across the generations. Okenyi *et al*. (2013) also observed a similar increase in heritability values of egg production from G_0 to G_2 (0.12, 0.19 and 0.48 respectively) in quails. Similar heritability estimates of egg number ranging from 0.05 to 0.29 have been reported by several authors (Aboul-Hassan, 2001; Adeogun and Adeoye, 2004; Momoh, 2005; Luciano *et al*., 2013; Okenyi *et al*., 2013; Kaye *et al*., 2016 and

Abbaya *et al*., 2017). However, higher heritability estimates of 0.40 to 0.88 for egg number in Japanese quails was reported by Helal, (1995), El-Fiky (1995) Aboul-Seoud (2008) and Kumari *et al*. (2009).

Wide range of heritability estimates in different studies supports the fact that egg number could be greatly influenced under environmental conditions and population size. The increase in heritability estimates for egg number across generations indicates increasing additive genetic variance across generations (Falconer, 1989). Genetic estimates of egg production traits in different breeds and/or stains have been cited by many investigators who found that there were many variations in these estimates according to the differences of the genetic make-up (Khali *et al*., 2004; Nurgiartiningsih *et al*., 2004).

There was an increase in heritability estimates of egg weight in this study from moderate in G_1 (0.27) to high in G_3 (0.46) in Japanese quails. Abbaya *et al*. (2017) also observed a similar increase in trend from G_1 to G_2 (0.27 to 0.45). Comparable heritability estimates have been reported by a number of scientists regarding egg weight. Andre *et al*. (2011), Hussain *et al*. (2014) and Sari *et al*., (2016) recorded 0.54, 0.31 0.45 and 0.44 respectively. The values observed in heritability of egg weight in the present study is higher than 0.12, 0.25, 0.25 and 0.20 reported by Abdul-Mounsef (2005), Saatci *et al*. (2006), Ozsoy and Aktan (2011) and Daikwo *et al*. (2014) respectively in the same trait in quails but lower than 0.48, 0.51, 0.59 and 0.69 reported by Kocak *et al*. (1995), El-Fiky, (2002); Momoh *et al*. (2014) and Kaye (2014) in Japanese quail respectively. Luciano *et al*. (2013) reported 0.41 and 0.39 as heritability value for two quail strains in their study which is comparable to the result obtained in the present study while Minvielle (1998) reported 0.36 to 0.65 as heritability values for egg weight in quails.

The variations in the heritability estimates in this study and in literature might be attributed to method of estimation, strain, environmental effects and sampling error due to small data size or sample size (Prado-Gonzalez *et al*., 2003). The heritability of egg weight, which falls within the range of 0.27 (G_1) to 0.46 (G_3) indicate that the traits as they appeared in the index were most probably passed on from the parents to their progenies without much influence by the environment.

The heritability estimates of 0.21 to 0.34 for feed conversion ratio were recorded in the present study. There is dearth of information in literature regarding genetic parameters for individual feed conversion ratio of Japanese quails. However, the result obtained in this study are consistent with the range of 0.23 to 0.44 reported by Bahie El-Deen *et al*. (2008) in Japanese quails and in line with the corresponding values reported on chicken, which ranged from 0.14 to 0.56 by various authors.

Devi *et al*. (2012) recorded low to high (0.02 to 0.59) heritability estimates for feed conversion ratio in Japanese quail while Foomani *et al*. (2014) reported medium to high values (0.20 to 0.55) for the same trait. According to Varkoohi *et al*. (2010), heritability estimate of feed conversion ratio in quails is 0.46 while Liu *et al*. (2017) recorded 0.29 for the same trait in chicken. Wide variations in heritability of feed conversion ratio have been observed in this study and also in literature and it could be attributed to genetic makeup of the birds, feed intake and other management practices.

Table 2: Heritability Estimates of egg production traits and feed conversion ratio in Japanese quail in Generation one, two and three

	h^2 (± SE)		
Trait	**G_1**	**G_2**	**G_3**
ASM	0.21 ± 0.18	0.18 ± 0.16	0.29 ± 0.23
BWFE	0.20 ± 0.19	0.29 ± 0.17	0.34 ± 0.21
EN	0.11 ± 0.21	0.23 ± 0.32	0.26 ± 0.40
EWT	0.27 ± 0.11	0.34 ± 0.26	0.46 ± 0.19
FCR	0.26 ± 0.08	0.21 ± 0.12	0.34 ± 0.15

ASM: Age at Sexual Maturity; BWFE: Body Weight at First Egg; EN; Egg Number; EWT: Egg Weight; FCR; Feed Conversion Ratio; G_1; Generation 1; G_2 Generation 2; G_3; Generation 3; h^2: heritability; SE: Standard error of heritability.

Table 3 shows the repeatability estimates of egg production traits and feed conversion ratio in Japanese quail in Generation one, two and three. The repeatability estimates for Age at Sexual maturity (ASM), Body weight at first egg (BWFE), Egg number (EN), of Egg weight (EWT) and feed conversion ratio (FCR) ranged from moderate to high (0.32-0.74) in the three generations of selection.

Due to dearth of information available in literature regarding repeatability of egg production traits and feed

conversion ratio of quails, intensive comparison of the estimates obtained in the present study with previous reports on quails is not feasible. However, Amao *et al*. (2013) and Bisenan (2011) reported repeatability estimates of 0.76 to 0.87 and 0.99 for the body weight in Japanese quails respectively whereas 0.51 to 0. 65 was recorded in the present study for body weight at first egg. Akpa *et al*., (2006) recorded repeatability estimate of 0.85 and 0.99 for body weight of Japanese quail at 12 and 24 weeks respectively. Similar high estimates (0.83, 0.59, 0.60 and 0.55) were reported by Ojo *et al*. (2012) for body weight in Hubbard broiler chickens at week 2, 4, 6 and 8. Obike *et al*. (2016) also recorded high (0.52 - 0.99) repeatability estimates in three different lines of Japanese quails used in their study.

The repeatability estimates of 0.68 to 0.85 were recorded in the present study for egg weight in Japanese quails. Reports of repeatability estimate of Egg weight in quails are scarce but Akpa *et al*., (2006) recorded repeatability estimate of 0.77 and 0.92 for egg weight of Japanese quail at 12 and 24 weeks respectively, also Abanikannda *et al*., (2016) reported high repeatability co-efficient of 0.56 for egg weight in quails. These high estimates generally agreed with the report in literature on repeatability in chickens.

Akpa *et al*. (2008) and Amoa *et al*. (2013) reported high repeatability estimates for egg quality traits in quails (0.58 to 0.99 and 0.55 to 0.99) respectively. The high repeatability estimates reported for egg production traits in the present study could be attributed to genetic factors. Variations in observed values for repeatability may be due to the attributed influence of environmental and age-related factors (Falconer 1989) and due to different species of poultry involved. The high repeatability estimates recorded for egg production traits imply possible high expected genetic response from selection for the traits while fewer records will be required to characterize the inherent transmitting ability of individuals. This can lead to some savings in the cost of collecting additional data. The repeatability of the traits increased as selection progressed, thus indicating progressive parental influence on these traits according to generation.

Table 3: Repeatability Estimates of Egg Production Traits and FCR in Japanese quails for the selected populations

	Repeatability (R)		
	G_1	G_2	G_3
ASM	0.55 ± 0.20	0.48 ± 0.23	0.62 ± 0.15
BWFE	0.63 ± 0.15	0.67 ± 0.15	0.74 ± 0.14
EN	0.50 ± 0.12	0.73 ± 0.30	0.80 ± 0.28
EWT	0.57 ± 0.17	0.53 ± 0.18	0.71 ± 0.18
FCR	0.38 ± 0.23	0.32 ± 0.11	0.49 ± 0.17

ASM: Age at Sexual Maturity; BWFE: Body Weight at First Egg; EN; Egg Number; EWT: Egg Weight; FCR; Feed Conversion Ratio; G_1; Generation 1; G_2 Generation 2; G_3; Generation 3; R: Repeatability

CONCLUSION

Moderate to high values of heritability estimate of age at sexual maturity, body weight at first egg, egg number and egg weight indicate that the use of selection index could lead to genetic improvement. Also, the high Repeatability coefficient for egg production recorded in this study indicate that fewer records would be required to characterize the inherent transmitting ability of these quails

REFERENCES

Abbaya, H.Y., Akpa, G. N., Adedibu, I. I. and Attah, E. O. (2017). Heritability and Response to Selection for Egg Production and Egg Quality Traits in Japanese Quails. Nigerian Journal of Genetics, 31:13-22

Abdel-Mounsef, N.A. (2005). Non-genetic factors affecting some productive traits in Japanese quail. M.Sc. Thesis Faculty of Agriculture Al-Azhar University Cairo. Egypt. Pp 66

Aboul- Hassan M.A. (2001). Crossbreeding effects on some growth and egg production traits among two strains of Japanese quail. *Al–Azhar Journal Agricultural Resources,* 34: 41-57.

Aboul-Seoud, D.I. (2008). Divergent selection for growth and egg production traits in Japanese quail. M.Sc. Thesis, Faculty of Agriculture, Al-Azhar University, Cario, Egypt. Pp.159.

Adeogun, I.O. and Adeoye, A. A. (2004). Heritability and phenotypic correlations of growth performance traits in Japanese quails. *Livestock Research for Rural Development*, 16(12):102-106. Retrieved from http://www.lrrd.org/lrrd16/12/adeo16103.htm

Akpa, G. N., Asirbo, O. E., Oni, O.O., Alawa, J.P., Dim, N. I., Asinwo, O. A. and Abubakar, B. Y. (2002). Milk production by Agropastoral Red Sokoto Goats in Nigeria. *Topical Animal Health and Production*, 34(6):525-533.

Akpa, G.N., Kaye, J, Adeyinka, I.A. and Kabir, M. (2006). Repeatability of body weight and egg traits in Japanese quail (*Coturnix coturnix japonica*). *Savannah Journal of Agriculture*, Pp 118-129.

Akpa, G.N., Kaye, J, Adeyinka, I.A. and Kabir, M. (2006). Repeatability of body weight and egg traits in Japanese quail (*Coturnix coturnix japonica*). *Savannah Journal of Agriculture*, Pp 118-129.

Amao, S. R., Ojedapo, L.O. and Ogundipe, R. I. (2013). Repeatability estimates of egg quality traits of pharaoh quail *(Coturnix coturnix japonica)* reared in derived Savanna Zone of Nigeria. *International Journal of Agriculture and Bioscience,* 2(4): 156-159. www.ijagbio.com.

Andre*, M.*, Elais, N., Alexandre, S., Thays, C., Oliveira de Quandros, C., Ana, P.S.T. and Rafael, T. (2011). Genetic characterization of egg weight, egg production and age at fist egg in quails*. Revista Brasileira de Zootecnia*. 40; 95-99

Bahie El-Deen M., El-Tahawy, W.S., Attia, Y.A. and Meky, M.A. (2008). Inheritance of age at sexual maturity and its relationships with some production traits of Japanese quails. *Egyptian Poultry Science* 28(4): 1217-1232

Becker, W.A. (1984). Manual of Quantitative Genetics. 5th Edition, Pp 189. Academic Enterprises, Pullman, Washington, USA.

Bisesan, O. (2011). Estimate of repeatability of body weight and some growth traits in Japanese quail. M.Sc thesis, Department of Animal Production, Faculty of Agriculture, University of Agriculture, Ilorin, Nigeria.

Daikwo, S. I., Dim, N. I. and Momoh, O. M. (2014). Genetic parameters of some Egg Production traits in Japanese quail in a tropical Environment. *IOSR Journal of Agriculture and Veterinary Science,* 7(9): 2319-2380.

Devi, K.S., Gupta, B.R., Prakash, M.G and Reddy, R.A (2012). Genetic parameters of feed efficiency and Daily weight gain in Japanese quails. *Tamilnadu Journal Veterinary and Animal Sciences,* 8(1) 6-13.

Dogan, N., Ali, A. and Tolga. S. (2013). Effects of cage type and mating ratio on Fertility in Japanese quails (*Coturnix coturnix japonica*) eggs. *Agriculture Science Developments*, 2(1):4-7

El-Fiky, F.A. (1995). Effects of intensive inbreeding on some productive traits in Japanese quail. *Annals Agricultural Science Moshtohor Journal,* 34, 189- 202

El-Fiky, F.A. (2002). The effect of parental age on their productivity and progeny growth in Japanese quail. *Egypt Journal Applied science*, 17:13-27

Falconer, D. S. (1989). Introduction to Quantitative Genetics. 3rd Edition, Longhman Science and Technology, London.

Falconer, D.S. and Mackay, T.F.C. (1995). Introduction to Quantitative Genetics. Fourth edition. Addision Wesley Longman, New York.

Fayeye, T.R. (2014). Genetic principles and animal breeding. Happy Printing Enterprises, Ilorin, Nigeria

Foomani, N.N., Zerechdaran, S., Azari, M.A. and Lofti, E. (2014). Genetic parameters for feed efficiency and body weight traits in Japanese quail. *British Poultry science 55, 298-304.*

Havenstein, G.B., Nestor, K.E. and Bacon, W.L. (1988). Comparison of pedigreed random bred control systems for use with artificial selection in the Japanese quail. *Poultry science* 67: 357-366.

Helal, M.A. (1995). The Effect of Crossing on the Performance of Japanese Quail. M. Sc. Thesis, Veterinary Medical College, Alexandria University, Egypt. Pp 81

Hussain J., Akram, M., Sahota, A.W., Javed, K., Ahmad, H.A. and Mehmood, S. (2014). Selection for higher three-week body weight in Japanese quail: 2. Estimation of genetic parameters. *Journal of Animal and Plant Sciences,* 24(3): 869-873

IAR (2013), Metrological Service Unit.

Jadhav, N.V., and Siddiqui M.F. (2007). Handbook of poultry production and management, 2nd edition. *Jaypee Brothers medical Publishers, new Delhi, pp 383*

Kaye, J. (2014). Genetic parameters of body weight and some economic important traits in the Japanese Quails *(Coturnix coturnix japonica)*. Ph.D Thesis, Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria. Pp. 39-71

Kaye, J., Akpa, G. A., Alphonsus, C., Kabir, M., Zahraddeen, D. and Shehu, D. M. (2016). Response to Genetic Improvement and Heritability of Egg Production and Egg Quality Traits in Japanese Quail (*Coturnix coturnix japonica*). *American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS) 16 (1): 277-292*

Khadiga, A. G., Mahmoud, B. Y. F. and El-Full, E. A. (2016). Genetic evaluation of early egg production and maturation traits using two different approaches in Japanese quails. *Poultry Science* 95:774-779

Khaldari, M., Pakdel, A., Yegane, H. M., Javaremi, A. N. and Berg, P. (2010). Response to selection and genetic parameters of body and carcass weights in Japanese quail selected for 4-week body weight*. Poultry Science*, 89(9): 1834-1841.

Khalil, M.K., Al-Homidan, A.H., hermes, J.K. (2004). Cross breeding components in age at first egg and egg production for crossing Saudi Chickens with white Leghorn. *Livestock Research & Rural Development 16 (1).*

Kocak, C. Altan, O. and Akbas, Y. (1995). An investigation of different production traits of Japanese quails (*Coturnix coturnix japonica*). *Turkish Journal of Veterinary and Animal Science* 19(1): 65-71.

Kumari, P.B., Gupta, B.R., Prakash, M.G. and Reddy, A.R. (2009). Genetic study on body weights of Japanese quails. *Indian Journal Poultry Sci.* Vol: 44 Issue: 3(Abstract).

Liu, T., Luo, C., Wang, J., Ma, J., Shu, D., Lund, M.S. (2017). Assessment of the genomic prediction accuracy for feed efficiency traits in meat-type chickens. *PLOS ONE 12(3): e0173620*

Lotfi, E., Zerehdaran, S. and Ahani Azari, M. (2012). Estimation of Genetic parameters for egg production traits in Japanese quail (*Coturnix coturnix japonica). Archiv fur* Geflügelkunde*,* 76(2): 131-135. ISSN 0003-9098

Luciano P. S., Jeferson, C. R., Aline, C. C., Felipe, G. S., Cristina, M. B,. Fabyano, F. S. and Robledo, A. T. (2013). Genetic parameters of body weight and egg traits in meat-type quail. *Livestock Science*, 153(1–3):27-32

Mahmoud, B. Y. F., Khadiga, A. G. and El-Full, E. A. (2015). Correlated response to selection for some egg performance traits in egg line of Japanese quail. *Animal Production Science,* 56(5):913-917

Minvielle, F. (1998). Genetics and Breeding of Japanese quail for Production around the World. In: *Proceedings 6th Asian Pacific Poultry Congress, Japanese Branch of the WPSA, Nagoya, Japan*, Pp 122-127.

Minvielle, F., (2004). The future of Japanese quail for research and production. *World's Poultry Science Journal*, 60: 500-507.

Molino, A.B., Garcia, E.A., Santos, G.C., Vieira-Filho, J.A., Baldo, G.A.A., and Almeida-Paz, I.C.L. (2015). Photostimulation of Japanese quail. *Poultry Science* 94: 156-161

Momoh, O.M. (2005). Genetic and phenotypic evaluation of the Nigerian heavy chicken ecotype and its crossbreds with the light ecotype. Ph.D Thesis, Dept. Animal Breeding and physiology, University of Agriculture Makurdi. Pp 167.

Momoh, O.M., Gambo, D. and Dim, N. I. (2014). Genetic parameters of growth, body and egg traits in Japanese quails (*Coturnix coturnix japonica*) reared in Southern Guinea Savannah of Nigeria. *Journal of Applied Bioscience,* 79: 6947-6954. ISSN 1997-5902

Muthukumar, S.P., and Dev Roy, A. K. (2005). Alternate Poultry Production in India. An overview. Sanda Publishers. http://www.dairyyearbook.com/poultryNews1.aspx

Narinc D and Aksoy T. (2014). Effects of multi-trait selection on phenotypic and genetic changes in a meat type dam line of Japanese quail. *Journal of the Faculty of Veterinary Medicine Kafkas University,*20: 231-238.

Nurgriartiningsih, V.M., Mieluz, N., Preisinger, R., Schmutz, M., Schuelar, L. (2004). Estimation of genetic parameters based on individual and group mean records in laying hens. *British Poultry Science.* 5: 604-610.

Obike, O. M., Obi, O. C. and Onwuchekwa, J. U. (2016). Repeatability estimate of growth traits in Sigmond strain of Japanese quails reared in humid tropics. *Nigerian Journal of Agriculture, Food and Environment.* 12(1):99-102

Ojo, O. A., Akpa, G. N., Adeyinka, I. A., Iyiola-Tunji, A. O. and Mukasa, C. (2012). Estimates of repeatability for body weight and conformation traits in Hubbard broiler chickens reared in the tropics. *Proceedings of the 37th Annual Conference of the Nigerian Society for Animal Production*. Pp. 58-60.

Okenyi, N., Ndofor-Foleng, H. M., Ogbu, C. C. and Agu, C. I. (2013). Genetic parameters and consequences of selection for short-term egg production traits in Japanese quail in a tropical environment. *African Journal of Biotechnology,* 12(12): 1357-1362.

Oleforuh-Okoleh, V. (2011). Estimation of genetic parameters and selection of egg production traits in a Nigerian local chicken ecotype. ARPN, *Journal of Agriculture and Biological Sciences* 6: 54-57.

Ovimaps (2015). Ovimap location: ovi earth imagery date 23rd October, 2014

Özsoy, A. N. and Aktan, S. (2011). Estimation of Genetic Parameters for Body Weight and Egg Weight Traits in Japanese quails. *Trends Animal Veterinary Science,* 2 (1): 17-20

Peng, Y., Ping, D.J., Jie S., Song, P.J., Zuo, P.A., Hua, L.Z., Jin, P.Y. and Yan, W. (2010). The genetic parameter estimation of egg production related traits of yellow quail. *Journal of Anhui Agricultural Sciences*, 2: 742-745.

Prado-Gonzalez, E. A., Ramirez-Avila, L. and Segura-Correa, J. C. (2003). Genetic Parameters for body weight of Creole chickens from southeastern Mexico using an animal model. *Livestock Research and Rural Development,* 15: 1-7.

Saatci, M., Omed, H. and Dewi, I.A. (2006). Genetic parameters from univariate and Bivariate Analysis of egg and weight traits in Japanese quail. *Poultry Science* 85: 185-190.

Sari, M.T., Uci, M. and Saatci, M (2016). Genetic parameters of egg quality traits in long-term pedigree recorded Japanese quail. *Poultry Science, Volume 95, Issue 8;1743-1749*

SAS. (2004). SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC., USA. *series, 3rd edition*. McGraw Hill, New York. pp 433

Varkoohi, S., Moradi S. B. M., Pakdel, A., Nejati J. A., Zaghari, M. and Kause, A. (2010). Response to selection for feed conversion ratio in Japanese quail. *Poultry Science* 89: 1590-1598.

THE EFFECTS OF NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS (nsSNPs) of DQB1 GENE OF TROPICAL GOATS ON PROTEIN FUNCTION AND STABILITY

Abdulmojeed Yakubu[1,2]*, Adebowale E. Salako[3], Marcos De Donato[2,4] Clement E. Isidahomen[5] and Ikhide G. Imumorin[2,6,7*]

[1]Department of Animal Science, Nasarawa State University,
Keffi, Shabu-Lafia Campus, P.M.B., 135, Lafia, 950101, Nigeria.
[2]Animal Genetics and Genomics Laboratory, International Programs,
College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853. USA.
[3]Animal Breeding and Genetics Laboratory, Department of Animal Science,
University of Ibadan, Ibadan, Nigeria.
[4]Tecnologico de Monterrey, Escuela de Ingenieria y Ciencias, Mexico.
[5]Department of Animal Science, Faculty of Agriculture,
Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[6]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, 30332. USA
[7]African Institute for Bioscience Research and Training, Ibadan, Nigeria.
Corresponding Authors: abdulmojyak@gmail.com; igi2@cornell.edu

Abstract: DQBI gene is a member of the highly polymorphic MHC complex which is fundamental to the vertebrate immune system, encoding molecules that bind parasite-derived peptides for presentation to T-cells and subsequent initiation of an immune response. In this study, SNAP2, PROVEAN and PolyPhen-2 computational algorithms were used to identify non-synonymous or amino acid-changing SNPs (nsSNPs) that are deleterious to DQB1 structure and/or function in Nigerian goats. Genomic DNA in 60 animals (20 each of West African Dwarf, Red Sokoto and Sahel goats of both sexes) was utilized for polymorphism identification. There was consensus among the three algorithms in identifying D34R, Y64K, E68M, V70R, A81N and G86Y as being deleterious among the sixteen nsSNPs investigated. However, two nsSNPs (V70R and G86Y) showed altered protein-protein interaction patterns as they were found in the buried region. Their damaging status was verified by the values of total energy change after energy minimization (-6699.902 and 50.808kJ/mol, respectively) which were quite higher than that of the native DQB1 protein (-6793.618 kJ/mol). The two nsSNPs also had lower overall ERRAT quality factor values (84.1 versus 76.9 and 75.8%, respectively). Combined mutational analysis comparing the Amutant (beneficial nsSNPs) and Bmutant (deleterious nsSNPs) together with the native protein also showed that they all had a zero RMSD score and TM (1.00) but differed in terms of energy of minimization, Ramachandran plot and ERRAT protein structure quality evaluation. This study is the first computational analysis of the nsSNPs in highly polymorphic DQBI gene of Nigerian goats and will be a valuable resource for future population-based pathological association studies in the tropics.

Keywords: Tropics, non-synonymous substitutions, goats, DQBI gene, *in silico* tools

INTRODUCTION

The MHC gene is well known to be involved in the vertebrate immune system and encodes antigen recognition proteins used in the adaptive immune response. Polymorphism of this gene has become a topic of interest in the past decades (Shen *et al*., 2014; Shrivastava *et al*., 2015; Stear *et al*., 2005). The diversity of MHC genes may directly influence the survival of individuals against infectious diseases (Yasukochi *et al*., 2012). DQB1 gene is a member of the MHC class II genes. This gene has a single open reading frame of 786 bp, being organized in five exons and displaying 95–97% nucleotide identity with its bovine and ovine cDNA orthologous sequences (Amills *et al*., 2004**)**. DQB1 exon 2 genotypes have been found to be related to nematode resistance in Lubo hybrid goat in China (Feng, 2008). In a related study, Shen *et al*. (2014) reported that this gene plays an important role in the resistance to *Cystic echinococcosis* in Chinese Merino sheep. The large

amounts of data on Single Nucleotide Polymorphisms (SNPs) create new challenges for research on structures and interactions and is probably the most important problem of DNA variation studies, namely linking genetic variation with phenotypic variation and then with natural selection. Obviously, the phenotypic effect of a nucleotide substitution is always caused by structural or functional changes in DNA, RNA or protein (Sunyaev *et al*., 2001). The huge number of SNPs makes it difficult for researchers to plan costly population-based genotyping.

Nonsynonymous Single Nucleotide Polymorphisms (SNPs) are coding variants that introduce amino acid changes in their corresponding proteins (Kelly *et al*., 2014). Although many SNPs are phenotypically neutral, non-synonymous SNPs (nsSNPs) often have deleterious effects on protein structure or function. nsSNPs are located in protein coding regions and result in an amino acid substitution in the corresponding protein product. As such, nsSNPs can alter the structure, stability, or function of proteins, and are often associated with livestock diseases. Therefore, it is important to distinguish those nsSNPs that affect protein function from those that are functionally neutral. The advents in computational algorithms are useful for predicting the impact of amino-acid substitutions on protein structure and function. In order to gain functional insight into mutation caused by amino acid substitution to protein function and expression, special emphasis has been laid on molecular dynamics simulation techniques in combination with in silico tools such as PROVEAN, PolyPhen 2.0, I-Mutant 2.0, PANTHER, nsSNP Analyzer and SNAP (Kelly and Barr, 2014; Patel *et al*., 2015; Ugbo *et al*., 2015).

In Nigeria, sub-Saharan Africa, there is dearth of information on the functional effects of nsSNPs at the MHC-DQB1 locus of goats. Therefore, this work was undertaken mainly to perform a computational analysis of the nsSNPs in the DQB1 gene and to identify the possible deleterious mutations and to check the stability and functional activity of the modelled structures for the mutant proteins. This will possibly facilitate the conduct of association analysis and evaluation of the nsSNPs as genetic markers for disease resistance/susceptibility in farm animals.

MATERIALS AND METHODS

nsSNPs identification

Genetic variation of the DQB1 gene of Nigerian goats was analyzed from data obtained by sequencing of exonic region of this innate immune gene. Primers designed using data from Amills *et al*. (2004) were employed to amplify an exon 2 fragment of 228 bp of DQB1 from Genomic DNA in 60 animals (20 each of West African Dwarf, Red Sokoto and Sahel goats of both sexes) for polymorphism identification. The deduced amino acid sequences of the Nigerian goats were compared with the published caprine, ovine, bovine, and porcine sequences to obtain synonymous and nonsynonymous amino acid substitutions as reported in an earlier study (Yakubu *et al*., 2013).

Prediction of functional impact of nsSNPs

Functional effects of nsSNPs were predicted using the following in silico algorithms: SNAP2, PROVEAN and Polyphen-2. SNAP2 is used for the prediction of impact of missense mutation based on neural network and improved machine-learning methodologies (Bromberg and Rost, 2007). For each mutant, SNAP2 returns three values: the binary prediction (neutral/non-neutral), the reliability index (RI, range 0–9) and the expected accuracy that estimates accuracy on a large dataset at the given RI. PROVEAN is a software tool which predicts whether an amino acid substitution or indel has an impact on the biological function of a protein (Choi and Chan, 2015). PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) utilizes a combination of sequence and structure based attributes and uses naive Bayesian classifier for the identification of an amino acid substitution and the effect of mutation. The output levels of probably damaging and possibly damaging were classified as deleterious (≤ 0.5) and the benign level being classified as tolerated (≥ 0.51). Combined mutational analysis was also done incorporating all the nsSNPs found to be beneficial by SNAP2, PROVEAN and PolyPhen-2 (Amutant) and the deleterious ones (Bmutant) in order to reduce the possibility of false positives and exploit the effect of correlated mutations as these may enhance or detract the functional property of a protein as earlier reported (Yakubu *et al*., 2017b)

Protein stability analysis

I-Mutant version 2.0 was used to evaluate nsSNP induced changes in protein stability. It is a Support

Vector Machine-based web server for the automatic prediction of protein stability changes upon single-site mutations (Capriotti *et al*., 2005). I-Mutant2.0 correctly predicts whether the protein mutation stabilises or destabilises the protein in 80% of the cases when the three-dimensional structure is known and 77% of the cases when only the protein sequence is available (http://folding.biofold.org/cgi-bin/i-mutant2.0.cgi).

Homology modelling

The Phyre2 server was used to generate 3D structural models for native and each of the 16 nsSNPs including Amutant and Bmutant. This aligns an input target with pre-existing templates to generate a series of predicted models (Kelly *et al*., 2015). Upon successful modelling of the three-dimensional structure using Phyre2, RMSD was calculated by superimposing all the mutants over the native using TMalign. Tm-Align was used to calculate Tm-scores and Root Mean Square Deviation (RMSD) (http://zhanglab.ccmb.med.umich.edu/TM-align/). A TM-score of <0.2 is equivalent to a random structure from the PDB and a TM-score of 0.5 or greater indicates the proteins have a very high probability of being in the same SCOP/CATH fold (Zhang and Skolnick, 2005). An RMSD ≥2.0 has negative effect on stability and function of the protein (Han *et al*., 2006). Total energy after energy minimization was calculated for the native and each altered model using the GROMOS96 implementation of Swiss PDB viewer.

Validation of homology modeling

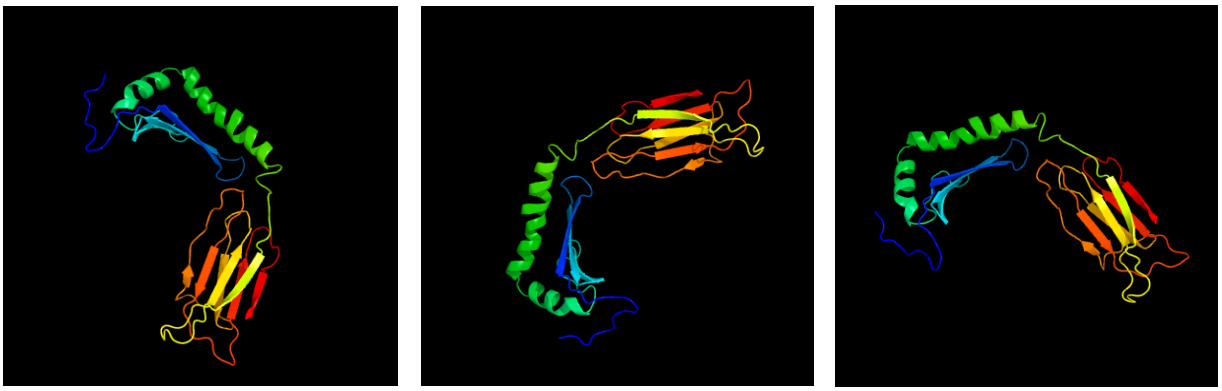
Model quality was checked for native and altered proteins by Ramachandran plot using software RAMPAGE (mordred.bioc.cam.ac.uk/~rapper/rampage.php) as well as ERRAT method. ERRAT is a program for verifying protein structures determined by crystallography. Error values are plotted as a function of the position of a sliding 9-residue window. The error function is based on the statistics of non-bonded atom-atom interactions in the reported structure (compared to a database of reliable high-resolution structures). http://services.mbi.ucla.edu/ERRAT/.

Prediction of protein-protein interaction sites

cons-PPISP is a consensus neural network method trained to predict whether or not a surface residue is in the interaction site (http://pipe.scs.fsu.edu/ppisp.html). Given the structure of a protein, cons-PPISP will predict the residues that will likely form the binding site for another protein. A residue contact could be Positive (P), Negative (N) and Buried and not predicted (-). The inputs to the neural network include position-specific sequence profiles and solvent accessibilities of each residue and its spatial neighbours. On a benchmark set of 22 protein complexes, cons-PPISP shows 71% prediction accuracy and 50% coverage of actual interface residues.

RESULTS

Out of a total of sixteen nsSNPs screened, eleven (D34R, G45D, L46K, Y64K, E68M, V70R, D75A, D77T, A81N, P84R and G86Y) were predicted to be deleterious in Nigerian goats by SNAP, while eight (D34R, D38V, Y64K, E68M, V70R, D75A, A81N and G86Y) (PROVEAN) and nine (E31F, D34R, D38V, G45D, Y64K, E68M, V70R, A81N and G86Y) (PolyPhen-2) were predicted to be deleterious in Nigerian goats (Table 1). However, there was consensus among the three algorithms in identifying D34R, Y64K, E68M, V70R, A81N and G86Y as being harmful.



a b c

Figure 1: Predicted 3D structures of native and mutant DQB1 proteins of Nigerian goats

a: Native DQB1 protein

b: Amutant DQB1 protein of Nigerian goats

c: Bmutant DQB1 protein of Nigerian goats

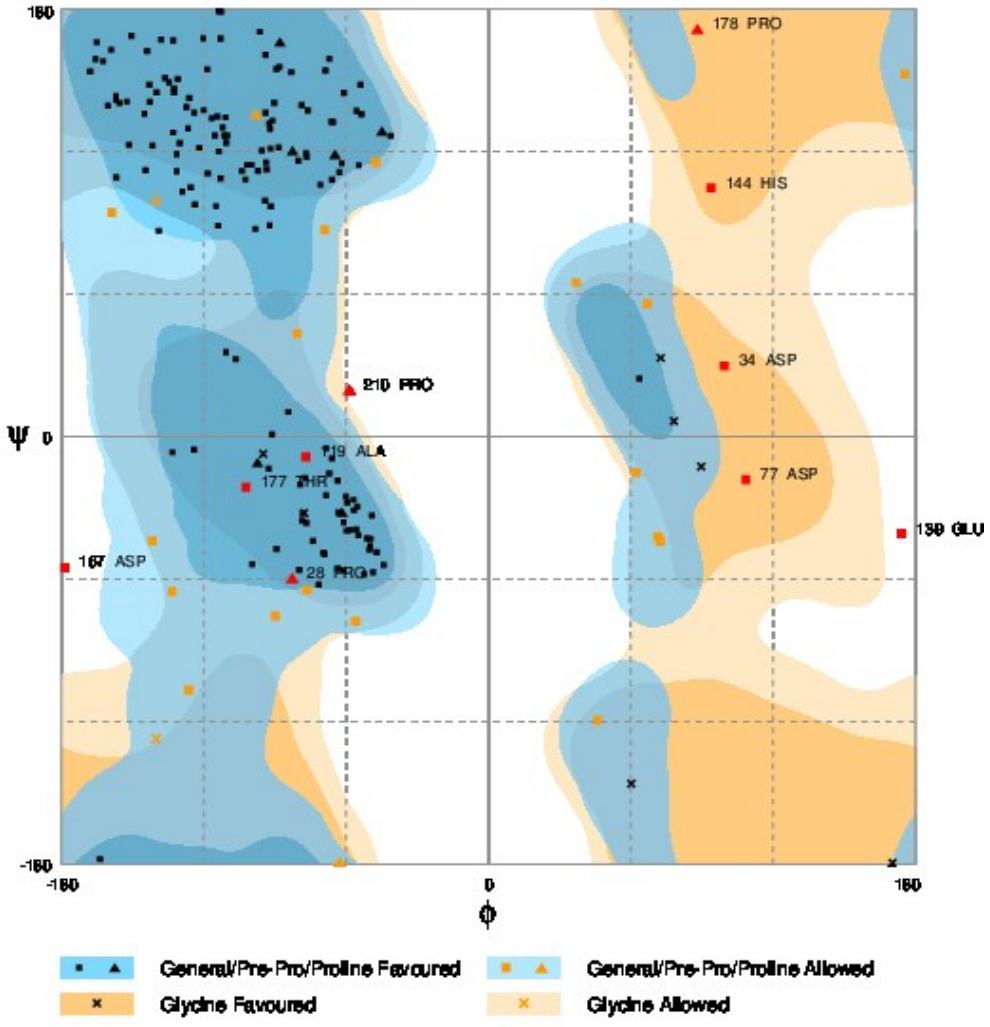


Figure 2: Ramachandran plot for native DQB1 protein

FNumber of residues in favoured region (~98.0% expected) : 164 (84.1%)

Number of residues in allowed region (~2.0% expected) : 21 (10.8%)

Number of residues in outlier region : 10 (5.1%)

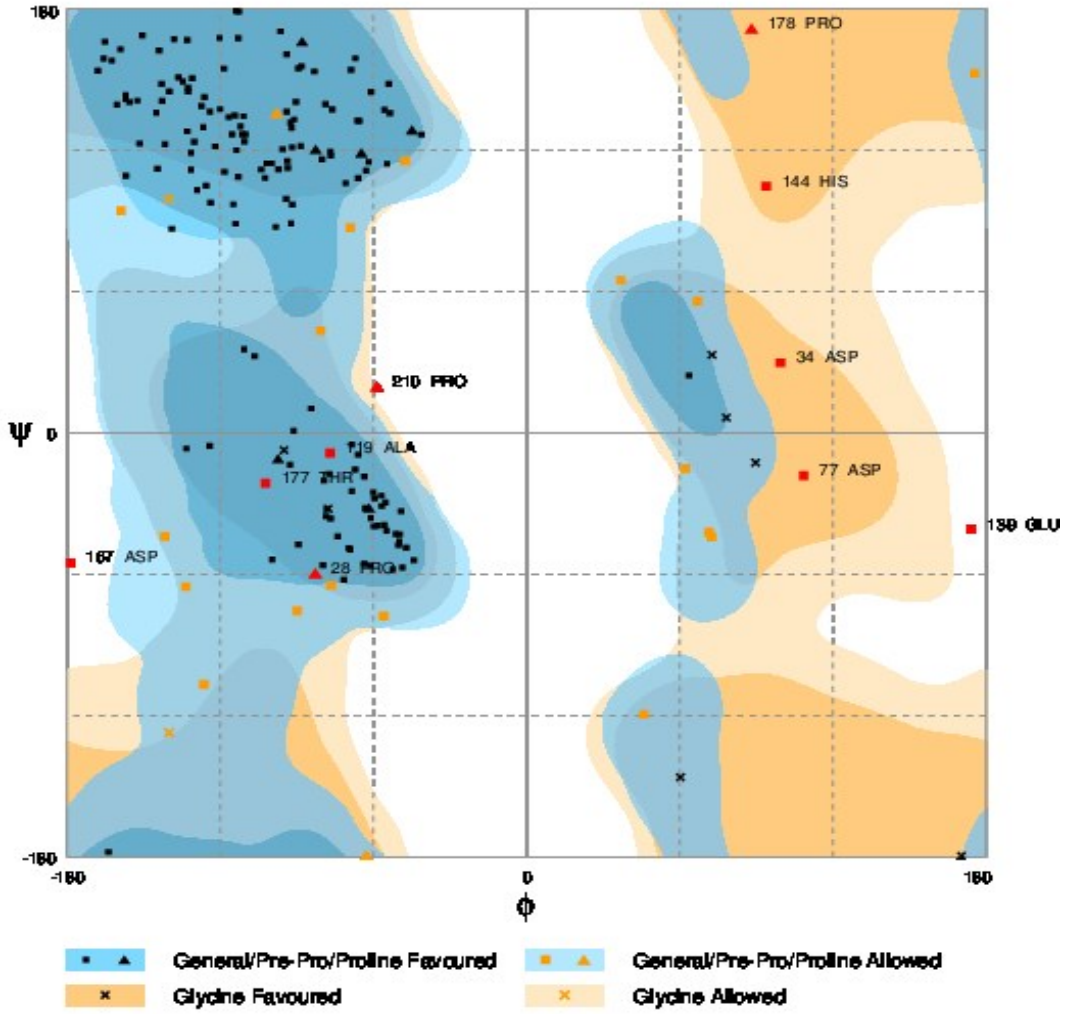
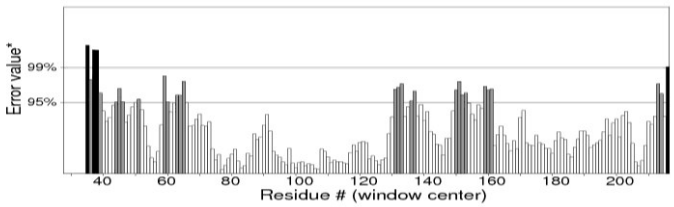


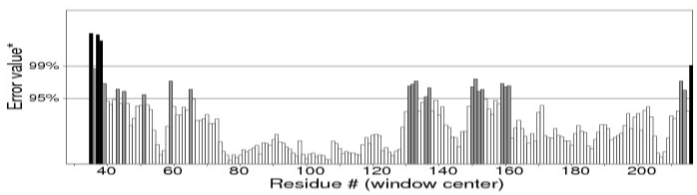
Figure 3: Ramachandran plot for Amutant DQB1 protein

Number of residues in favoured region (~98.0% expected) : 165 (84.6%)
Number of residues in allowed region (~2.0% expected) : 20 (10.3%)
Number of residues in outlier region : 10 (5.1%)

A



B



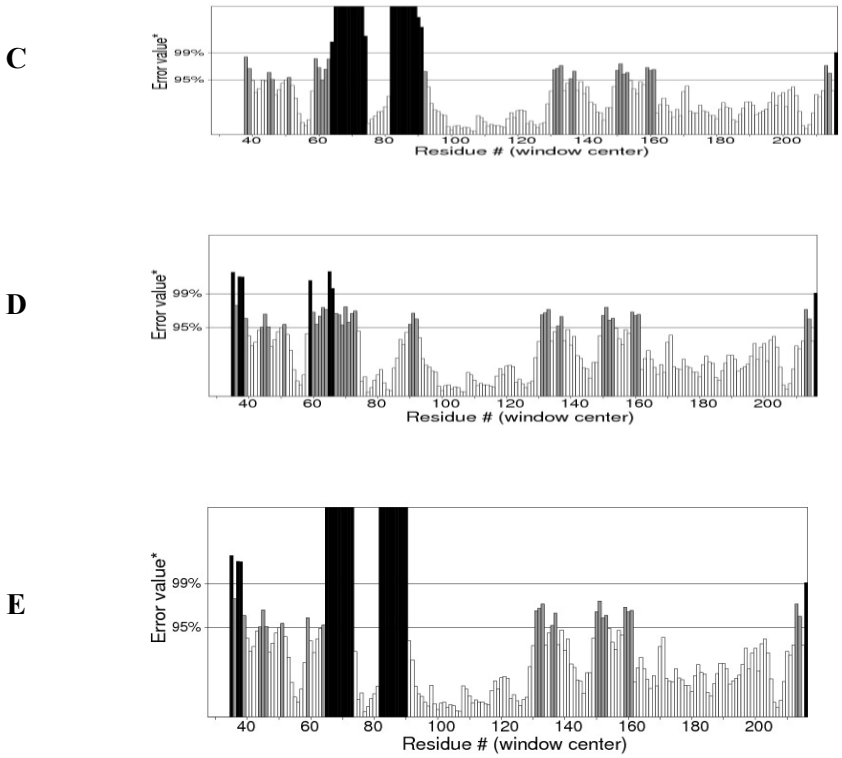


Figure 4: The ERRAT histograms for the native, Amutant, Bmutant, V70R mutant and G86Y mutant DQB1 proteins of Nigerian goats

A: The ERRAT evaluation method for the native DQB1 protein gave 84.1% as the overall quality factor

B: The ERRAT evaluation method for the Amutant DQB1 protein gave 86.3% as the overall quality factor.

C: The ERRAT evaluation method for the Bmutant DQB1 protein gave 73.7% as the overall quality factor.

D: The ERRAT evaluation method for the V70R mutant of DQB1 protein gave 76.9% as the overall quality factor.

E: The ERRAT evaluation method for the G86Y mutant DQB1 protein gave 75.8% as the overall quality factor.

In the ERRAT histogram, the correct regions are shown in black, and the incorrect regions are shown in grey.

Table 1: Functional validations of nsSNPs in DQB1 gene of Nigerian goats using SNAP2, PROVEAN and Polyphen-2

Variants	SNAP2		PROVEAN		Polyphen-2	
	Score	Prediction	Score	Prediction	Score	Prediction
S26G	-34	Neutral	-1.673	Neutral	0.012	Benign
E31F	-16	Neutral	-1.096	Neutral	0.980	Probably damaging
D34R	8	Effect	-3.374	Deleterious	0.990	Probably damaging
D38V	-33	Neutral	-4.403	Deleterious	1.000	Probably damaging
G45D	17	Effect	-2.225	Neutral	0.961	Probably damaging
L46K	44	Effect	0.935	Neutral	0.067	Benign
Y64K	90	Effect	-8.106	Deleterious	0.999	Probably damaging
E68M	28	Effect	-6.815	Deleterious	1.000	Probably damaging
V70R	58	Effect	-4.688	Deleterious	0.998	Probably damaging
D75A	8	Effect	-7.312	Deleterious	0.034	Benign
D77T	6	Effect	-0.961	Neutral	0.020	Benign
R80K	-20	Neutral	-2.061	Neutral	0.348	Benign
A81N	22	Effect	-5.460	Deleterious	0.845	Possibly damaging
P84R	49	Effect	-1.511	Neutral	0.004	Benign
G86Y	72	Effect	-8.763	Deleterious	1.000	Possibly damaging
R88P	-88	Neutral	6.113	Neutral	0.000	Benign

Table 2: Effects of mutations on protein stability of DQB1 gene of Nigerian goats

Wild type amino acid	Position	Variant Amino acid	Reliability index	DDG value	Stability prediction
S	26	G	8	-1.83	Decrease
E	31	F	3	-0.25	Decrease
D	34	R	4	-0.97	Decrease
D	38	V	1	-1.03	Decrease
G	45	D	7	-1.81	Decrease
L	46	K	9	-3.25	Decrease
Y	64	K	5	-1.70	Decrease
E	68	M	2	0.58	Increase
V	70	R	4	-0.21	Decrease
D	75	A	6	-1.91	Decrease
D	77	T	6	-2.03	Decrease
R	80	K	9	-2.21	Decrease
A	81	N	6	-1.06	Decrease
P	84	R	6	-0.48	Decrease
G	86	Y	6	-0.57	Decrease
R	88	P	4	-0.46	Decrease

DDG (free energy change value): DG(NewProtein)-DG(WildType) in Kcal/mol

DDG<0: Decrease Stability

DDG>0: Increase Stability

Table 3: Total energy after minimization of altered model of DQB1 protein

Wild type amino acid	Position	Variant Amino acid	Total energy after energy minimization (kJ/mol)
Native protein	-	-	-6793.618
S	26	G	-6786.792
E	31	F	-6801.268
D	34	R	-6825.370
D	38	V	-6793.618
G	45	D	-6809.822
L	46	K	-6795.894
Y	64	K	-6775.062
E	68	M	-6783.539
V	70	R	-6699.902
D	75	A	-6776.254
D	77	T	-6765.484
R	80	K	-6798.099
A	81	N	-6715.215
P	84	R	-6790.800
G	86	Y	50.808
R	88	P	-6796.044
AMutant(S,E,D,G,L,D,D,R,P,R)	26,31,38,45,46,75,77,80,84,88	G,F,V,D,K,A,T,K,R,P	-6770.789
BMutant (D,Y,E,V,A,G)	34,64,68,70,81,86	R,K,M,R,N,Y	546.414

Table 4: Prediction of protein-protein interaction site using cons-PPISP

Wild type amino acid	Position	Variant Amino acid	Neural network Score	Prediction
S	26	G	0.994	Positive
E	31	F	0.182	Negative
D	34	R	0.102	Negative
D	38	V	0.062	Negative
G	45	D	0.361	Negative
L	46	K	0.643	Negative
Y	64	K	0.986	Positive
E	68	M	0.954	Positive
V	70	R	0.000	Buried
D	75	A	0.738	Negative
D	77	T	0.121	Negative
R	80	K	0.488	Negative
A	81	N	0.261	Negative
P	84	R	0.992	Positive
G	86	Y	0.000	Buried
R	88	P	0.277	Negative

All the nsSNPs which were predicted to be deleterious by SNAP2, PROVEAN and PolyPhen-2 were also found to be involved in decreasing protein stability except E68M (which could be doubtful due to very low reliability index of 2) (Table 2).

197 residues (or 75% of the DQB1 sequence) were modelled with 100.0% confidence by the single highest scoring template for the native, Amutant and Bmutant (Figure 1). A zero RMSD score and TM (1.00) were returned for all the sSNPs including Amutant and Bmutant. The energy of minimization varied from one nsSNP to another (Table 3). The values recorded for V70R, G86Y and Bmutant (6699.902, 50.808, 546.414 kJ/mol, respective were quite higher than that of the native (-6793.618 kJ/mol). Other mutant models had energy values close to the native structure.

Ramachandran plot of native protein (Figure 2) was similar to that of the Bmutant and other nsSNPs. It showed I64 residues (84.1%) in favoured region, 21 residues (10.8%) in allowed region and 10 residues (5.1%) in outlier region. However, in Amutant protein, I65 residues (84.6%) were found in favoured region, 20 residues (10.3%) in allowed region and 10 residues (5.1%) in outlier region (Figure 3). The ERRAT procedure (Figure 4) revealed that the native and Amutant protein structures were of a very high quality (84.1 and 86.3%, respectively) compared to V70R (76.9%), G86Y (75.8) and Bmutant (73.7%). The values for other mutants ranged from 81.32-86.81%.

The cons-PPISP prediction of whether the residue contacts (P = Positive; N = Negative; - = Buried and not predicted) showed that only the mutants V70R and G86Y were buried (found in the core region of the protein).

DISCUSSION

Mutations can change the phenotype and have a beneficial, deleterious or neutral effect on the fitness of the individual organism as observed in the present study. Phenotypes and the corresponding mutations are then acted on by selection (natural or artificial) at the population level (Studer *et al*., 2013). Due to the fact that nsSNPs can affect protein function, they are believed to have the largest impact on disease manifestation in livestock species compared with SNPs in other regions of the genome. Xing *et al*. (2008) linked some DQB1 genotypes with some immune traits. Therefore, the probability of their involvement in disease predisposition increases. Similarly, Hui *et al*. (2012) reported that variation in DQB1 gene, may impact immune responses to pathogens, which may lead to variation in disease resistance. This was buttressed by the findings of Yakubu *et al*. (2017a) that amino acid substitutions may be used in search of disease resistant genotypes in MHC-DRB locus of goats.

The TM scores and RMSD values observed in the present study imply that there exists no greater deviation between the structures of the native and the mutants. This could be due to very high level of structural conservation among them. This is consistent

with the findings of Yakubu *et al*. (2017b) in MHC DQA1 gene of goats. However, the reverse was the case in respect of energy of minimization [the lower (more negative) its value, the stronger] as V70R, G86Y and Bmutant impacted negatively on DQB1 protein structure. This was consolidated by the lower Overall ERRAT quality factor values compared to the native structure. It could therefore be said that correlated mutations appeared to be in favour of Amutant compared to Bmutant.

Variants at different interfaces can, in some cases, lead to different phenotypes by affecting different pathways and complexes (Yates *et al*., 2014). V70R in the present study is a change from valine (hydrophobic) to arginine (Basic) while G86Y is a change from Glycine (aliphatic) to tyrosine (hydrophobic) and were both found in the buried region. According to Razali (2015), most non-synonymous changes in the core region (buried) were expected to cause damage to the stability of the protein structure, which may in turn affect the function or interactions with other proteins, potentially leading to disease. Such Protein-protein interactions of the missense variants in the present study, can thus, facilitate study of genotype/phenotype relationships, which can, in turn, shed light on the problem of multigenic phenotypes (Sunyaev *et al*., 2001). This could be exploited in a wide range of biological and pharmaceutical applications (Yan *et al*., 2002)

Structure and stability of a protein are strongly dependent on the arrangements and compositions of the hydrophobic core. Many studies have suggested that hydrophobic core residues are likely sites of deleterious mutations (David and Sternberg, 2015; Sahoo *et al*., 2015; Zhangab and Wanga, 2014; Yakubu *et al*., 2017b). Hence, change from an exposed to buried state could be considered functionally significant in the mutant protein at structural level (Doss *et al*., 2008). This is because it can result in loss of hydrogen bonds and disturbs correct folding. Accordingly, change in amino acid may affect polar–polar interactions within the protein molecule itself which further altered energy of stabilization and further destabilized the protein (Peng *et al*., 2005). It is most likely that the difference in charge between hydrophobic and basic residues as observed in the present study will disturb the ionic interaction made by the original wild type residue.

CONCLUSION

The present study revealed that two nsSNPs (V70R and G86Y) showed altered interaction patterns as they were found in the buried region and was verified by calculating total energychange after energy minimization which confirmed them as damaging. This was in addition to lower overall ERRAT quality factor. Combined mutational analysis comparing the Amutant (beneficial nsSNPs) and Bmutants (deleterious nsSNPs) together with the native also showed varying and similar changes. Thus, analysis of 'molecular phenotypes', that is, allele specific features in structure, folding, binding or stability, can help to explain the biological mechanism of phenotypic effect or even to predict this effect. These predictions can be used for prioritisation of candidates for pathological association studies using a large population at the DQB1 locus in tropical breeds of goats. This would lay a theoretical foundation for breeding of disease resistant individuals in the future.

ACKNOWLEDGEMENT

Funding for this study was provided by the College of Agriculture & Life Sciences and Mario Einaudi Center for International Studies of Cornell University, Ithaca, NY. Additional support from the Nigerian Government Education Trust Fund (now TETFUND) fellowship to the first author for a research visit to Cornell is gratefully acknowledged.

REFERENCES

Amills, A., C. Sulas, A. Sanchez, G. Bertoni, R. Zanoni and G. Obexer-Ruff (2004). Structural characterization of the caprine major histocompatibility complex class II DQB1 (Cahi-DQB1) gene. *Mol. Immunol*., 41: 843–846.

Bromberg, Y. and B. Rost (2007). SNAP: predicts effect of non-synonymous polymorphisms on function. *Nucleic Acids Research*, 35: 3823-3835.

Capriotti, E., P. Fariselli and R. Casadio (2005). I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. *Nucl. Acids Res*., 33: 306-310.

Choi, Y. and A.P. Chan (2015). PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*, 31 (16): 2745-2747.

David, A. and M. J. E. Sternberg (2015). The contribution of missense mutations in core and rim residues of

protein–protein interfaces to human disease. *Journal of Molecular Biology*, 427 (17): 2886–2898.

Doss, C.G.P., R. Rajasekaran, C. Sudandiradoss, K. Ramanathan, R. Purohit and R. Sethumadhavan (2008). A novel computational and structural analysis of nsSNPs in CFTR gene. *Genomic Med*., 2: 23–32.

Feng, X. (2008). Studies on polymorphisms analysis in Exon2 of DQA1、DQB1、DQA2 gene and their relationship with Nematode resistance and immune traits. MSc. Thesis, Shandong Agricultural University, (2008) S827.7

Han, J.H., N. Kerrison, C. Chothia and S.A. Teichmann (2006). Divergence of interdomain geometry in two-domain proteins. *Structure*, 14: 935–945.

Hui, W., H. Shen, S. Jiang and B. Jia (2012). MHC-DQB1 variation and its association with resistance or susceptibility to Cystic Echinococcosis in Chinese Merino Sheep. *Asian-Australasian Journal of Animal Sciences*, *25*(12), 1660–1666.

Kelly, L.A., S. Mezulis, C.M. Yates, M.N. Wass and M.J.E. Sternberg (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, 10: 845-858.

Kelly, J.N. and S.D. Barr (2014). In silico analysis of functional single nucleotide polymorphisms in the Human TRIM22 gene. *PLoS ONE*, 9(7): e101436. doi:10. 1371/journal.pone.0101436.

Patel, S.M., P.G. Koringa, B.B. Reddy, N.M. Nathani and C.G. Joshi (2015). *In silico* analysis of consequences of non-synonymous SNPs of Slc11a2 gene in Indian bovines. *Genomics Data*, 5: 72–79.

Peng, Y., L. Zhaolong and M. John (2005). Loss of protein structure stability as a major causative factor in monogenic disease. *J. Mol. Biol*., 353: 459–473.

Razali, R. B. M. (2015). Studies on the relationship between single nucleotide polymorphisms and protein interactions. A dissertation submitted in fulfilment for the degree of Doctor of Philosophy, Division of Molecular Biosciences, Faculty of Natural Sciences, Imperial College of London (United Kingdom).

Sahoo, A., S. Khare, S. Devanarayanan, P. C. Jain and R. Varadarajan (2015). Residue proximity information and protein model discrimination using saturation-suppressor mutagenesis. *ELife*, 4: e09532. 10.7554/eLife.09532.

Shen, H., G. Han, B. Jia, S. Jiang and Y. Du (2014). MHC-DRB1/DQB1 gene polymorphism and its association with resistance/susceptibility to Cystic Echinococcosis in Chinese Merino sheep. *Journal of Parasitology Research*, Article ID 272601. http://dx.doi.org/10.1155/2014/272601.

Shrivastava, K., P. Kumar, N. R. Sahoo, A. Kumar, M. F. Khan, A. Kumar, A. Prasad, B. H. M. Patel, A. Nasir, B. Bhushan and D. Sharma (2015). Genotyping of major histocompatibility complex Class II DRB gene in Rohilkhandi goats by polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing. *Vet. World*, 8 (10): 1183–1188.

Stear, M.J., G.T. Innocent and J. Buitkamp (2005). The evolution and maintenance of polymorphism in the major histocompatibility complex. *Vet. Immunol. Immunopathol*., 108: 53-57.

Studer, R.A., B.H. Dessailly and C.A. Orengo (2013). Residue mutations and their impact on protein structure and function: detecting beneficial and pathogenic changes. *Biochem. J*., 449: 581–594.

Sunyaev, S., W. Lathe and P. Bork (2001). Integration of genome data and protein structures: prediction of protein folds, protein interactions and 'molecular phenotypes' of single nucleotide polymorphisms. *Current Opinion in Structural Biology*, 11: 125–130.

Ugbo, S.B., A. Yakubu, J.N. Omeje, B.S. Bibinu, I.S. Musa, J.O. Egahi and N.I. Dim (2015). Assessment of genetic relationship and application of computational algorithm to assess functionality of non-synonymous substitutions in DQA2 gene of cattle, sheep and goats. *Open Journal of Genetics,* 5: 145-158.

Xing, F., P.P. Li, J. Li, C.B. Li, and J.M. Wang (2008). Association of polymorphisms of exon 2 of GOLA-DQB1 gene with immune traits in goats. *Yi Chuan*., 30 (7): 870-876.

Yakubu, A., A.E. Salako, M. De Donato and I.G. Imumorin (2017a). Application of computational algorithms to assess the functionality of non-synonymous substitutions in MHC DRB gene of Nigerian goats. *Genetika,* 49 (1): 63-76.

Yakubu, A., M. De Donato and I.G. Imumorin (2017b). Modelling functional and structural impact of non-synonymous single nucleotide polymorphisms of the DQA1 gene of three Nigerian goat breeds. *South African Journal of Animal Science,* 47 (2): 146-156.

Yakubu, A., A.E. Salako, M. De Donato, M.I. Takeet, S.O. Peters, M.A. Adefenwa, M. Okpeku M. Wheto, B.O. Agaviezor, T.M. Sanni, O.O. Ajayi, G.O. Onasanya, O.J. Ekundayo, B.M. Ilori, S.A. Amusan and I.G. Imumorin (2013). Genetic diversity in Exon 2 at the major histocompatibility complex DQB1 locus in Nigerian indigenous goats. *Biochemical Genetics*, 51: 954-966.

Yan, C., V. Honavar and V.D. Dobbs (2002). Predicting protein-protein interaction sites from amino acid sequence. Computer Science Technical Reports. Paper 225 (2002). http://lib.dr.iastate.edu/cs_techreports/225

Yasukochi, Y., T. Kurosaki, M. Yoneda, H. Koike and Y. Satta (2012). MHC class II *DQB* diversity in the Japanese black bear, *Ursusthibetanus japonicas*. *BMC Evolutionary Biology*, 12: 230 DOI: 10.1186/1471-2148-12-230.

Yates, C.M. and M.J.E. Sternberg (2014). Impact of missense variants on protein–protein interactions. John Wiley & Sons, Ltd., DOI: 10.1002/9780470015902.a0025699

Zhangab, J. and F. Wanga (2014). A survey and a molecular dynamics study on the (central) hydrophobic region of prion proteins. http://arxiv.org/pdf/1409.6104.pdf

Zhang, Y. and J. Skolnick (2005). TM-align: a protein structure alignment algorithm based on the TM-score. *Nucleic Acids Res*., 33: 2302–2309

CHARACTERIZATION OF ADULT DONKEYS IN NORTH WEST NIGERIA USING MORPHOMETRIC TRAITS

[1]John, P. A. and [2]Iyiola-Tunji, A. O.

[1]Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria.

[2]National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria, Nigeria.

Corresponding Author: johnpaulapagu@gmail.com

Abstract: A total of 280 adult donkeys were used to evaluate the morphometric traits among the five strains in North West Nigeria. The five strains of donkeys are Red (Auraki), Black (Duni), White (Fari), Brown (Idabari) and Brown-white (Idabari-fari). Morphometric measurements taken were head length, head width, ear length, neck length, neck circumference, shoulder width, height at withers, heart girth, body length and tail length. Body weight was derived from the prediction equation. Data obtained were subjected to statistical analyses to determine the distribution of measured morphometric traits among the five types of donkeys. The effect of strain, sex, location and interaction on the morphometric measurements were estimated using the GLM procedure of SAS. The mean adult BWT, BL, HG, HW and SW were 142.6kg, 106.6cm, 111.0cm, 110.5cm, 22.8cm, respectively. Values obtained for the NC, NL, HL, HWD, EL, and TL were 62.7cm, 46.9cm, 47.4, 15.0cm, 25.0cm and 57.8cm, respectively. There were variations in the morphometric traits of the donkeys due to strain, sex and location effects with brown donkeys having the heaviest body weight while males from Kebbi State exhibited the heaviest adult body weight (176.67kg). Large variation exists among donkeys in North West Nigeria (70.5% CV). Sexual dimorphism exists in the body size measures of donkeys with females having heavier BWT, larger HWD, HG and longer body while the males have longer head, taller height and wider neck in the adult donkeys. Zoometric phenotypic differentiations exist among the observed strains of donkeys in the Northwestern Nigeria. BWT was significantly correlated with HW ($p<0.01$; $r=0.72$), HWD ($p<0.05$, $r=14$), EL ($p<0.05$, $r=0.10$) and HG ($p<0.05$; $r=0.13$).

Keywords: Characterization, Adult donkeys, North West, Nigeria, Morphometric traits

INTRODUCTION

Morphometric characterization involves a baseline surveys to establish identities and characteristics of individual populations; range of existing breeds and strains, productivity and management systems (ILRI, 2011). Primary characterization refers to activities that can be carried out in a single field visit, for example, measurements of morphological features, interviews with livestock keepers and measures of geographical distribution. The specific conditions of the African continent enabled donkeys to develop a typical habit, which play a key role in order to survive in dry locations. Phenotypic characterizations (an aspect of primary characterization) involves skeletal and tissue measurements. Skeletal aspect includes height and length measurements; while tissue assessment involves heart girth, chest depth, punch girth, width of hips among others (Udeh *et al.,* 2011; Essien and Adesope, 2013). The understanding of developmental patterns of breeds can be achieved through phenotypic characterization (Orheruata and Olutogun, 1994). Phenotypic characterization is also useful for documenting diversity within and between breeds (CGRFA, 2011). It further allows conclusions to be drawn on proportionality of traits and maturity (Bene *et al.,* 2007). It has been suggested that before attempting any genetic improvement, animals must first be characterized (Mbap, 1985). A comparison of values before and after could be used to assess breeding goals (Zechner *et al.,* 2001). Furthermore, until there is proper information on phenotypic and genetic characterization of indigenous breeds, no rational decision can be made regarding their utilization and conservation (ILRI, 2009). The aim of this study is to characterized adult donkeys in North West Nigeria on the basis of their morphometric traits in other to establish the relationships among donkeys, which can be used for future selection.

MATERIALS AND METHODS

Experimental Sites

The study was conducted in the semi-arid zone of Nigeria in Sokoto, Jigawa, Kano, Katsina, Kaduna, Zamfara and Kebbi States respectively. The semi-arid

zone of Nigeria starts from about 11ºN latitude and ends at the Nigeria-Niger frontier. It encompasses the Sudan and Sahel Savanna and part of the Northern Guinea Savanna. The mean annual temperature runs between 26 and 28ºC. There is a single rainy season from May to October, with mean annual rainfall ranging from 1016mm in the wettest parts to less than 508mm in the driest parts. The length of growing period is about 100-150 days which makes it possible to cultivate a wide variety of crops (Ogungbile *et al*., 1998). The semi-arid zone has a land mass of 113,530km^2 and a population of over 35 million people (NPC, 2006). This part of Nigeria has very low level of infrastructure and roads which render it difficult for the people to have access to both rural and urban markets. The major inhabitants of this area are Hausa and Fulani who are predominantly mixed crop-livestock farmers and livestock herders, respectively.

Sampling Size and Sampling Structure

Two hundred and eighty (280) adult donkeys were sampled from Sokoto, Jigawa, Kano, Katsina, Kaduna, Zamfara and Kebbi State. North West Nigeria was selected for the study because of the existence of high population of donkeys in the area. All the three senatorial zones in each of the seven States were covered in this study. Donkeys within the age category of above 3 years were classified as adults. The age of the donkeys were determined using teeth count in combination with the information provided by the donkey owners. A total of 14 adult donkeys were sampled from two senatorial zones while the other 12 were also sampled from the third senatorial zone per state, making a total of 40 donkeys in each of the seven (7) State using random sampling technique. Visibly pregnant donkeys were exempted from this study.

Data Collection

Biometric traits measured

A flexible tailors measuring tape was used to take the body linear measurement. During body measurement, animals were made to stand upright and restrained by assistants in such a way that their necks, heads, tails and ears were stretched almost in a straight line. Each measurement was taken for at least two times and recorded in centimeter.

Body measurements of two hundred and eighty (280) adult donkeys of various strains were taken for phenotypic characterization. Reference marks for body measurements were according to the method of Searle *et al*. (1989a and b) and Salako (2006).

- Head Length (HL): Measured as the distance between the ears to the upper lip (cm).
- Head Width (HDW): Measured as the distance between the outer ends of both eyes (cm).
- Ear length (EL): Measured as the distance from the base to the zygomatic arch of the ear (cm).
- Neck length (NL): Measured as the distance from the base of the cervical vertebra to the base of the top shoulder (cm).
- Neck circumference (NC): Taken as the circumference of the neck at the midpoint (cm).
- Shoulder width (SW): Measured as the horizontal distance between the two shoulders or distance between the lateral tuberisities of the humeri which is also described as the widest point over the intraspinus muscle (cm).
- Height at Wither (HW): Vertical distance from ground to the point of withers measured vertically from the ridge between the shoulder bones to the fore hoof (cm).
- Heart girth (HG): Measured as the circumference of the body at the narrowest point just behind the shoulder perpendicular to the circumference of the body, just in front of the hind leg perpendicular to the body axis (cm).
- Body length (BL): Distance between points of shoulder to point of hip i.e the distance from the first thoracic vertebrae to base of tail. This is also described as the distance between the most cranial palpable spinosus process of thoracic vertebrae and either sciatic tubers or distance between the tops of the pelvic bone (cm).
- Tail length (TL): Measured from the base of the tail to the tip (cm).

Statistical Analysis

The effects of strain, sex, location and interaction on linear body measurement were estimated using the GLM procedure of the statistics analysis software SAS (2004) statistical package. These were computed on the basis of interaction with age groups. Statistical significant means were compared using Duncan Multiple Range Test Duncan, (1955).

$Y_{ijkl}=\mu+S_i+L_j+V_k+(V\times S)_{ik}+(L\times V)_{jk}+\varepsilon_{ijkl}$

Where Y_{ijkl}= overall observation.

μ= population mean
S_i=fixed effect of the i^{th} sex (males and females)
L_k=effect of k^{th} location (Kaduna, Kano, Kebbi, Katsina, Sokoto, Jigawa and Zamfara State)
V_l= fixed effect of l^{th} strain (Auraki, Fari, Duni, Idabari and Idabari-Fari)
$V \times S_{(li)}$= The effect of interaction of l^{th} level of strain, with i^{th} level of sex
$L \times V_{(kl)}$= The effect of interaction of k^{th} location, with l^{th} level of strain.
ε_{ijkl}= residual error.

RESULTS AND DISCUSSION

The least square means of morphometric traits of adult donkeys in North West Nigeria is presented in Table 1. Generally, there were high variations in body weight (70.5%) and height at withers (70.1%). The variations in head length, head width, ear length, neck circumference, shoulder width, heart girth, body length and tail length were 3.9%, 6.7%, 3.6%, 5.1%,7.1%, 11.0%, 3.9%, 3.6% and 11.7% in adult donkeys were generally low. Patterns of growth of body parts based on age have been highlighted by Mavule *et al*. (2013); they stated that body parts developed at a different rate at different age groups. Some morphometric parameters were early maturing and stopped growing before others.

Table 1: Least square means of morphometric traits of adult donkeys

Characteristics	Adult donkeys (N=280)	CV%
BWT(kg)	142.6±6.01	70.5
HL(cm)	47.4±0.11	3.9
HWD(cm)	15.0±0.06	6.7
EL(cm)	25.0±0.05	3.6
NL(cm)	46.9±0.14	5.1
NC(cm)	62.7±0.27	7.1
SW(cm)	22.8±0.15	11.0
HW(cm)	110.5±4.63	70.1
HG(cm)	111.0±0.26	3.9
BL(cm)	106.6±0.22	3.6
TL(cm)	57.8±0.41	11.7

BWT: body weight; HL: head length; HWD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length; CV: Coefficient of variation, %; percent.

The effect of strain on morphometric characteristics of adult donkeys are shown in Table 2. Strain affected ($P<0.01$) neck length, neck circumference and shoulder width. The brown-white (Idabari-Fari) donkey strain had the longest neck length (52.0cm) compared to other strains. The neck circumference of adult donkeys was the widest for red (63.9±1.17cm). The narrowest neck circumference was however obtained in brown-white (57.0cm) donkeys. The shoulder width was longer for the solid strains (Red, Black, Brown and White) compared to the mixed strain (brown-white). The results of this study is similar with the findings of (John *et al*., 2017) who reported significant differences in neck length, neck circumference and shoulder width in donkeys. The significant effect of strain on morphometric traits of donkeys observed could be because of the differences in the experimental site.

Table 2: Effect of strain on morphometric characteristics of adult donkeys (N=280)

Adult donkeys	Red (N=3)	Black (N=10)	White (N=11)	Brown (N=184)	Brown-white (N=2)	SEM	LOS
BWT(kg)	139.3±4.29	137.4±3.90	134.9±4.51	143.2±6.77	147.4	45.35	NS
HL(cm)	47.4±0.36	46.9±0.49	47.6±0.68	47.4±0.12	46.0	0.84	NS
HWD(cm)	15.3±0.26	15.0±0.27	14.5±0.38	15.0±0.06	16.0	0.45	NS
EL(cm)	25.3±0.22	24.7±0.27	25.1±0.52	25.0±0.06	26.0	0.41	NS
NL(cm)	46.7±0.48^{b}	46.5±0.55^{b}	46.5±0.86^{b}	46.9±0.15^{b}	52.0^{a}	1.06	**
NC(cm)	63.9±1.17^{a}	59.5±1.78^{b}	60.5±1.84^{a}	62.8±0.27^{a}	57.0^{c}	1.97	**
SW(cm)	22.8±0.75^{a}	22.2±0.84^{a}	20.6±0.94^{a}	22.9±0.16^{a}	17.0^{b}	1.11	**
HW(cm)	104.1±0.84	103.6±0.92	102.8±0.70	111.4±5.23	107.0	34.89	NS
HG(cm)	110.1±1.37	109.0±1.30	110.5±1.79	111.2±0.27	115.0	1.94	NS
BL(cm)	107.4±1.01	105.3±1.04	107.8±0.92	106.6±0.25	108.0	1.72	NS
TL(cm)	58.9±1.38	53.3±2.28	56.0±3.38	57.9±0.43	60.0	3.03	NS

BWT: body weight; HL: head length; HWD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length, **P<0.01, NS: Not significant, N= number; SEM= Standard Error Mean, LOS= Level of significance.

Effect of sex on morphometric traits of adult donkeys are shown in Table 3. Sex of adult donkeys affected (p<0.01) (BWT), (HL), (HWD), (NC), (HG), (BL). Sex has no significant effect (p>0.05) on other morphometric traits (p>0.05). Adult females were superior to males for BWT (149.68±24.25), HWD (15.46±0.19cm) HG (112.08±0.96cm) and BL (107.87±0.91cm), while the male donkeys tended to be taller than their female counterpart (107.26±18.69cm versus 106.93±18.76cm). The larger BWT of female could be attributed to pregnancy as some of the females could be pregnant during the course of the study. Though visibly pregnant donkeys were not sampled for this study. Higher values (P<0.05) were observed in males for HL (47.64±0.42cm) compared with female (46.82±0.42cm). This is in agreement with the findings of Yilmaz and Ertugrul (2011) that the HL (48.8±0.48) for male is higher than females (48.0±0.49) in adult donkeys. This confirmed earlier findings of Folch and Jordana (1997), who had suggested sexual dimorphism in Catalonian donkeys. The sex differences obtained in the morphometric traits of donkeys could be attributed to sexual dimorphisms (Yakubu and Akinyemi, 2010; Carneiro *et al*., 2010).

Table 3: Effect of sex on morphometric traits of adult donkeys (N=280)

Traits	N	Male (N=140)	Female (N=140)	Overall	SEM	LOS
BWT (kg)	280	135.2±24.17^{b}	149.6±24.25^{a}	142.60	6.01	**
HL (cm)	280	47.6±0.42^{a}	46.8±0.42^{b}	47.37	0.11	**
HWD (cm)	280	15.3±0.19^{b}	15.4±0.19^{a}	15.01	0.06	**
EL (cm)	280	25.1±0.22	25.2±0.22	25.00	0.05	NS
NL (cm)	280	47.5±0.58	47.8±0.57	47.69	0.14	NS
NC (cm)	280	61.3±1.04^{a}	60.3±1.02^{b}	60.83	0.26	**
SW (cm)	280	22.0±0.46	22.0±0.46	22.83	0.15	NS
HW (cm)	280	107.2±18.69	106.9±18.76	110.51	4.63	NS
HG (cm)	280	110.2±0.95^{b}	112.0±0.96^{a}	111.03	0.26	**
BL (cm)	280	106.4±0.91^{b}	107.8±0.91^{a}	106.62	0.23	**
TL (cm)	280	56.5±1.39	57.1±1.39	57.79	0.41	NS

BWT: body weight; HL: head length; HWD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length, **P<0.01, NS: Not significant, N= number; SEM= Standard Error Mean, LOS= Level of significance.

The effect of location on morphometric traits of adult donkeys is shown in Table 4. All the traits (body weight and linear body measurements) of adult donkeys were significantly (P<0.01) affected by location. Adult donkeys with the biggest body weight were recorded from Kebbi State (176.67±28.08kg). However, the least body weight was recorded for donkeys sampled in Jigawa (129.80±24.77kg) state. The highest and lowest HL and HWD were obtained in Kebbi (47.90±0.49cm and 16.46±0.22cm, respectively) and Sokoto states (46.53±0.45cm, 14.54±0.20cm respectively). The The highest and lowest ear length and neck length were obtained for donkeys in Zamfara state (25.42±0.25cm and 48.31±0.66cm, respectively) and Jigawa (25.03±0.25cm and 47.7±0.65 cm, respectively) states. Generally, high values obtained in adult donkeys in Kano for neck circumference, shoulder width, body length and tail length were (62.06±1.19cm), (21.73±0.54cm), (107.13±1.06cm) and (57.78±1.63cm). However, the neck circumference of adult donkeys from Kano was similar (P>0.05) to those from Katsina. Tail length of adult donkeys from Kano state was also similar (P>0.05) to those from Katsina (57.78±1.63cm), Kebbi (59.11±1.61cm), Sokoto (59.52±1.48cm) and Zamfara state (59.84±1.61cm). Wilson (1981) reported that there was little physical variation in donkeys found throughout Africa and that donkeys rarely exceed a height at withers of 105cm. This is not in agreement with the result of this study. According to this study, maximum wither height for adult donkeys were obtained from Kaduna (132.51±13.16cm) and Kebbi (123.68±21.73cm). This is not in agreement with the findings of Aganga and Maphorisa (1994) who reported maximum of 110cm withers heights for Botswana donkeys, and 100.7cm for Igdir donkeys at 6-8years (Yalmaz and Ertugrul, 2011) which means those donkeys were somewhat smaller than Nigerian donkeys. Barzev (2004) reported withers height of 100-120cm from Bulgaria, 140cm from Cyprus. Pearson and Ouassat (1996) reported 129cm from Morocco which are in the range of values obtained in this study.

Table 4: Effect of locations on morphometric traits of adult donkeys (N = 280)

Traits/ Location	Jigawa	Kaduna	Kano	Katsina	Kebbi	Sokoto	Zamfara	SEM	LOS
BWT(kg)	129.8±24.77^{c}	136.4±28.00^{b}	139.2±28.21^{b}	137.4±28.33^{b}	176.6±28.08^{a}	136.6±25.76^{b}	140.9±28.08^{b}	6.01	**
HL(cm)	46.7±0.48^{bc}	47.6±0.49^{b}	46.7±0.49^{a}	46.7±0.49^{bc}	47.9±0.49^{a}	46.5±0.45^{c}	47.3±0.49^{b}	0.11	**
HWD(cm)	15.0±0.22^{c}	15.5±0.22^{c}	15.7±0.22^{b}	15.7±0.22^{b}	16.4±0.22^{a}	14.5±0.20^{d}	15.7±0.22^{d}	0.06	**
EL(cm)	25.0±0.25^{b}	25.2±0.25^{ab}	25.0±0.25^{b}	25.0±0.25^{b}	24.9±0.25^{b}	25.3±0.23^{ab}	25.4±0.25^{a}	0.05	**
NL(cm)	47.7±0.65^{b}	47.1±0.66^{b}	47.7±0.67^{b}	47.3±0.66^{b}	47.7±0.66^{b}	47.7±0.60^{b}	48.3±0.66^{a}	0.14	**
NC(cm)	57.9±1.16^{c}	60.8±1.17^{b}	62.0±1.19^{a}	62.0±1.19^{a}	60.9±1.18^{b}	60.9±1.08^{b}	61.5±1.18^{ab}	0.26	**
SW(cm)	22.3±0.53^{b}	22.0±0.53^{b}	21.7±0.54^{a}	21.7±0.54^{a}	23.1±0.54^{a}	18.3±0.49^{c}	23.2±0.52^{a}	0.15	**
HW(cm)	98.6±21.48^{c}	123.3±21.66^{a}	100.6±21.80^{b}	100.8±21.92^{b}	123.6±21.73^{a}	101.4±19.93^{b}	101.1±21.72^{b}	4.63	**
HG(cm)	107.8±1.10^{c}	111.6±1.11^{ab}	111.7±1.12^{ab}	111.7±1.13^{ab}	110.6±1.12^{b}	111.6±1.02^{ab}	112.8±1.12^{a}	0.26	**
BL(cm)	106.9±1.04^{ab}	107.3±1.05^{a}	107.1±1.06^{a}	107.7±1.07^{a}	106.4±1.06^{b}	106.6±0.97^{ab}	107.8±1.06^{a}	0.23	**
TL(cm)	52.1±1.59^{b}	50.5±1.61^{b}	57.7±1.63^{a}	57.7±1.63^{a}	59.1±1.61^{a}	59.5±1.48^{a}	59.8±1.61^{a}	0.41	**

BWT: body weight; HL: head length; HWD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length; **P<0.01, NS: Not significant; SEM: Standard Error Mean; LOS: Level of significance. Equal number of 40 adult donkeys were sampled in each of the seven States.

The effect of strain and sex interaction on morphometric traits of adult donkeys are shown in Table 5. Body weight, head length, head width ear length, neck circumference, neck length, heart girth and body length were significantly affected (P<0.01) by strains and sex interaction whereas other morphometric traits were not affected (P>0.05). Female brown adult donkeys had high values for BWT (151.00±9.03kg), HWD (15.08±0.67cm), EL (25.06±0.08cm), NL (47.05±0.21cm), HG (112.04±0.34cm) and BL (107.41±0.33cm) compared to male brown donkeys. The result of this study is similar with the findings of (John *et al*., 2017) who reported significant differences between strains and sex in donkeys. The significant effect of strain and sex observed on morphometric traits of donkeys could be attributed to differences in geographical location.

Table 5: Effect of strain and sex (interaction) on biometric traits of adult donkeys

Strain	Sex	BWT(kg)	HL(cm)	HWD (cm)	EL(cm)	NL(cm)	NC(cm)	SW(cm)	HW(cm)	HG(cm)	BL(cm)	TL(cm)
Idabari	Male	134.2±9.94^{b}	47.8±0.16^{a}	14.8±0.08^{b}	24.9±0.09^{b}	46.6±0.23^{b}	63.4±0.39^{a}	22.8±0.18	111.9±7.68	110.0±0.38^{b}	105.6±0.36^{b}	57.6±0.55
	Female	151.0±9.03^{a}	47.0±0.14^{b}	15.0±0.67^{a}	25.0±0.08^{a}	47.0±0.21^{a}	62.2±0.34^{b}	22.9±0.17	111.1±6.97	112.0±0.34^{a}	107.4±0.33^{a}	58.2±0.49
	Overall mean	142.60	47.37	15.01	25.00	47.68	60.83	22.83	110.51	111.03	106.62	57.79
	SEM	6.01	0.11	0.06	0.05	0.14	0.26	0.15	0.63	0.26	0.23	0.41
	LOS	**	**	**	**	**	**	NS	NS	**	**	NS

BWT: body weight; HL: head length; HWD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length; **P<0.01, NS: Not significant; SEM: Standard Error Mean; LOS: Level of significance; ab: Means with different superscripts along same row shows significant differences (P<0.01).

The effect of locations and strains on morphometric traits of adult donkeys are shown in Table 6 (a and b). All the traits (body weight and linear body measurements) were significantly affected (P<0.01) by location and strain interaction. The biggest body weight (179.06±17.53kg) was recorded in Idabari from Kebbi state while the least body weight (120.78±74.29kg) was recorded in Auraki from Kebbi state. Longest head length (50.00±1.18cm) was observed in Fari from Kaduna whereas the shortest HL (45.00±1.18cm) was in Duni strain from Kaduna state. Auraki donkey strain from Kebbi had the widest head width (16.50±0.57cm) while Fari strain from Sokoto had the least HWD (13.50±0.49cm). Ear length (26.00±0.64cm) was longest in Fari strain from Kaduna whereas the shortest EL (24.50±0.64cm) was obtained in Duni from Kaduna state. Neck length was highest in Auraki Auraki (48.00±1.72) strain. The widest neck circumference was obtained in Auraki (67.25±2.53cm) strains from Kano, while the lowest NC (51.50±2.92cm) were recorded in Duni donkey strain from Kaduna state. Shoulder width (SWD) was widest in Auraki (24.50±1.18cm) strain from Kano while the shortest SWD (18.50±1.36cm) was observed in Fari strain from Kaduna state. The height at wither (132.51±13.61cm) was highest in Idabari strain while, the lowest height at wither (HW) was recorded in Idabari (101.87±14.14cm) from Jigawa. Large heart girth (HG) were obtained in Fari (115.50±2.83cm) from Kaduna and Auraki (115.25±2.45cm) strains from Kano state while the smallest HG (104.50±2.83cm) was observe in Auraki adult donkey from Kebbi state. Highest body length (108.00±2.33cm) was obtained in Auraki donkeys from Kano state whereas the adult donkey with the lowest BL (104.00±2.69cm) was observed in Duni strain from Kaduna state. Adult donkey strain that had the longest tail length (62.00±3.56cm) was recorded in Auraki strain from Kano state. However, the shortest TL (42.50±4.11cm) was recorded in Fari strain from Kaduna state. The

report of 130kg average body weight reported by Ebangi and Vall (1998) was similar to the body weight (130.4kg) of brown (Idabari) donkeys from Jigawa State recorded in this study. However, the same authors recorded a somewhat higher height at wither (125cm) than the average of 100cm recorded for brown (Idabari) donkeys in this study. Wilson (1981) reported that there was little physical variation in donkeys found throughout Africa and that donkey rarely exceed a height at withers of 105cm. The result of this study revealed that matured adult donkey attained a maximum height at withers of about 132.51±13.16cm in Idabari strain from Kaduna state which can be attributed to differences in locations and managements.

Table 6a: Effect of location and strains (interactions) on biometric traits of adult donkey

State	Strain	BWT(kg)	HL(cm)	HWD(cm)	EL(cm)	NL(cm)	NC(cm)
Jigawa	**Idabari**	130.4±18.31^{de}	46.8±0.29^{e}	14.6±0.14^{f}	24.8±0.16^{e}	46.6±0.43^{de}	60.0±0.72^{g}
Kaduna	**Duni**	135.0±74.29^{cbd}	45.0±1.18^{g}	15.5±0.57^{c}	24.5±0.64^{f}	44.5±1.72^{h}	51.5±2.92^{i}
	Fari	142.4±74.29bc	50.0±1.18^{a}	15.5±0.57^{c}	26.0±0.64^{a}	45.5±1.72^{g}	67.0±2.92^{a}
	Idabari	135.6±17.62^{cbd}	48.0±0.28^{c}	15.1±0.13^{e}	25.0±0.15^{d}	46.4±0.41^{ef}	63.1±0.69^{de}
Kano	**Auraki**	148.1±64.33^{b}	48.5±1.03^{b}	15.0±0.49^{e}	25.7±0.55^{b}	46.2±1.49^{f}	67.2±2.53^{a}
	Idabari	138.5±17.53^{bcd}	47.7±0.28^{d}	14.2±0.13^{g}	24.8±0.15^{e}	46.5±0.41^{ef}	63.2±0.69^{cd}
Katsina	**Idabari**	137.3±17.43^{bcd}	46.9±0.28^{e}	15.3±0.13^{d}	24.8±0.15^{e}	47.0±0.40^{c}	64.0±0.68^{b}
Kebbi	**Auraki**	120.7±74.29^{e}	47.5±1.18^{d}	16.5±0.57^{a}	25.0±0.64^{d}	48.0±1.72^{a}	59.0±2.92^{h}
	Idabari	179.0±17.53^{a}	48.1±0.28^{c}	16.0±0.13^{b}	24.7±0.51^{e}	46.8±0.41^{d}	63.2±0.69^{cde}
Sokoto	**Fari**	137.9±64.33^{bcd}	46.0±1.03^{f}	13.5±0.49^{h}	25.0±0.55^{d}	46.5±1.49^{ef}	59.0±2.53^{h}
	Idabari	136.4±18.30^{bcd}	46.7±0.29^{e}	14.1±0.14^{g}	25.3±0.16^{c}	47.0±0.42^{c}	62.7±0.72^{ef}
Zamfara	**Auraki**	138.8±74.29^{bcd}	48.0±1.18^{c}	15.5±0.57^{c}	25.0±0.64^{d}	48.0±1.72^{a}	62.5±0.92^{f}
	Idabari	140.7±17.62^{bcd}	47.5±0.28^{d}	15.3±0.13^{d}	25.2±0.15^{c}	47.5±0.41^{b}	63.6±0.69^{c}
	SEM	6.01	0.11	0.06	0.05	0.14	0.26
	LOS	**	**	**	**	**	**

BWT: body weight; HL: head length; HWD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length; **P<0.01, NS: Not significant; SEM: Standard Error Mean; LOS: Level of significance; abc; Means with different superscripts along same row shows significant differences (P<0.01).

Table 6b: Effect of location and strains (interactions) on biometric traits of adult donkey

State	Strain	SW(cm)	HW(cm)	HG(cm)	BL(cm)	TL(cm)
Jigawa	**Idabari**	23.0±0.34^{c}	101.8±14.14^{g}	107.7±0.69^{h}	106.2±0.66^{ghi}	52.7±1.01^{ef}
Kaduna	**Duni**	21.0±1.36^{e}	101.5±57.38^{g}	105.5±2.83^{i}	104.0±2.69^{j}	47.5±4.11^{g}
	Fari	18.5±1.36^{g}	102.0±57.38^{fg}	115.5±2.83^{a}	107.5±2.69^{bc}	42.5±4.11^{h}
	Idabari	23.2±0.32^{c}	132.5±13.61^{a}	111.4±0.67^{de}	106.6±0.64^{efg}	52.1±0.97^{f}
Kano	**Auraki**	24.5±1.18^{a}	106.0±49.69^{c}	115.2±2.45^{a}	108.0±2.33^{a}	62.0±3.56^{a}
	Idabari	24.0±0.32^{b}	104.3±13.54^{de}	111.3±0.67^{e}	106.5±0.64^{fgh}	59.8±0.97^{c}
Katsina	**Idabari**	22.5±0.32^{d}	104.3±13.46^{de}	111.6±0.66^{cde}	106.9±0.63^{de}	58.9±0.96^{d}
Kebbi	**Auraki**	23.0±1.36^{c}	103.5±57.46^{de}	104.5±2.83^{j}	103.5±2.69^{k}	53.0±4.11^{e}
	Idabari	24.1±0.32^{b}	128.4±13.54^{b}	110.7±0.67^{f}	105.9±0.64^{i}	60.4±0.97^{bc}
Sokoto	**Fari**	19.2±1.18^{f}	103.2±49.69^{ef}	109.5±2.45^{g}	107.7±2.33^{ab}	60.0±3.56^{c}
	Idabari	19.2±0.34^{f}	104.4±14.33^{d}	111.7±0.69^{cde}	106.0±0.66^{hi}	60.5±1.01^{bc}
Zamfara	**Auraki**	22.5±1.36^{d}	104.5±57.38^{d}	112.0±2.83^{c}	107.5±2.69^{bc}	61.0±4.11^{b}
	Idabari	24.0±0.32^{b}	104.6±13.61^{d}	112.8±0.67^{b}	107.1±0.64^{cde}	60.8±0.97^{b}
	SEM	0.15	0.63	0.26	0.23	0.41
	LOS	**	**	**	**	**

BWT: body weight; HL: head length; HWD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length; **P<0.01, NS: Not significant; SEM: Standard Error Mean; LOS: Level of significance; abc; Means with different superscripts along same row shows significant differences (P<0.01).

The correlation among morphometric traits of adult donkeys are shown in Table 7. There was negligible correlation between body weight and shoulder width, and between shoulder width and height at withers (-0.04). There was negligible correlation between height at withers and body length (-0.01) and between body weight and head length, neck length, neck circumference, shoulder width, body length and tail length (r=0.02-0.09). However, body weight was significantly correlated with height at withers (0.72; P<0.01;), head width (0.14; P<0.05,), ear length (0.10; P<0.05) and heart girth (0.13; P<0.05;). The relationships between shoulder width and ear length; height at withers with head length, head width, ear length, neck length and circumference; tail length with ear length, shoulder width and height at withers were non-significant (0.02-0.09; P>0.05). Other relationships between the body dimensions were ranged from low to moderate (0.11-0.53; P<0.05). The recurring negative relationships between BWT and SW (-0.04), SW and HW (-0.04), HW and BL (-0.01) in adult donkeys were at variance with literature reports among this traits (Cam *et al*., 2010; Semakula *et al.*, 2010; and Okpeku *et al*., 2011). This implies that body weight had no or less relationship with body linear measurements.

Table 7: Correlated relationships between morphometric traits of adult donkeys

Traits	BWT(kg)	HL(cm)	HWD(cm)	EL(cm)	NL(cm)	NC(cm)	SW(cm)	HW(cm)	HG(cm)	BL(cm)
HL(cm)	0.08^{NS}	-								
HWD(cm)	0.14*	0.25**	-							
EL(cm)	0.10*	0.21*	0.20*	-						
NL(cm)	0.03^{NS}	0.20*	0.16*	0.11*	-					
NC(cm)	0.02^{NS}	0.37**	0.24**	0.23**	0.21*	-				
SW(cm)	-0.04^{NS}	0.41**	0.32**	0.01^{NS}	0.19*	0.27**	-			
HW(cm)	0.72**	0.07^{NS}	0.09^{NS}	0.06^{NS}	0.01^{NS}	0.03^{NS}	-0.04^{NS}	-		
HG(cm)	0.13*	0.27**	0.24**	0.38**	0.29**	0.53**	0.21*	0.02^{NS}	-	
BL(cm)	0.08^{NS}	0.24**	0.20*	0.27**	0.30**	0.29**	0.27**	-0.01^{NS}	0.52**	-
TL(cm)	0.09^{NS}	0.13*	0.16*	0.07^{NS}	0.24**	0.27**	0.08^{NS}	0.03^{NS}	0.32**	0.21*

BWT: body weight; HL: head length; HD: head width; EL: ear length; NL: neck length; NC: neck circumference; SW: shoulder width; HW: height at withers; HG: heart girth; BL: body length; TL: tail length, **P<0.01, *P<0.05, SEM= Standard Error of Mean, LOS= Level of significance.

CONCLUSION AND RECOMMENDATIONS

Donkeys in North West Nigeria varied in adult body size depending on the strain, location and sex. The zoometric variation that exist among strains of donkeys in North West Nigeria should be exploited for genetic improvement of the species.

REFERENCE

Aganga, A.A. and Maphorisa, K. (1994). Characteristics and uses of donkeys in Botswana. Pp146-149 in: Starkey P, Mwenya E and Stares J (eds), Improving animal Traction technology. Proceedings of the first workshop of the Animal Traction Network for Eastern and Southern Africa (ATNESA) held 18-23 January 1992, Lusaka, Zambia. Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, the Nertherlands.

Barzev, G. (2004). Donkey Utilization in Bulgaria.www.atnesa.org/donkeys/donkeys-barzev-utilisation-BG.pdf. (accessed 20th January, 2018).

Bene, S., Nagy, B.Kiss, B., Polgar, J.P. and Szabo, F.(2007). Comparison of body measurements of beef cows of different breeds. *Arch. Tierz. Dummerstof*, 50(4): 363-373

Cam, M.A., Olfaz, M. and Soydan, E. (2010). Possibilities of Using Morphometrics Characteristics as a tool for Body Weight Prediction in Turkish Hair Goats (Kilkeci). *Asian Journal of Animal and Veterinary Advances*. 5: 52-59. http://scialert.net/abstract/?do=ajava.2010.52.59.

Carneiro, H., Louvandini, H., Paiva, S. R., Macedo, F., Memies, B., and Mcmanus, C. (2010). Morphological characterization of sheep breeds in Brazil, Uruguay and Colombia, *Small Ruminant Research,* 94: 58-65.

Commission on Genetic Resource for Food and Agriculture (2011). Draft Guideline on Phenotypic Characterization of Animal Genetic resources. 13th Regular Session, Rome Pp. 18th–22ndJuly2011, Retrieved on 24th November, 2017; from: http://www.Foa.org/againfo/pregramme/en/A5.html/.

Duncan, D. B. (1955). New Multiple F-test. *Biometrics*, 11: 1-42.

Ebangi L and Vall E. (1998). Phenotypic characterization of draft donkeys within the Sudano-sahelian zone of Cameroon. Revue Elev. Med. Vet. Pays trop. 51, 327-334.

Essien, A. and Adesope, N.C. (2013). Linear body measurements of Ndama calves at 12 months in a South Western zone of Nigeria. *Livestock Research for Rural Development*, 15(4): 1-4.

Folch, P and Jordana, J. (1997). Characterization, reference ranges and the influence of gender on morphological parameters of the endangered Catalonian donkey breed. *Journal of Equine Veterinary Science*, 17(2): 102-111.

International Livestock Research Institute (2009). On farm Phenotypic characterization of Kenyan Zebu cattle: Animal Genetic Training Resource. Retrievedon30th October, 2014;from: http://www.dagris.ilri-cgiar.org

International Livestock Research Institute (2011). Ndama Ancient West African cattle. Retrieved on 16th November, 2014; from: http://www.blogs.wordwatch.org/nourishingtheep.

John, P.A., Akpa, G.N. and Iyiola-Tunji, A.O. (2017). Characterization of Young Donkeys in North West Nigeria using Morphometric Traits. *Nigerian Journal of Animal Science*, 19(1): 1-12

Mavule, B.S., Muchenji, V., Bezuidenhout, C.C. and Kunene, N.W. (2013). Morphological structure of Zulu sheep based on principal component analysis of body measurements. *Small Ruminant Research,* 111: 23-30

Mbap, S.T. (1985). The performance of exotic and hybrid cattle at Ibadan and Vomin Nigeria. *Ph.D. Thesis*: University of Ibadan, Ibadan, Nigeria.

NPC, (2006). National Population Commission, Abuja, Nigeria. www. Nigeriannews.com/census/census2006.htm (Accessed on 25th January, 2018).

Ogungbile, A.O., Tabo, R., VanDuivenbooden, N. and Debrah, S.K. (1998). Analysis of constraints to agricultural production in the Sudan Savanna Zone of Nigeria using multi-scale characterization. *Nigerian Journal of Animal Science*, 590: 1998.

Okpeku, M., Yakubu, A., Peters, S.O., Ozoje, M.O., Ikeobi, C.O.N., Adebambo, O.A. and Imumorin, I.G. (2011). Application of multivariate principal component Analysis to morphological characterization of indigenous goats in Southern Nigeria. *Acta argiculturae Slovenica,* 98(2): 101-109.

Orheruata, A.M. and Olutogun, O. (1994). Pre and post weaning phenotypic relations between some N'dama cattle linear measurements in the tropics. *Nigerian Journal of Animal Production,* 22: 76-82.

Pearson, R. A. and Ouassat, M. (1996). Estimation of weight and a body condition scoring system for working donkeys in Morocco. *Veterinary Record*, 138: 229-233.

Salako, A. E. (2006). Application of morphological indices in the Assessment of Type and Function in Sheep. *International morphology*, 24: 13-18.

SAS, (2004). SAS/STAT user guide: Statistics, Version 8.1, SAS. Institute Inc; Cary, Nc

Searle, T.M., McGraham, N. and Donnely, J.B. (1989a). Change of Skeletal Dimensions During growth in Sheep: The effect of Nutrition. *Journal of Agricultural Science*, 112: 321-327.

Searle, T. M., McGraham, N., Donnely, J.B and Makgari, D.E. (1989b). Breed and Sex Differences in Skeletal Dimentions of Sheep in the first year of Life. *Journal of Agricultural Science*, 113: 349-354.

Semakula, J., Mutetikka, D., Kugonza, R.D. and Mpairwe, D. (2010). Variability in body morphometric measurements and their application in predicting live body weight of Mubende and Small East African Goat Breeds in Uganda. Middle-East *Journal of Scientific Research* 5(2):98-105.ISSN 1990-9233 (C) IDOSI Publications.

Udeh, I., Akporhuarho and Onogbe, C.O. (2011). Phenotypic correlations among body measurements and physiological parameters in Muturu and Zebu cattle. *Journal of Agricultural and Biological Sciences,* 6(4): 1-4.

Wilson R. T., (1981). Distribution and importance of the domestic donkey in circumsaharan Africa. *Singapore Journal of Tropical Geography,* 2(2): 136-143.

Yakubu, A. and Akinyemi, M.O. (2010). An evaluation of sexual size dimorphism in Uda sheep using multifactorial discriminant analysis, Acta Agriculturae Scandinavica, section A-*Animal Science,* 60: 74-78.

Yilmaz, O and Ertugrul, M. (2011). Some Morphological Traits of Donkeys Raised in Igdir, Turkey. Arastirma.Makalesi/ *Research Article.Igdir University Journal of Institute of Science and Technology.* 1(2): 113-116, 2011.

Zechner, P., Zohman, F., Solkner, J., Bodo, I., Habe F. and Marti, E. (2001). Morphological description of the Lipizzan horse population. *Livestock Production Science*, 69(2):163-177.

PERFORMANCE OF SORGHUM (*Sorghum bicolor* (L.) MOENCH) CROSSES DERIVED FROM STERILE AND MAINTINER LINES

Lawan, Z. M.[1*], M. Y. Yeye[2], M. F. Ishiyaku[2], S. M. Bugaje[2], H. O. Ahmed[2] and A. S. Shaibu[1]

[1]Department of Agronomy, Faculty of Agriculture, Bayero University, Kano.
[2]Department of Plant Science, Faculty of Agriculture, Ahmadu Bello University, Zaria.
*Corresponding Author: asshuaibu.agr@buk.edu.ng

Abstract: Performance of sorghum (*Sorghum bicolor* (L.) Moench) crosses derived from A (Sterile) and B (Maintainer) lines were evaluated in the field using a randomized complete block design. Six pairs of A and B sorghum lines were used for the study. The B lines were the recurrent parent, which were backcrossed to the A lines (non - recurrent parent), and BC_1F_1, BC_2F_1 and BC_3F_1 populations were generated. There was data collection on the following characters: days to 50% heading, days to maturity, plant height, panicle appreciation and panicle weight. The results from the analysis of variance indicated significant differences for some agronomic traits including days to 50% heading and days to maturity. There was evidence of genetic variability, thus genetic improvement can be achieved for the characters studied. There was significant variation among the genotypes as indication of the superiority of some of the genotypes over others. The crosses 85 x 86, 159 x 160 and 421 x 422 have been identified as the best A/B pairs, in the light of the performance and stability of the cross. It is recommended that those pairs of A and B lines can be used to develop sorghum hybrid in Nigeria.

Keywords: Backcrosses; Grain yield; Maintainer line; Sorghum; Sterile line

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in the world after rice, wheat, maize and barley [1]. Sorghum is indigenous to semi-arid tropics of Africa [2]. Typically, it is an annual crop, but some varieties are perennial. Sorghum is a very versatile and hardy crop that is adapted to varying locations, from the wet Guinea Savanna through to the vast dry Sudan Savanna and semi-arid Sahel zones in Nigeria. The cultivated area in Nigeria lies between latitudes 4˚ to 14˚ N and longitudes 2˚ 2' to 14˚ 30' E of the country [3]. Sorghum is a C4 species with higher photosynthetic ability, greater nitrogen and water-use efficiency, and it is suited to hot and dry agro-ecologies where it is difficult to grow other food crops, due to frequent droughts.

Worldwide sorghum production in 2014 was 63.7 million tons with United States leading with 14.5 million metric tons and Nigeria in the 4th position with 6.2 million metric tons [4]. It is also the second cheapest source of energy and micronutrients after pearl millet with a vast majority of the population in Africa and India depending on sorghum for their dietary energy and micronutrient requirements [5]. The increased uses of sorghum as food in sub-tropical Africa could alleviate the problem of chronic undernourishment, as the air-dried whole grain sorghum contains approximately 8-19% moisture, 68-74% carbohydrates, 8-15% protein, 2-5% fats, 1-3% fibre and 1.5-2.0% ash [6]. Appreciable quantities of sorghum are being used by commercial food industries in the production of flour, alcoholic and malted beverages.

The yield and quality of sorghum is affected by a wide array of biotic - pests and diseases and abiotic - drought, extreme temperatures and soil/nutrient deficiency/imbalance. The world sorghum productivity is dismally low (0.7 t ha^{-1}) because of production constraints, use of traditional cultivars (low-yielding) and traditional production methods [7]. Farmers in Nigeria still use open pollinated varieties and local land races. It is a common knowledge that open pollinated varieties (OPVs) are generally lower yielding than hybrids. The demands for cereals, including grain sorghum is increasing due to population growth and total production is not sufficient to cover the local demands [7]. Demand for

higher productivity over a unit area, and increasing population growth with corresponding decrease in arable land because of other use to which land is put are some of the reasons that necessitate hybrid production in order to increase yield of sorghum.

The first step in hybrid development using male sterility is the development of the A and B lines. The discovery of cytoplasmic male-sterility in sorghum [8] and [9] facilitated cross pollination and thus the commercial utilization of hybrid vigor. Hybrid cultivars make use of male sterility to enhance the combining ability of the parent lines, resulting in heterosis and significant increases in phenotypic traits such as plant height and days to flowering [10]. Use of cytoplasmic male sterility (CMS) is an important method for production of hybrid seed. The objective of the study was to evaluate the performance of sorghum crosses derived from A and B lines and identify the best combination of A and B sorghum lines adapted to savanna agro-ecology of Nigeria.

MATERIALS AND METHODS

The experiment was carried out at Institute for Agricultural Research (IAR), Ahmadu Bello University, Samaru. Evaluation was conducted at two locations; IAR, Ahmadu Bello University, Samaru, Zaria (11^0 $11^{'}$ N, 7^0 $38^{'}$ E, 600 m above sea level) in the Northern Guinea savannah, and IAR Irrigation Research Station, Kadawa (11^0 $39^{'}$ N, 08^0 $2^{'}$ E, and 469 m above sea level) in the Sudan savanna.

The genetic materials for this study comprised six A/B pairs (Table 1), which were selected based on their potential as good source of A and B lines obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Mali. The F_1s were developed at ICRISAT, Mali. The backcross one (BC_1) population was developed during 2013 wet season at I.A.R Farm. The A and the corresponding B line were grown each on a single row, side by side in a plot. At anthesis, 100% female sterile plants were identified among the A-lines together with their corresponding B lines, and were bagged separately. Pollen was taken from a single B line plant to pollinate the F_1 plant which gave rise to BC_1F_1 plants. The BC_1F_1 was advanced to BC_2F_1 and BC_3F_1 during the 2014 dry and wet season, respectively. A total of 24 combinations of A and B lines were made as shown in Table 2.

Twenty-four entries derived from four populations (F_1, BC_1F_1, BC_2F_1, BC_3F_1) of each of the 6 crosses involving the six different A- and B- lines combinations were evaluated during 2015 dry season. The treatments were laid out in a Randomized Complete Block Design (RCBD) with two replications at Samaru and Kadawa. Each plot consisted of a single 5m long row spaced 75cm and 30cm inter- and intra-row spacing. The plots were sown with 5 seeds/hill and later thinned to one seedling per stand two weeks after sowing, during which time missing hills were compensated for by transplanting. Split application of compound 80 kg/ha NPK fertilizer (20:10:10) was done at three (2) and six (6) weeks after sowing (WAS). Weeds were controlled manually by hoeing and birds were scared off the plants from flowering time to harvest.

The following data were collected; days to 50% heading recorded as the number of days from planting to when about 50 per cent of the plants in the plots headed. Days to maturity was taken as the number of days from sowing to when about 50% of the plants in a plot attained physiological maturity. Plant height was recorded as the average distance in cm from the ground to the tip of the panicle at maturity of five randomly selected plants in each plot. Panicle appreciation: This is the desirability of panicle form and appropriate density observed on standing plot on a scale of 1-5, where 5 = excellent, 4 = good, 3 = acceptable, 2 = poor, and 1 = reject. Panicle weight per plot: All the panicles in a plot were harvested and sun dried. The dried panicles were weighed to the nearest gram using a Mettler balance. Grain yield: All panicles harvested (at maturity) in each plot where allowed to sun dry and threshed. Weights where recorded in grams for each plot after threshing using a Mettler balance. All grain yields were converted to kg/ha. 1000 grain weight (gm.): One thousand grains were randomly selected, counted and then weighted and recorded in grams.

The data collected from each location were subjected to combined analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure of the Statistical Analysis System [11].

RESULTS AND DISCUSSION

Table 1: Pedigree of the lines used in the study

Entry	A Lines (Sterile)	Entry	B Lines (Fertile)
67	ISX-09001-11-2-2-BC-6	68	ISX-09001-11-2-2-6
85	ISX-09002-4-1-2-BC-7	86	ISX-09002-4-1-2-7
157	ISX-09003-8-5-1-BC-10	158	ISX-09003-8-5-1-10
159	ISX-09003-8-5-2-BC-12	160	ISX-09003-8-5-2-12
421	ISX-09005-11-7-1-BC-6	422	ISX-09005-11-7-1-6
477	ISX-09001-7-1-1-BC-13	478	ISX-09001-7-1-1-13

Table 2: Development of populations using A- and B-lines

Pairs	A-line	B-line	F_1	BC_1F_1	BC_2F_1	BC_3F_1
1	67	68	AxB	(AxB)xB	[(AxB)xB]xB	[(AxB)xBxB]xB
2	85	86	AxB	(AxB)xB	[(AxB)xB]xB	[(AxB)xBxB]xB
3	157	158	AxB	(AxB)xB	[(AxB)xB]xB	[(AxB)xBxB]xB
4	159	160	AxB	(AxB)xB	[(AxB)xB]xB	[(AxB)xBxB]xB
5	421	422	AxB	(AxB)xB	[(AxB)xB]xB	[(AxB)xBxB]xB
6	477	478	AxB	(AxB)xB	[(AxB)xB]xB	[(AxB)xBxB]xB

Table 3: Mean square for grain yield and agronomic traits of different generation of backcross across locations during 2014/2015 dry season

Source of Variation	df	50% H	PH (cm)	DTM	PA	PW (g)	GW (g)	1000GW (g)	GY(kg/ha)
Loc	1	1846.26**	1240.56*	1107.04**	1.50	267780.50*	6537.30	0.01	29281.60
Rep(Loc)	2	33.68	758.61	26.02	2.85	105374.80	14120.82	0.43	63249.37
Genotype	23	38.39**	273.00	16.93**	1.72*	65150.02	16099.11	2.61	72110.44
Loc*genotype	23	53.89**	266.92	25.28**	0.65	32227.72	12664.22	2.08	56725.06
Error	46	5.72	225.48	6.56	0.77	54376.36	13937.80	3.51	62429.59

*, and ** Significant at 0.05 and 0.01 probability levels, respectively. Loc = Location. 50% H - Days to 50% heading; PH - Plant height; DTM - Days to maturity; PA - Panicle appreciation; PW - Panicle weight; GW - Grain weight; 1000GW - 1000 grain weight; GY - Grain yield.

Table 4: Mean performance for agronomic traits in different generation of backcross across locations during 2014/2015 dry season

Genotype	Days to 50% heading	Plant height (cm)	Days to maturity	Panicle Appreciation	Panicle Weight (g)	Grain weight (g)	1000 grain weight (g)	Grain yield (kg/ha)
67 x 68F_1	89.0	156.6	135.0	3.5	298.5	151.3	28.0	320.2
85 x 86F_1	89.0	156.1	136.0	3.8	569.2	299.0	27.3	632.8
157 x 158F_1	85.0	145.9	131.0	4.0	502.1	244.5	28.0	517.4
159 x 160F_1	90.0	153.3	136.0	4.0	487.7	242.6	26.3	513.4
421 x 422F_1	85.0	146.9	133.0	4.0	563.7	242.9	26.5	514.1
477 x 478F_1	91.0	156.4	137.0	3.3	364.3	168.1	27.5	355.8
67 x 68BC_1	87.0	150.0	134.0	4.3	537.5	301.6	28.5	638.3
85 x 86BC_1	82.0	147.4	131.0	2.8	343.5	162.5	27.8	344.0
157 x 158BC_1	85.0	155.3	135.0	4.5	622.1	196.1	28.8	414.9
159 x 160BC_1	80.0	159.1	131.0	3.8	421.1	204.3	26.8	432.4
421 x 422BC_1	84.0	142.2	133.0	3.0	445.9	232.2	26.3	491.5
477 x 478BC_1	81.0	150.9	130.0	2.8	253.5	99.3	27.5	210.1
67 x 68BC_2	86.0	167.2	134.0	3.5	497.2	237.7	27.0	503.1
85 x 86BC_2	85.0	151.0	134.0	3.3	402.9	190.5	27.0	403.1
157 x 158BC_2	90.0	155.2	137.0	2.5	247.0	93.1	26.3	197.0
159 x 160BC_2	87.0	164.2	135.0	3.0	261.0	131.1	27.8	277.5

421 x 422BC_2	83.0	127.0	131.0	2.0	184.2	95.6	27.3	202.4
477 x 478BC_2	83.0	150.5	134.0	3.0	447.0	220.7	27.8	467.1
67 x 68BC_3	84.0	143.9	134.0	2.3	188.6	95.7	26.5	202.4
85 x 86BC_3	82.0	153.2	131.0	3.8	410.4	192.2	26.0	406.8
157 x 158BC_3	82.0	149.7	133.0	3.5	477.7	210.0	28.3	444.5
159 x 160BC_3	85.0	155.2	133.0	3.0	485.5	242.7	27.5	513.5
421 x 422BC_3	86.0	141.0	133.0	2.8	299.6	133.5	26.5	282.4
477 x 478BC_3	87.0	158.9	136.0	2.5	242.0	108.6	28.5	229.8
Means	85.00	151.50	134.00	3.30	398.00	187.30	27.30	396.40
SE(±)	1.55	3.88	1.60	0.94	15.27	10.87	1.37	15.81

Table 5: Genotype by location interaction for agronomic traits of different backcross generations (BCF's) of A lines x B lines of Sorghum

Genotype	Days to 50% Heading			Days to Maturity		
	Samaru	Kadawa	Means	Samaru	Kadawa	Means
67 x 68F_1	97.0(2)	80.0(14)	89.0	142.0(1)	128.0(13)	135.0
85 x 86F_1	90.0(6)	88.0(8)	89.0	137.0(6)	135.0(7)	136.0
157 x 158F_1	79.0(14)	91.0(5)	85.0	128.0(13)	135.0(7)	131.0
159 x 160F_1	97.0(2)	84.0(11)	90.0	141.0(2)	130.0(11)	136.0
421 x 422F_1	85.0(10)	85.0(10)	85.0	134.0(8)	131.0(10)	133.0
477 x 478F_1	98.0(1)	83.0(12)	91.0	142.0(1)	133.0(9)	137.0
67 x 68BC_1	98.0(1)	76.0(17)	87.0	140.0(3)	128.0(13)	134.0
85 x 86BC_1	85.0(10)	79.0(14)	82.0	133.0(9)	129.0(12)	131.0
157 x 158BC_1	89.0(7)	80.0(14)	85.0	138.0(5)	133.0(9)	135.0
159 x 160BC_1	84.0(11)	75.0(18)	80.0	134.0(8)	128.0(13)	131.0
421 x 422BC_1	89.0(7)	78.0(15)	84.0	138.0(5)	129.0(12)	133.0
477 x 478BC_1	88.0(8)	74.0(19)	81.0	134.0(8)	125.0(15)	130.0
67 x 68BC_2	88.0(8)	84.0(11)	86.0	135.0(7)	133.0(9)	134.0
85 x 86BC_2	89.0(7)	81.0(13)	85.0	138.0(5)	130.0(11)	134.0
157 x 158BC_2	92.0(4)	89.0(7)	90.0	139.0(4)	135.0(7)	137.0
159 x 160BC_2	86.0(9)	89.0(7)	87.0	135.0(7)	135.0(7)	135.0
421 x 422BC_2	88.0(8)	78.0(15)	83.0	135.0(7)	128.0(13)	131.0
477 x 478BC_2	90.0(6)	76.0(17)	83.0	140.0(3)	128.0(13)	134.0
67 x 68BC_3	92.0(4)	77.0(16)	84.0	140.0(3)	128.0(13)	134.0
85 x 86BC_3	85.0(10)	79.0(14)	82.0	133.0(9)	130.0(11)	131.0
157 x 158BC_3	89.0(7)	75.0(18)	82.0	139.0(4)	127.0(14)	133.0
159 x 160BC_3	91.0(5)	80.0(14)	85.0	138.0(5)	129.0(12)	133.0
421 x 422BC_3	93.0(3)	78.0(15)	86.0	138.0(5)	129.0(12)	133.0
477 x 478BC_3	91.0(5)	83.0(12)	87.0	140.0(3)	133.0(9)	136.0
Means	90.00	81.00	85.00	137.00	130.00	134.00
S. E. (±)	1.54	1.55	1.55	1.65	1.55	1.60

The result of mean squares (Table 3) shows that 50% heading, and days to maturity were highly significant ($p \leq 0.01$). Panicle appreciation showed significant difference ($p \leq 0.05$), while no significant differences were observed for the rest of the traits studied. The result also showed highly significant ($p \leq 0.01$) interaction between genotype and environment for 50% heading and days to maturity. The significant differences observed for 50% heading and days to maturity indicate significant genetic variability within the mentioned traits in each location, thus genetic improvement can be achieved for the characters studied. This is similar to the findings of Ramesh et al. [12] who also reported significant difference for agronomic traits among A/B-lines. In addition, the amount of genetic improvement that can be achieved among a given set of genotypes depends on the amount of genetic diversity that is present within the population [13].

The mean performance of the genotypes across the two locations for all the traits measured was presented in Table 4. Significant differences were observed for Days to 50% heading with values ranging from 80 to 91 days, genotype 477 x 478F_1 recorded the highest mean, while 159 x 160BC_1 recorded the lowest mean. For days to maturity the mean performance ranged from 130 days (477 x 478BC_1), which was the earliest, to 137 days (477 x 478F_1 and 157 x158BC_2) which took the longest time to mature. The mean performance for panicle appreciation ranged from 2.0 to 4.5, genotype 157 x 158BC_1 recorded the highest mean while genotype 421 x 422BC_2 had the lowest mean.

The mean performances for genotype by location interaction for days to 50% heading and days to maturity were presented in Table 5. The mean performance for days to 50% heading across the genotypes for both the locations ranged from 74 to 98 days, genotype 477 x 478F_1 and 67 x 68BC_1 recorded the highest mean, genotype 477 x 478BC_1 recorded the lowest mean. The mean performance for days to maturity for both locations ranged from 125 to 142days. Genotype 477 x 478F_1 and 67 x 68F_1 took longest time to mature (142 days) while 477 x 478BC_1 was the earliest to mature (125 days). There was significant variation among the genotypes such that cross 85 x 86 recorded the highest yield and matured early. Based on genotype by location interaction, for Samaru, genotype 157 x 158F_1 was the best in terms of days to 50% heading, while 477 x 478BC_1 was the best at Kadawa. Also genotype 85 x 86BC_1 was the best in terms of days to maturity at Samaru, while 157 x 158BC_3 was the best at Kadawa.

In conclusion, the crosses 159 x 160 and 421 x 422 have been identified as the best A/B pairs. In the light of the performance of the cross, it is recommended that those pairs of A and B line can be used to develop sorghum hybrid in Nigeria.

REFERENCES

[1] Food and Agriculture Organization (2015) Sorghum and millets in human nutrition. Retrieved from http://www.fao.org/docrep/T0818e/T0818E01.htm.

[2] Dewar, J. (2003) Fermented Products: Beverages and Porridges. In: *Sorghum Origin, History, Technology, and Production.* (C. Wayne Smith and R.A. Frederickson, Eds), John Wiley and Sons, New York (2000). Pp. 751-795.

[3] Food and Agriculture Organization / Global Information and Early Warning System on Food and Agriculture (2005) Food Outlook Number 5. Food and Agriculture Organization of the United Nations, Rome, Italy. 28p.

[4] WSP, 2015. World Sorghum Production 2014. https://www.wsp.com

[5] Parthasarathy, R.P., Basavaraj, G., Ahmad, W., Bhagavatula, S. (2006) An analysis of availability and utilization of sorghum grain in India. Journal of SAT Agriculture Research 8: 54 – 63.

[6] Dasai, B. B., Inamdar, D. G., Chavan, V.D., Naik, R. M. (1992) Proximate Composition and Protein fractions of some promising sorghum cultivars. Journal of Maharashtra Agricultural Universities, 17:307-308.

[7] Ali, M.A., Mahdy, E.E., Mahmoud, A.M. (2011) The effect of environment on combining ability and heterosis in grain sorghum. Asian Journal of Crop Science, 3: 1-15.

[8] Stephens, J.C., Holland, P.F. (1954) Cytoplasmic male sterility for hybrid sorghum seed production. Agronomy Journal, 46: 20 – 23.

[9] Dogget, H. (1969) Yield of Hybrid Sorghum. Experimental Agriculture, 5: 1-8.

[10] Reddy, B.B.S., Ramesh, S., Reddy, P.S. (2006) Sorghum Genetic Resources, Cytogenetic and Improvement. In: *Cereals*, Singh, R.J. and Jauhar, P.P. (Editors.). CRC Taylor and Francis, Boca Raton. Pp.286-312.

[11] SAS Institute (2015) Statistical Analysis Software (SAS). Users' Guide. SAS Inst., Cary, NC. UK.

[12] Ramesh, S., Reddy, B.B.S., Reddy, P.S., Ramaiah, B. (2006) Influence of Cytoplasmic-nuclear Male Sterility on Agronomic Performance of Sorghum Hybrids. SAT eJournal, 2: 112 - 127

[13] Falconer, D.S., Mackey, T.F.C. (1996) Introduction to quantitative genetics, 4th ed. *Longman Scientific and Technical*, New York.

HETEROTIC PATTERN AND INBREEDING DEPRESSION AMONG CROSSES OF EIGHT MAIZE (*Zea mays* L.) VARIETIES

Ibrahim, A. K.[1]*, Ado, S. G[2]., Mohammed, S. G.[1] and Dawaki, K. D.[3]

[1]Department of Agronomy, Bayero University, Kano, Nigeria, PMB 3011, Kano, Nigeria.

[2]Department of Plant Science, Ahmadu Bello University, Zaria, PMB 1044, Kaduna, Nigeria.

[3]Department of Crop Production Technology,

Audu Bako College of Agriculture, Dambatta, PMB 3159, Kano, Nigeria.

Corresponding Author Email: aikurawa.agr@buk.edu.ng

Abstract: A full-diallel cross comprising eight varieties of maize were developed and studied for some selected characters to determine heterosis and inbreeding depression. Eighty-one genotypes comprising the crosses, reciprocals, self's, parents and nine checks were evaluated at Kadawa under irrigation in two different sowing dates serving as two environments. Heterotic patterns observed revealed that at environment one, eighteen hybrids out of twenty-eight showed heterosis for grain yield which ranged from 1.6% to 927.0%. The hybrid Sammaz 36 x Sammaz 35 exhibited highly significant heterobeltiosis for grain yield. Fifteen hybrids out of twenty-eight exhibited heterosis for grain yield in environment two, which ranged from 17.8% to 397.1%. Sammaz 19 x Sammaz 27 exhibited highly significant heterobeltiosis for grain yield. High percent heterosis was observed among crosses involving the parents Sammaz 35, Sammaz 36, and Sammaz 27. Results indicate that inter variety hybrid development programme can be an effective breeding approach to exploit the heterotic potential of these varieties. High reduction in yield with self-fertilization was observed in Sammaz 19, Sammaz 35 and Sammaz 28 for environments one, two and combined data. In environment one, the hybrid Sammaz 36 x Sammaz 35 recorded high inbreeding depression estimate for grain yield (-1260.70%), thus indicating accumulation of recessive genes in the progeny. Similarly, in environment two, Sammaz 19 x Sammaz 27 had a high inbreeding depression value (-491.39%). For the combined data across environments, Sammaz 36 x Sammaz 29 had highest estimate of inbreeding depression (-162.16%).

Keywords: Crosses, Heterotic pattern, Inbreeding depression, Progenies and Maize Varieties

INTRODUCTION

Maize (*Zea mays* L.) is a major food item in Nigeria and it is consumed in many forms. It is consumed as green maize when the ear is boiled or roasted. When dry, the grain may be processed into different forms of products such as pap (*ogi*), starch and it is also an industrial crop in Nigeria [1]. It is an important food crop in Africa, providing 50% of the calories in diets in Southern Africa, 30% in Eastern Africa and 15% in West and Central Africa [2]. Due to increasing interest in hybrid maize development in the National Agricultural Research Programmes and private seed companies conducting various researches related to hybrid development, information about heterotic patterns and inbreeding depression among crosses of parent populations of maize will play an important role.

The performance of a hybrid is a function of the genes it received from its parents but can be judged by its phenotypic performance in terms of the amount of heterosis expressed. Many breeders and geneticists believe that the magnitude of heterosis is directly related to the degree of genetic diversity between the parents. In other words, it is assumed that the more the parents are genetically different, the greater the heterosis will be. It is common in most hybrid breeding programmes to maintain two or more distinct germ plasm as heterotic groups.

The counterpart to heterosis, inbreeding depression, is generally attributed to the abundance of unfavourable recessive alleles and so it is argued that heterosis should simply reflect the converse effect [3]. The unfavourable recessive alleles in one line would be masked in the cross between them by dominant alleles from the other leading to heterosis. The immediate task in hybrid production programmes in Research Institutes is where to obtain information on heterotic patterns and inbreeding depression of various germplasm materials. Diallel mating design

provides the basis for generating information regarding heterotic patterns and inbreeding among maize populations, and also can be used in estimating the efficiency of transferring alleles as widely used in breeding programs [4]. Diallel crosses are also important in providing information on dominant gene action in inheritance of the traits to be improved as well as estimates of combining ability of the parents [5]. This study was carried out to determine heterotic patterns for grain yield and other agronomic traits in maize, and identify appropriate germplasm for hybrid development.

MATERIALS AND METHODS

The parent materials comprised of eight varieties consisting of Sammaz 19, Sammaz 17 and Sammaz 36 obtained from IAR-Zaria, Sammaz 27, Sammaz 37, Sammaz 35, Sammaz 29 and Sammaz 28 from International Institute of Tropical Agriculture. They were crossed in a complete diallel pattern to generate crosses, reciprocals and self's at Samaru-Zaria in April, 2010. Eighty-one varieties comprising the crosses, reciprocals, self's, parents and nine checks were evaluated at Kadawa in two sowing dates at three months interval in January, 2011. The sowing dates were considered as separate environments. The 81 entries were arranged in 9 x 9 lattice design with three replications in each environment. One row of 5m long spaced at 0.75m apart was used as a plot. Three seeds were planted at intra row spacing of 50cm and later thinned to two plants per hill. Three hoe weedings were carried out, first one at two weeks after planting, second at four weeks after planting and earthing up at six weeks after planting. There was split fertilizer application of compound fertilizer (NPK 20:10:10) as basal dressing and urea (46 % N) as top dressing, giving a total plant nutrient of 120 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare. Data were collected for: Days to 50% pollen shed (Daf), days to 50% silking (Das), plant height (PH) (cm), days to maturity (DM), kernels per row (KPR) and grain yield per hectare (Gy) (kg).

Heterosis for individual crosses was estimated based on the difference between F_1 and high parent: High parent heterosis (H_{PH})

$$H_{PH} = \frac{F_1 - H_P}{H_P} \times 100$$

Where:

F_1 = Average performance of hybrids formed between j^{th} and i^{th} parents

H_P = Average performance of the high parent

The significance of heterosis was tested as described by Pascal and Wilcox [9]:

$$SD = \sqrt{2MSE/r}$$

Where

SD = Standard difference between F_1 and High parent required for significance at 5% and 1% probability levels.

MSE = Mean square of error

r = number of replications

INBREEDING DEPRESSION

Inbreeding depression was calculated as described by Halauer et al. [7]:

$$\text{ID relative to self} = (s_o - p)/s_o X100$$

Where:

ID= Inbreeding depression percentage

s_o = Mean of the progeny from selfin

p = Parent mear

$$\text{ID relative to } F_1 = (x_o - x_f)/x_o) X100$$

Where:

x_o = Mean of the parent

x_f = Mean of the hybric

RESULTS AND DISCUSSION

Table 1: Percent heterosis of five characters in Kadawa environment one

Genotypes	NL	PH	EH	Cw	Gy
Sammaz 19 x Sammaz 37	4.8	-8.2	-5.9	-16.1	-33.7
Sammaz 19 x Sammaz 17	-4.8	-10.2	10.9	-29.9	-40.8
Sammaz 19 x Sammaz 36	0	-5.2	12.6	28.0	49.6
Sammaz 19 x Sammaz 27	0	-19.8	-10.3	16.8	29.2
Sammaz 19 x Sammaz 35	0	10.6	50	38.5	86.0
Sammaz 19 x Sammaz 29	0	-10.0	4.0	-7.7	14.2

Sammaz 19 x Sammaz 28	-14.3	-19.8	-1.2	-39.4	-59.1
Sammaz 37 x Sammaz 17	10.0	1.0	-8.3	4.1	1.6
Sammaz 37 x Sammaz 36	5.0	-13.5	-13.2	-23.0	-27.4
Sammaz 37 x Sammaz 27	0	-10.5	-25	-30.3	-28.2
Sammaz 37 x Sammaz 35	10.0	1.9	-1.0	-28.4	-44.2
Sammaz 37 x Sammaz 29	5.0	-4.5	-3.4	27.2	28.9
Sammaz 37 x Sammaz 28	-5.0	-1.2	-7.4	15.5	-30.6
Sammaz 17 x Sammaz 36	0	-15.9	-4.1	-26.3	-39.2
Sammaz 17 x Sammaz 27	-5.0	0.5	17.1	74.8	75
Sammaz 17 x Sammaz 35	10.0	19.9	37.4	68.5	68.5
Sammaz 17 x Sammaz 29	5.3	5.9	-5.8	86.8	92.3
Sammaz 17 x Sammaz 28	10.5	4.6	0	39.8	22.4
Sammaz 36 x Sammaz 27	10.0	-7.0	-6.3	3.9	1.7
Sammaz 36 x Sammaz 35	5.0	-9.2	14.7	862.5**	927.0**
Sammaz 36 x Sammaz 29	13.2	0.9	35.2	91.5	74.8
Sammaz 36 x Sammaz 28	10.5	-6.1	9.4	968	-4.8
Sammaz 27 x Sammaz 35	15	17.7	40.0	64.2	91.0
Sammaz 27 x Sammaz 29	5.0	-3.2	-2.3	1.8	0.7
Sammaz 27 x Sammaz 28	0	3.9	2.3	34.4	23.1
Sammaz 35 x Sammaz 29	-0.0	-6.5	4.1	205.1	196.8
Sammaz 35 x Sammaz 28	10.0	13.9	11.8	22.5	13.1
Sammaz 29 x Sammaz 28	5.3	1.2	1.3	-0.9	-7.6
SE±	1.34	20.26	13.35	0.84	1710.20

Key: **,* significant at 1% and 5% levels, respectively, NL: Number of leaves, PH: Plant height, EH: Ear height, Cw: Cob weight and Gy: Grain yield/ha

Table 2: Percent heterosis for two characters in Kadawa environment two

Genotype	Cw	Gy
Sammaz 19 x Sammaz 37	198.8	160.9
Sammaz 19 x Sammaz 17	86.1	85.5
Sammaz 19 x Sammaz 36	-55.9	-61.3
Sammaz 19 x Sammaz 27	480**	397.1**
Sammaz 19 x Sammaz 35	-85.5	-89.7
Sammaz 19 x Sammaz 29	-35.7	-45.3
Sammaz 19 x Sammaz 28	-46.6	40.6
Sammaz 37 x Sammaz 17	254.7	244.6
Sammaz 37 x Sammaz 36	-44.7	-38.7
Sammaz 37 x Sammaz 27	298.3	344.7
Sammaz 37 x Sammaz 35	-8.1	-25.3
Sammaz 37 x Sammaz 29	45.4	122.7
Sammaz 37 x Sammaz 28	216.0	261.3
Sammaz 17 x Sammaz 36	-29.3	-7.3
Sammaz 17 x Sammaz 27	82.7	91.1
Sammaz 17 x Sammaz 35	-63	-65.2
Sammaz 17 x Sammaz 29	-62.2	-65.3
Sammaz 17 x Sammaz 28	36.4	53.2
Sammaz 36 x Sammaz 27	-23.4	-9.7
Sammaz 36 x Sammaz 35	-86.5	-85.4
Sammaz 36 x Sammaz 29	88.3	123.3
Sammaz 36 x Sammaz 28	1.1	17.8
Sammaz 27 x Sammaz 35	9.1	-6.3
Sammaz 27 x Sammaz 29	74.0	80.0
Sammaz 27 x Sammaz 28	276.2	353.2
Sammaz 35 x Sammaz 29	-26.9	-35.6
Sammaz 35 x Sammaz 28	-46.1	-50.6
Sammaz 29 x Sammaz 28	75.1	73.3
SE±	0.47	1028

Key: **,* significant at 1% and 5% levels, respectively, Cw: Cob weight and Gy: Grain yield/ha

Table 3: Percent inbreeding depression in Kadawa environment one

Genotype	Status	Daf	Das	PH	DM	GY
Sammaz 19 x Sammaz 19	Self	-15.43	-15.26	-24.93	6.25	-182.10
Sammaz 37 x Sammaz 37	Self	4.99	6.05	-9.35	0.00	-63.77
Sammaz 17 x Sammaz 17	Self	7.81	6.57	-7.18	5.73	-5.06
Sammaz 36 x Sammaz 36	Self	-1.15	-2.12	-0.22	-2.12	82.64
Sammaz 27 x Sammaz 27	Self	1.63	-0.38	0.25	1.08	24.60
Sammaz 35 x Sammaz 35	Self	-6.25	-3.83	-6.05	-2.73	81.34
Sammaz 29 x Sammaz 29	Self	4.82	3.37	2.50	3.43	28.63
Sammaz 28 x Sammaz 28	Self	-5.15	-3.97	16.93	-0.28	-51.34
Sammaz 19 x Sammaz 37	Hybrid	3.78	3.72	0.93	-3.68	26.50
Sammaz 19 x Sammaz 17	Hybrid	2.92	3.42	-0.51	-9.45	29.95
Sammaz 19 x Sammaz 36	Hybrid	8.49	7.72	1.63	-1.02	-169.15
Sammaz 19 x Sammaz 27	Hybrid	9.21	8.41	12.53	-2.44	-48.73
Sammaz 19 x Sammaz 35	Hybrid	8.45	7.61	-21.81	-2.13	-205.12
Sammaz 19 x Sammaz 29	Hybrid	3.47	6.21	-5.15	-4.66	-43.48
Sammaz 19 x Sammaz 28	Hybrid	9.34	8.25	1.92	-0.63	50.41
Sammaz 37 x Sammaz 17	Hybrid	-5.18	-8.08	-5.07	-1.51	-9.36
Sammaz 37 x Sammaz 36	Hybrid	2.54	4.16	9.90	1.73	-33.33
Sammaz 37 x Sammaz 27	Hybrid	-4.70	-5.50	9.48	-3.96	25.08
Sammaz 37 x Sammaz 35	Hybrid	0.40	-0.56	-4.13	1.75	5.12
Sammaz 37 x Sammaz 29	Hybrid	-4.96	-5.36	-4.15	-0.68	-74.64
Sammaz 37 x Sammaz 28	Hybrid	0.00	-3.04	-13.20	0.27	23.26
Sammaz 17 x Sammaz 36	Hybrid	0.80	1.28	8.99	-3.21	-12.87
Sammaz 17 x Sammaz 27	Hybrid	-5.86	-7.51	-3.50	-2.47	-80.75
Sammaz 17 x Sammaz 35	Hybrid	-3.87	-5.12	-22.13	-0.54	-192.77
Sammaz 17 x Sammaz 29	Hybrid	-3.59	-2.34	-11.23	-1.10	-173.11
Sammaz 17 x Sammaz 28	Hybrid	-6.03	-4.63	-15.67	-2.62	-26.07
Sammaz 36 x Sammaz 27	Hybrid	-1.58	-2.54	2.07	0.27	-87.93
Sammaz 36 x Sammaz 35	Hybrid	2.51	5.71	3.37	2.10	-1260.70
Sammaz 36 x Sammaz 29	Hybrid	-0.60	5.30	-14.21	-0.53	-194.04
Sammaz 36 x Sammaz 28	Hybrid	0.59	1.63	-11.39	1.47	-77.43
Sammaz 27 x Sammaz 35	Hybrid	2.62	1.12	-19.02	8.89	-228.92
Sammaz 27 x Sammaz 29	Hybrid	-3.97	-4.03	-4.59	-1.37	-40.15
Sammaz 27 x Sammaz 28	Hybrid	1.44	-0.19	-17.91	-1.78	-30.76
Sammaz 35 x Sammaz 29	Hybrid	-0.81	-1.70	0.00	0.54	-333.23
Sammaz 35 x Sammaz 28	Hybrid	1.20	3.19	-28.03	-0.14	-97.91
Sammaz 29 x Sammaz 28	Hybrid	-0.83	0.00	-6.76	0.14	-33.48

Key: Das: Days to flower, Das: Days to silk, PH: Plant height, DM: Days to maturity and Gy: Grain yield/ha

Table 4. Percent inbreeding depression in Kadawa environment two

Genotype	Status	Daf	Das	PH	DM	GY
Sammaz 19 x Sammaz 19	Self	-1.54	-0.46	-6.32	-0.36	50.36
Sammaz 37 x Sammaz 37	Self	-0.48	0.44	-28.57	-2.50	65.38
Sammaz 17 x Sammaz 17	Self	2.90	2.62	-2.86	0.35	-9.82
Sammaz 36 x Sammaz 36	Self	-7.46	-7.73	-8.79	-6.03	-188.38
Sammaz 27 x Sammaz 27	Self	-7.92	-6.79	-23.16	-9.52	41.25
Sammaz 35 x Sammaz 35	Self	4.19	4.64	-71.48	3.73	-1707.80
Sammaz 29 x Sammaz 29	Self	1.03	0.47	23.00	4.67	-20.00
Sammaz 28 x Sammaz 28	Self	6.31	5.33	-4.22	-3.61	36.74
Sammaz 19 x Sammaz 37	Hybrid	1.72	0.22	8.78	-2.84	-275.01
Sammaz 19 x Sammaz 17	Hybrid	1.25	-4.09	-11.85	0.71	-104.81
Sammaz 19 x Sammaz 36	Hybrid	-2.90	-1.32	16.39	1.04	50.25
Sammaz 19 x Sammaz 27	Hybrid	11.06	9.49	14.57	8.68	-491.39
Sammaz 19 x Sammaz 35	Hybrid	-4.45	-4.29	19.26	-5.17	83.85
Sammaz 19 x Sammaz 29	Hybrid	-1.28	-0.70	-13.76	3.37	25.11
Sammaz 19 x Sammaz 28	Hybrid	-8.44	-6.98	9.24	-4.26	-48.09
Sammaz 37 x Sammaz 17	Hybrid	3.90	2.00	-8.23	1.40	-365.06
Sammaz 37 x Sammaz 36	Hybrid	-1.18	-2.38	4.45	-1.02	-0.67
Sammaz 37 x Sammaz 27	Hybrid	1.17	0.00	23.64	-0.34	-464.85
Sammaz 37 x Sammaz 35	Hybrid	-1.20	-1.77	18.20	0.88	-35.00
Sammaz 37 x Sammaz 29	Hybrid	0.00	-0.46	13.89	1.92	-277.41
Sammaz 37 x Sammaz 28	Hybrid	-3.48	-5.24	0.27	-0.35	-403.38
Sammaz 17 x Sammaz 36	Hybrid	3.12	5.22	9.12	2.06	-27.78
Sammaz 17 x Sammaz 27	Hybrid	-4.53	-6.32	18.64	-3.78	-107.75
Sammaz 17 x Sammaz 35	Hybrid	0.25	-1.11	5.72	-2.29	43.04
Sammaz 17 x Sammaz 29	Hybrid	-9.64	-7.09	-10.40	-2.99	49.52
Sammaz 17 x Sammaz 28	Hybrid	-4.57	-3.67	-33.00	-2.11	-61.01
Sammaz 36 x Sammaz 27	Hybrid	4.15	4.44	15.28	7.02	-31.00
Sammaz 36 x Sammaz 35	Hybrid	-3.32	-1.94	15.09	-6.00	80.37
Sammaz 36 x Sammaz 29	Hybrid	9.53	9.53	-5.54	9.40	-144.53
Sammaz 36 x Sammaz 28	Hybrid	-6.11	-4.89	0.00	-0.34	-57.00
Sammaz 27 x Sammaz 35	Hybrid	2.36	1.30	9.63	0.86	-58.00
Sammaz 27 x Sammaz 29	Hybrid	-4.62	-3.56	-30.22	0.85	-174.11
Sammaz 27 x Sammaz 28	Hybrid	-0.24	-0.67	-5.26	1.02	-415.60
Sammaz 35 x Sammaz 29	Hybrid	-8.27	-7.27	9.73	-5.26	19.11
Sammaz 35 x Sammaz 28	Hybrid	-2.76	-2.96	14.80	2.98	20.64
Sammaz 29 x Sammaz 28	Hybrid	-2.07	-1.17	-28.77	5.41	-145.29

Key: Das: Days to flower, Das: Days to silk, PH: Plant height, DM: Days to maturity and Gy: Grain yield/ha.

Table 5: Percent inbreeding depression combined across environments

Genotype	Status	Daf	Das	PH	DM	GY
Sammaz 19 x Sammaz 19	Self	-5.10	-4.75	-13.49	5.77	-9.66
Sammaz 37 x Sammaz 37	Self	2.56	3.54	-18.00	-1.08	-22.00
Sammaz 17 x Sammaz 17	Self	5.62	4.77	-2.77	3.44	-5.76
Sammaz 36 x Sammaz 36	Self	-3.90	-4.57	-3.81	-3.78	18.18
Sammaz 27 x Sammaz 27	Self	-2.68	-3.31	-10.34	-3.42	27.47
Sammaz 35 x Sammaz 35	Self	-1.32	0.20	-33.23	0.15	-12.78
Sammaz 29 x Sammaz 29	Self	3.15	2.07	13.40	3.98	8.58
Sammaz 28 x Sammaz 28	Self	0.23	0.42	7.43	-1.72	-24.05
Sammaz 19 x Sammaz 37	Hybrid	0.86	0.32	3.61	-4.54	21.59
Sammaz 19 x Sammaz 17	Hybrid	0.11	-1.80	-7.26	-6.24	-14.90
Sammaz 19 x Sammaz 36	Hybrid	1.68	2.04	7.22	-1.28	-55.10
Sammaz 19 x Sammaz 27	Hybrid	8.14	7.15	12.60	1.36	-24.58
Sammaz 19 x Sammaz 35	Hybrid	0.96	0.61	-2.66	-4.68	-159.38
Sammaz 19 x Sammaz 29	Hybrid	-0.66	1.34	-10.36	-2.34	-59.95
Sammaz 19 x Sammaz 28	Hybrid	-0.22	-0.18	4.27	-3.45	-1.17
Sammaz 37 x Sammaz 17	Hybrid	-1.01	-3.41	-7.09	-0.23	-54.01
Sammaz 37 x Sammaz 36	Hybrid	0.86	1.18	7.49	0.52	-22.17
Sammaz 37 x Sammaz 27	Hybrid	-1.96	-2.93	16.53	-2.35	-32.28
Sammaz 37 x Sammaz 35	Hybrid	-0.33	-1.11	7.33	1.37	-13.80
Sammaz 37 x Sammaz 29	Hybrid	-2.71	-3.12	4.51	0.46	-137.47
Sammaz 37 x Sammaz 28	Hybrid	-1.57	-4.04	-6.51	0.00	-32.07
Sammaz 17 x Sammaz 36	Hybrid	1.85	3.08	8.55	-0.90	-18.92
Sammaz 17 x Sammaz 27	Hybrid	-5.24	-6.95	6.40	-3.05	-84.68
Sammaz 17 x Sammaz 35	Hybrid	-2.00	-3.28	-9.13	-1.30	-83.51
Sammaz 17 x Sammaz 29	Hybrid	-6.34	-4.52	-11.53	-1.93	-101.87
Sammaz 17 x Sammaz 28	Hybrid	-5.37	-4.19	-24.49	-2.39	-31.49
Sammaz 36 x Sammaz 27	Hybrid	1.06	0.68	8.31	3.26	-67.87
Sammaz 36 x Sammaz 35	Hybrid	-0.11	2.25	9.09	-1.42	-132.13
Sammaz 36 x Sammaz 29	Hybrid	3.96	7.21	-10.30	3.81	-162.16
Sammaz 36 x Sammaz 28	Hybrid	-2.40	-1.30	-6.06	0.67	-70.33
Sammaz 27 x Sammaz 35	Hybrid	2.50	1.20	-4.12	5.36	-148.59
Sammaz 27 x Sammaz 29	Hybrid	-4.27	-3.81	-17.10	-0.38	-83.09
Sammaz 27 x Sammaz 28	Hybrid	0.67	-0.41	-11.52	-0.53	-88.22
Sammaz 35 x Sammaz 29	Hybrid	-4.15	-4.23	4.92	-1.98	-87.18
Sammaz 35 x Sammaz 28	Hybrid	-0.56	0.41	-5.67	1.22	-43.93
Sammaz 29 x Sammaz 28	Hybrid	-1.38	-0.53	-17.47	2.46	-68.91

Key: Das: Days to flower, Das: Days to silk, PH: Plant height, DM: Days to maturity and Gy: Grain yield/ha

Based on high-parent heterosis in environment one (Table 1), eighteen hybrids out of twenty-eight showed heterosis for grain yield which ranged from 1.63% to 927.03%. Sammaz 36 x Sammaz 35 exhibited highly significant positive heterobeltiosis for cob weight and grain yield. Fifteen hybrids out of twenty-eight exhibited heterosis for grain yield in environment two (Table 2) which ranged from 17.75% to 397.1%. Sammaz 19 x Sammaz 27 exhibited highly significant heterobeltiosis for cob weight and grain yield. Ryo et al. suggested that heterosis may lead to increases in genetic diversity as new genotypes with high level of performance or fitness are obtained [8]. The high per cent heterosis manifested in this study by crosses Sammaz 36 x Sammaz 35, and Sammaz 19 x Sammaz 27 corroborated the fact that wide genetic distances on the basis of origin, adaptation and maturity contribute to genetic diversity and to higher heterosis. Similar findings were reported by Leta who worked with seven East African maize populations that were crossed in diallel series [9]. These parents-in-crosses are therefore suitable for further genetic improvement of cob weight and grain yield. High reduction in yield with self-fertilization was observed in Sammaz 19, Sammaz 35 and Sammaz 28 for environments one, two and combined data (Tables 3, 4 and 5). According to Falconer, increasing homozygosity due to selfing,

vigour and productiveness was reduced by 50% in each generation due to inbreeding depression [10]. In environment one, the hybrid Sammaz 36 x Sammaz 35 recorded high inbreeding depression estimate for grain yield (-1260.70%), indicating accumulation of debilitating or recessive genes. Similar findings were recorded by Kumari et al. who recorded high inbreeding depression for number of bolls in F2 [11]. Similarly, in environment two, Sammaz 19 x Sammaz 27 had a high inbreeding depression value (-491.39%). For the combined data across environments, Sammaz 36 x Sammaz 29 had highest estimate of inbreeding depression (-162.16%). These results suggest that the parents may have recessive genes in common resulting in the reduction in yield of the hybrid.

CONCLUSION

Sammaz 36 x Sammaz 35 and Sammaz 19 x Sammaz 27 exhibited highly significant heterobeltiosis for cob weight and grain yield. These parents are the best for improvement of grain yield through recurrent selection. Inter variety hybrid development programme is recommended as an effective breeding approach to exploit heterotic potential of these varieties in the following crosses: Sammaz 36 x Sammaz 35, Sammaz 19 x Sammaz 27 and Sammaz x 37 x Sammaz 17.

ACKNOWLEDGMENT

The authors wish to acknowledge the AGRA-ABU-MSc Plant Breeding Training Project for sponsoring the project. The supervisory team (Prof. S. G. Ado, Prof. Aba and Dr. Katung), Prof. M. F. Ishiyaku, Prof. S. G. Mohammed and Dr. Alhassan Usman are also acknowledged for their guidance and assistance.

REFERENCES

1. Fakorede, M. A. B., Oluwaranti, A., Badu-Apraku, B. (2008) Grain yield of maize varieties of different maturity groups under marginal rainfall conditions. Journal of Agricultural Science, 53(3): 97-107
2. Vivek, S. B., Crossa, J., Alvarado, G. (2009) Heterosis and combining ability among CIMMYT's mid-altitude early to intermediate maturing maize populations. Maydica, 54:97-107.
3. Brown, J., Caligari, D.S. (2008) *An introduction to plant breeding*. Blackwell Publishing LTD. Pp. 48-49.
4. Miranda-Filho, J. B., Gorgulho, E. P. (2001) Cruzamentos com testadores e dialelos. In: L. L. Nass, A. C. C. Valois, I. S. Melo, M. C. Valadares (Eds.) Recursos genéticos e melhoramento de plantas (p. 649-672). Rondonópolis: Fundação Mato Grosso.
5. Cruz, C. D., Regazzi, A. J., Carneiro, P. C. S. (2012) Modelos biométricos aplicados ao melhoramento genético. Viçosa: UFV, 514 p.
6. Paschal, E. H., Wilcox, J. R. (1975) Heterosis and combining ability in exotic soyabean germplasm. Crop Sci., 15: 344-349.
7. Halauer, A.R., Carena, M.T., Miranda, J.B. (1988) *Quantitative genetics in maize breeding*. Second edition, Iowa State University Press. 443p.
8. Ryo, F., Kosuke, U., Sonoko, I., Kenji, O., Peacock, W. J., Dennis, E. S. (2018) Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. Breeding Science, 68: 145–158.
9. Leta, T. (2001) Heterosis and genetic diversity in crosses of seven East African maize (*Zea mays L.)* populations. *Seventh Eastern and Southern African Regional Maize Conference,* 11th -15th February, 2001. Pp. 125-129.
10. Falconer, D. S. (1989) *Introduction to Quantitative Genetics*, 3rd Ed. Longmans Green/John Wiley & Sons, Harlow, Essex, UK/New York. Pp. 200-210.
11. Kumari, B., Kajjidoni, S. T., Salimath, P.M., Patil, M. (2009) Heterosis and inbreeding depression for economic traits in desi cotton. Electronic Journal of Plant Breeding, 1: 47-51.

GENETIC DIVERSITY IN MORPHOLOGICAL TRAITS AMONG NIGERIAN VARIETIES OF GARDEN EGG (*Solanum gilo*)

Onwubiko, N. C. and Ohuba, C.O.
Department of Crop Science and Technology, Federal University of Technology, Owerri.
Email: nwakuche.onwubiko@futo.edu.ng

Abstract: Variations in 21 morphological (quantitative and qualitative) characters of four popular Nigerian varieties of garden egg were investigated. All vegetative pre-flowering qualitative traits of leaves (shape, colour, lobe, and hairs), stems (colour and hairs), and fruit (color, and shape) showed a reasonable level of variation among the four garden egg varieties except for shape of leaf blade, leaf margin, colours of margins, and corolla at flowering. The analyses of variance (ANOVA) for the eleven quantitative morphological characters were all statistically significant ($P< 0.05$) except for petiole length, internodal length, leaf length, leaf breadth, and mean number of days to flowering. Broad sense heritability estimates for all the traits were high with values > 60% in each case, except for mean days to flowering, leaf length, petiole length and leaf breath that had moderate ratings of between 30% to 59% estimates.

Keywords: Garden egg, Genetic variability, Quantitative and qualitative characters, *Solanum gilo*.

INTRODUCTION

Diversity of forms that exit in nature among an organism is the basis for speciation. Principally the biological concept of speciation is based on variations in physiology, chromosome and morphology. In fact, evolutions of species are phenotypically expressed by morphological variations. Some developments or morphological variations like changes in colour and shapes are strictly genetic, and this is essential in explaining the developmental basis of gene-to-form relationship. Morphological variations have been used to assess the genetic variations and relationships among populations of different plant species, as reported in mungbean (Arshad *et al*., 2009; Tantasawat *et al*., 2010). Furthermore, assessment of morphological variations of vegetative and reproductive organs is a useful tool in the characterization and evaluation of plant genetic resource, which is very essential in germplasm characterization and conservation. Invariably this will ensure the continued maintenance and improvement of agricultural production and, equally to sustainable development and poverty alleviation (Karp *et al*., 1997).

Garden egg (*Solanum gilo*) is an important food crop in several African countries, where it originated from, precisely Tropical Africa (Norman, 1992). Garden egg derived its name from the shape of its fruit -shaped like chicken eggs (Chen *et al.,* 2001).

This vegetable in recent times is gaining increasing popularity in the world (Pessarakli and Dris, 2003), because of its economic, nutritional and medicinal importance. It is the main source of income for producing households in West Africa and it is consumed on daily basis by urban families (Danquah-Jones, 2000). While garden egg is one of the three most consumed vegetables in Ghana, it was reported to be the second most important vegetable crop after okra in Côte d'Ivoire, (Siemonsma, 1981). In Nigeria, Garden egg is called "gauta" in Hausa, "afufa or anara" in Igbo and "igba" in Yoruba. It is cultivated extensively in the North (Chinedu *et al.,* 2011) while in other parts of the country (Nigeria), it is massively eaten raw or cooked; very popular in mixed and rich dishes such as stews and soups (Edem *et al.,* 2009). In recent times, several studies like Aliero (2007), Sabo and Dia (2009), Okon *et al*., 2010, and Chinedu *et al.,* 2011 reported some works carried out by scholars on the improvement of this crop and agronomic practices to increase its propagation. However, there is paucity of information on the morphological variation that exists on popular grown species of garden egg in Nigeria. The importance of information on genetic variability in efficient germplasm management which includes evaluation, utilization and genetic enhancement is crucial in crop breeding programmes. Therefore, this study was set up to assess genetic

variations in qualitative and quantitative traits of four popular Nigerian garden egg varieties.

MATERIALS AND METHODS

Materials and study area

Matured fruits of four popular varieties of garden egg were collected from various localities in Nigeria (Table 1). The study was carried out at the screen house and teaching field of the Department of Crop Science and Technology, Federal University of Technology Owerri (FUTO), that is located between longitudes 70^0 00E- 07^0 07E and latitudes 05^0 20N – 05^0 27N and have altitude of 55 m above sea level. The mean annual rainfall of the study area is 2000 mm and has a relative humidity of between 89-93% (Owerri meteorological center). Other materials used for this study include top soil, river sand, poultry manure, perforated nursery bags, hand trowel, shovel, watering can, meter rule and weighing balance.

Method and experimental design

The seeds of the four varieties of garden egg used for this study were sown in the nursery polybags filled with a mixture of topsoil, river sand and poultry manure prepared in the ratio of 3:2:1. Watering was done once a week and other standard cultural phyto-sanitory practices were carried out in order to raise healthy seedlings. This first phase of the experiment was conducted in the screen house. Healthy seedlings raised in the nursery were transplanted into flat seed beds in the field at 6 weeks after germination. Two seedlings were planted into each hole at 60 cm x 60 cm spacing which was later thinned down to one. The experimental design used in the study was Randomized Complete Block Design (RCBD) with four treatments (varieties) and five replications, giving a total of 20 experimental plots. Morphometric measurements were taken mostly at flowering on 10 qualitative characters and 11 quantitative (Table 2) directly in the field on ten plants per each variety on each plot, giving a total of 200 samples. All data collected were subjected to analysis of variance (ANOVA) and means separated using Fisher's least significance difference at 5% level of probability.

Table 1: Passport data of the garden egg varieties used for the study

Variety (local name)	Local Govt. Area	State
Yalo	Jama'a	Kaduna
Anyara	Nsukka	Enugu
Mkpuruofe	Owerri	Imo
Mikimiki	Isiala Ngwa	Abia

Estimation of genetic and phenotypic variance components

Using the expected mean square for ANOVA, the variance components were calculated as suggested by Uguru (1996).

Genetic variance = $\sigma^2e + r\,\sigma^2g$

$$\sigma^2g = Mg - \sigma^2e/r$$

Phenotypic variance (σ^2p) = $\sigma^2e + \sigma^2g$

Where, σ^2e = Error variance = Mse

σ^2g = Genetic variance

r = Number of replication

Heritability estimates

Broad Sense Heritability (BSH) gives an estimate of the presence of the proportion of the total variance (phenotypic) that is due to genetic effect and calculated as follows:

$$BSH(h^2) = \sigma^2g/\ \sigma^2g + \sigma^2e$$

but $\sigma^2p = \sigma^2F_2 = \sigma^2e + \sigma^2g$

$$h^2 = \sigma^2g/\ \sigma^2F_2$$

where, σ^2F_2 = total or phenotypic variance

σ^2g = genetic variance

$$h^2(\%) = \sigma^2g/\ \sigma^2F_2 \times 100/1$$

Table 2: Morphological characters of *Solanum gilo* evaluated

Quantitative characters	Qualitative characters
Plant height	Shape of leaf blade
Petiole length	Leaf margins
Number of branches	Presence of hairs on stem
Leaf length	Presence of hairs on leaves
Leaf breadth	Stem colour
Number of leaf lobes	Fruit colour
Days to flowering	Leaf colour at flowering
Internodes length	Corolla colour
Number of fruits	Colour of margins
Fruit weight	Fruit shape
Number of leaves	

RESULTS

Variation in qualitative Traits

Table 3 below shows the phenotypic differences that exist on qualitative characters among the four varieties of garden used for the study. The level of variation observed clearly differed from one character to another. Characters like leaf and stem colour at flowering, and fruit colour at harvest displayed wide range of variation among the varieties, while other traits like presence of hairs on stem, presence of hairs on leaves, and fruit shape showed moderate variation. There were no differences on characters like shape of leave blade, leaf margins, colour of margins and corolla colour among the four garden egg varieties.

Table 3: Result on qualitative characters of the garden egg varieties studied

Character	Yalo	Mkpuruofe	Anyara	Mikimiki
Leaf colour at flowering	Dark green	Green	Light green	Light green
Stem colour at flowering	Green	Light green	Green	Light green
Presence of hairs on stem	+	-	+	+
Presence of hairs on leaves	+	-	+	+
Fruit colour	Strip cream	Strip white	Strip green	Stripe green
Shape of leaf blade	Rhomobiod	Rhomobiod	Rhomobiod	Rhomobiod
Leaf margins	Serrated	Serrated	Serrated	Serrated
Colour of margins	White	White	White	White
Corolla colour	White	White	White	White
Fruit shape	Oval shaped	Round	Round	Round

+ = presence, - = absent

Quantitative morphological characters variability

The range, mean, standard deviation and coefficient of variation values for the eleven quantitative characters of the four varieties of garden egg evaluated are shown in Table 4 below. Broad variability was observed for most of the quantitative characters. Apparently, number of fruits per plant exhibited the highest variability among all the traits with a range value of 110.40, standard deviation of 39.80 and coefficient of variation of 74%. Other traits that displayed a considerable level of variability were leaf breadth, number of leaves, internodal length and number of branches. On the other hand, leaf length recorded the least level of variation amongst all the traits; had range of 4.5, standard deviation of 1.91 and coefficient of variation of approximately 8%. However, petiole length and number of leave lobes were traits that showed low variability.

The analyses of variance (ANOVA) for the eleven quantitative morphological traits of the four varieties of garden egg as shown in Table 5 below were all statistically significant ($P< 0.05$) except for petiole length and intermodal length, leaf length and leaf breath, and mean number of days to 50% flowering.

Table 4: Statistical parameters to measure variability on quantitative traits of the four garden egg varieties used for the study

Quantitative traits	Range	Mean	S.D.	C.V.
Plant height	58.30	75.32	26.00	34.51
Number of branches	14.80	14.90	6.21	41.67
Petiole length	2.30	8.22	1.05	12.77
Internodal length	5.70	5.65	2.40	42.47
Leaf length	4.50	23.90	1.91	7.99
Leaf breadth	31.96	26.95	15.59	57.84
Number of leave lobes	1.50	10.17	2.07	20.35
Number of leaves	60.00	54.55	26.56	48.68
Days to 50% flowering	12.4	55.10	5.31	9.63
Number of fruits per plant	110.40	39.80	53.64	74.19
Fruit weight per plant	138.86	292.16	26.56	9.09

S.D. = Standard deviation, C.V. = Coefficient of variation

Table 5: Analysis of variance (ANOVA) result on quantitative traits of the four garden egg varieties

Treatment	PH (cm)	NOB	PL (cm)	IL (cm)	LL (cm)	LB (cm)	NOL	NOLL	DTF	NOF/P	WOF/P (g)
Yalo	38.98**	7.61**	7.30	2.30	24.50	20.64	39.42**	8.60**	50.00	14.00**	273.40**
Mkpuruofe	74.66**	16.62**	7.51	6.40	21.72	18.54	62.41**	12.31**	52.80	120.22**	386.86**
Anyara	90.36**	22.40**	9.64	5.91	26.20	50.30	88.24**	11.60**	55.21	15.21**	258.41**
Mikimiki	97.28**	13.00**	8.50	8.00**	23.21	18.34	28.20**	8.20**	62.42	9.80	250.00**
$LSD_{(0.05)}$	9.92	3.41	1.34	1.29	4.87	3.38	7.17	1.55	5.92	12.41	14.90

** Significant at P = 0.05/ Where PH, NOB, PL, IL, LL, LB, NOL, NOLL, DTF, NOF/P, WOF/P represent Plant height, Number of branches, Petiole length, Internode length, Leaf length, Leaf breadth, Number of leaves, Number of leaf lobes, Days to flowering, Number of fruits per plant, weight of fruits per plant

Variance components and heritability estimates

Genotypic variance estimates for the all the traits as displayed in Table 6 below were generally less than the phenotypic variances. Generally, genotype by environment variance (G x E) estimates were the lowest and negligible in all cases. Broad sense heritability estimates for almost all the traits were high with values > 60% in each case. However, leaf length, leaf breath, petiole length and days to flowering recorded moderate heritability ratings of between 30% to 59% estimates.

Table 6: Variance components and heritability estimates of the 11 quantitative characters of the four varieties of garden egg used for the study

Sources of variation	σ^2g	σ^2p	σ^2ge	h^2 (%)
Number of fruits	2815.51	2896.60	0.00	97.19
Number of leaves	698.47	725.42	0.00	96.27
Plant height	666.13	717.98	0.01	92.77
Number of branches	37.45	43.57	0.03	85.95
Internode length	4.47	5.35	0.01	83.56
Fruit weight	3877.77	4802.66	0.00	80.74
Number of leaf lobes	3.91	5.186	0.06	75.51
Days to 50% flowering	24.49	42.93	0.01	57.04
Leaf length	3.00	5.36	0.03	55.97
Petiole length	0.68	1.64	0.00	41.46
Leaf breadth	0.92	2.85	0.01	32.28

σ^2g = genetic variance, σ^2p = phenotypic variance,
σ^2ge = genotype X environment variance, h = percentage heritability

DISCUSSION

In crop breeding programmes, investigations to identify the available level of diversity is an essential step in crop improvement, and this can be achieved by collection and classification of germplasm. In this study, analysis of morphological characters of the four varieties of garden egg revealed that the varieties differed considerably in both qualitative (Table 3) and quantitative (Table 4) traits. However, a wide range of morphological differences were observed more in the quantitative than the qualitative characters. The pattern of the spread of variation (Tables 3-5) in all the characters evaluated were not uniform.

The quantitative characters studied were mostly significantly different (P< 0.05) and proved useful in examining diversity and discriminating among the varieties. The quantitative trait that showed the highest variation was the number of fruits per plant. Other quantitative traits that showed pronounced variation among the garden egg varieties were leaf breadth, number of leaves, internodal length and number of branches (Table 5). Apparently these

agronomic and vegetative growth parameters significantly revealed a distinct and wide range of variation among the varieties, which have some implication in selection for any crop breeding improvement programme of this crop. The finding of this study is in agreement with the report of wide range of variation in some morphological characters observed by other workers in pepper (Sood *et al.,* 2009, Farhed *et al.,* 2008), and solanum species (Tumbilen *et al.,* 2011, Aliero, 2007).

The result on qualitative characters showed that only two characters exhibited a pronounced variation among the garden egg varieties (Table 3). Leaf colour at flowering discriminated them into green, light and dark green while fruit colour at harvest distinctly differentiated the four garden egg varieties into strip cream, strip white and strip green types. Other qualitative characters that showed moderate variation were stem colour, fruit shape, presence of hairs on stems and leaves. These qualitative traits that discriminated among the garden egg varieties fell among the classical Mendelian traits of kinds such as form (e.g., round or wrinkle seeds of pea); which are usually under genetic control of two or many alleles of a single gene with little or no environmental modifications to obscure the gene effects; inferring that genetic differences may exist among these four garden egg varieties. This result is in line with the findings of some studies on other varieties of eggplant (Islam and Uddin, 2009; Naik *et al*., 2010; Hitomi *et al*., 1998; Vadivel and Bapu, 1990a). On the other hand, qualitative characters like shape of leaf blade, leaf margins, colour of margins and corolla colour were the same for all the varieties. Certainly, closely related species or varieties will definitely share some similarities amongst them as a mark of distinction and identity. Infact similarities exist in subspecies. The four varieties of garden egg evaluated in this study were varieties or cultivars of the specie *gilo*, hence, may not differ completely in all the traits. These results are in line with findings of Osei *et al*., (2010), who observed distinct and wide variation, as well as a lot of similarities in quantitative traits among the varieties of eggplant they evaluated for morphological characterization.

In all the traits, phenotypic variance (σ^2p) were slightly higher than the genotypic variance (σ^2g), which suggests low environmental effects (Ashok *et al*, 2000). In addition, highly significant genotypic variances as were observed in all the traits are indicative of the magnitude of variation that exists among the varieties, thus offering a wide range of opportunity to carry out selection of desirable traits. The estimated heritability ranges from 32.28% for leaf breadth to 97.19% for the number of fruits, which were observed to be generally high for all the traits. Heritability estimates classified by John *et al* (1955) suggests that estimates (0% to 30%) low, (above 30% to 60%) moderate, and above 60% high, and the effectiveness of selection depends on heritability. Invariably the observed high heritability values in this study are satisfactory and can possible be used for crop improvement with regards to these traits through selection.

CONCLUSION

The significant variations observed in the performances of both the quantitative and qualitative traits of the four varieties of garden egg studied shows that they differ considerably. The result from the estimates of genotypic and phenotypic variance as well as the heritability suggests that these traits were under genetic control and therefore can be used for genetic improvement of the crop. This provides opportunity to improve desirable morphological traits of garden egg.

REFERENCES

Ashok,S., M.N. Sheriff, and S.L.Narayanam (2000). Combining ability study on sun flower (Helianthus annus L.), Crop Research 20(3). 457-462.

Chen N.C., Li H.M. and Kail, T. (2001). Eggplant Production, AVRD, http://www.av.rdc.org/LC/eggplant/production/oltitle.html

Chinedu, S.N., Olasumbo, A.C., Eboji, O.K., Emiloju, O.C., Arinola, O.K. and Dania, D.I.(2011). Proximate and Phytochemical analyses of *Solanum aethiopicum* L. and *Solanum macrocarpon* L. Fruits. Research Journal of Chemical Sciences *1*(3), 63-71.

Danqua-Jones, A**.** (2000). Variation and correlation among agronomic traits of Garden egg (*Solanium gilo* Radii). Department of Crop Science, Accra, University of Ghana, Lgon 30.

Edem, C.A., Dounmu, M.I., Bassey, F.I., Wilson, C. and Umoren, P., (2009). A comparative Assessment of the Proximate Composition, Ascorbic Acid and heavy metal content of two Species of Garden egg (*Solanum*

gilo and *Solanum aubergine*). Pakistan Journal of Nutrition, 8(5): 582-84.

Farhad M., Hasanuzzaman, M., Biswas, B.K. Azad, A. K and Arifuzzaman, M. (2008). Reliability of yield contributing characters for improving yield potential in chilli. International Journal of Sustainable Crop Production 3 (3), 30-38.

Hitomi, A., Amagal, H. and Ezura, H. (1998) The influence of auxin type on the array of somaclonal variants generated from somatic embryogenesis of egg plant, *Solanum melongena*. Plant Breeding 117,379-383.

Islam M.S. and Uddin, M.S, (2009). Genetic variation and trait relationship in the exotic and local eggplant germplasm. Bangladesh Journal of Agriculture Research 34(1), 91-96.

John, H.W., Robinson, H.F. and Comstock, R.E. (1955). Estimates of genetic and environmental variability in soya bean. Agronomy Journal.,47: 318-324.

Karp, A., Kresovich, S., Bhat, K. V., Ayad, W. G., & Hodgkin, T. (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. International Plant genetic Resources Institute (IPGRI). *IPGRI* Technical Bulletin No. 2.

Naik K., Sreenivasulu, G.B., Prashanth, S.J., Jayaprakashnaraya, R.P., Nataraj S.K. and Mulge, R. (2010) Genetic variability in eggplant (*Solanum melongena L.*). International Journal of Agriculture Science 6**(**1), 229-231.

Norman, J.C**.** (1992). Tropical Vegetable Crops. Arthur stockwell Ltd, Devon.

Okon, U.E., Enete, A.A. and Bassey, N.E. (2010) Technical efficiency and its determinants in Garden Egg (*Solanum spp*.) production in Uyo Metropolis, Akwa Ibom State, Nigeria. Field Action Science Report, Special Issues (1)

Osei, M. K., Banful, B., Osei, C. K., & Oluoch, M. O. (2010). Characterization of African Eggplant for Morphological Characteristics. Journal of Agricultural Science and Technology, 4,33-38.

Pessarakli, M.M., and Dris, R., (2003). Effects of pruning and spacing on the yield and quality of eggplant. Food Agric. Environ. 1(2): 215-216.

Sabo, E., and Dia, Y. Z., (2009): Awareness and Effectiveness of Vegetable Tech. Information packages by vegetable Farmers in Adamawa State, Nigeria. J. Agric. Res. 4(2): 65-70.

Siemonsma, J. S (1981). A survey of indigenous vegetables in Ivory Coast Proc. 6th African Symposium on Horticultural crops, Ibadan, Nigeria.

Sood, S., Sood, R., Sagar, V. and Sharma, K.C, (2009). Genetic variation and association analysis for fruit yield, agronomic and quality characters in Bell pepper. International Journal of Vegetable Science 15(3), 272-284.

Tantasawat, P., Trongchuen, J., Prajongjai, T., Thongpae, T., Petkhum, C., Seehalak, W., & Machikowa, T. (2010). Variety identification and genetic relationships of mungbean and blackgram in Thailand based on morphological characters and ISSR analysis. Afr. J. Biotechnol*, 9*(27), 4452-4464.

Tumbilen Y., Frary, A., Mutlu, S. and Doganlar, S. (2011). Genetic diversity in Turkish eggplant (*Solanum melongena*) varieties as determined by morphological and molecular analyses. International Research Journal of Biotechnology 2(1), 016-025.

Uguru, M.I. (1996). Crop production: tools, techniques and practice. Fulladu publishing company. 153pp.

Vadivel, E. and Bapu, J.R.K, (1990a).Variability and correlation coefficient in F2 generation of egg plant. South India Horticulture 36(3):153-155.

MULTIVARIATE EVALUATION OF VARIATIONS IN FRUIT AND SEED YIELD COMPONENTS OF DIFFERENT GENOTYPES OF CAYENNE PEPPER (*Capsicum frutescens*)

[1*]Abdul-Rafiu, A. M., [2]Adebisi, M. A., [2]Oduwaye, O.O. [3]Idowu-Agida, O. O, and [1]Olomide, O.A.K.

[1]Seed Technology Unit, [3]Vegetable Research Program,
National Horticultural Research Institute (NIHORT), PMB 5432, Ibadan, Oyo State, Nigeria.
[2] Department of Plant Breeding and Seed Technology,
Federal University of Agriculture, Abeokuta, PMB 2240, Abeokuta, Ogun State, Nigeria.
***Corresponding Author:** monsurayoola@yahoo.co.uk

Abstract: Sixteen genotypes of *Capsicum frutescens* were evaluated for fruit and seed yield characters in late cropping seasons of 2010 and 2011 at the Teaching and Research Farms, Federal University of Agriculture, Abeokuta. The genotypes were grown in a randomized complete block design with three replicates under rain-fed field conditions. Data collected on fruit and seed yield components were subjected to principal component (PCA), single linkage cluster, FASTCLUS and path-coefficient analysis. PCA indicated that three components accounted for 86.25% of the total variation among traits. The first principal component (PC) was assigned 50.16 % while the second and third PC recorded 25.23 and 10.86 % respectively. Seven characters (unit fruit weight, fruit yield per plant, fruit length, fruit yield per hectare, number of seeds per fruit, seed weight per fruit and seed production efficiency) significantly contributed to the total variation in the first PC implying that these characters were largely responsible for variation among the 16 genotypes. Single Linkage Cluster Analysis (SLCA) summarized the accessions into three distinct clusters at 40.3% level of similarity. However, the FASTCLUS analysis segregated the genotypes into four groups, showing the fruit and seed yield potentials of each group and the degree of resemblance. The correlation between other traits on fruit yield and seed yield revealed significant association between number of fruits, number of seeds per fruit, seed weight, SPE, fruit yield and seed yield. Direct selection for number of fruits per plant and number of seeds per fruit can be considered for simultaneous improvement of fruit yield and seed yield in cayenne pepper. Genotypes PP9955–15 and PP0337–7562 were identified as top fruit yielding while Chilli 14 was the most distinct with averagely high fruit yield and highest seed yield. The characters identified by PCA could be included in the crop improvement scheme for improved fruit and seed yield within the evaluated pepper germplasm. These cayenne peppers are therefore suitable for breeding program.

Keywords: Chilli Pepper, FASTCLUS Analysis, Fruit Yield, Principal Component Analysis, Seed Yield.

INTRODUCTION

The cayenne pepper is a popular chilli pepper used for flavouring and medicinal purposes. Availability of diverse genetic base provides means for selection in crop improvement. The assessment of available genetic materials through multivariate analysis could provide essential information towards identification of suitable genotype for crop improvement.

Multivariate analysis using clustering and principal component analysis of morphological traits has been reported to be most widely used and cost effective in germplasm classification and evaluation (Smith *et al.,* 1991; Mohammadi and Prasanna, 2007). The use of FASTCLUS analysis is also significant in identifying desirable characters associated with cluster groups among genotypes (Oduwaye, 2013: Abdul-Rafiu, 2015). The technique of path analysis has been helping breeders to develop appropriate strategies to select superior genotypes of different crops, such as tomato (Sobreira *et al.,* 2009; Rodrigues *et al.,* 2010) and peppers (Luitel *et al.,* 2013, Moreira *et al.,* 2013, Rohini and Lakshmanan, 2015).

Furthermore, multivariate analysis could also help in identifying traits for selection purposes among genotypes. According to Beiragi *et al.* (2012), selection based on the result of Multivariate analysis can be more effective than when such traits are studied alone. In 2012, Maga *et al*. utilized multivariate analysis to study agro-morphological traits in aboriginal Nsukka yellow pepper (*Capsicum annumm*) genotypes and observed wide diversity for all the characters studied. Similarly, Adebisi (2004) and Adekoya *et al.* (2011) utilized

multivariate techniques to explain the extent of variation in sesame and okra genotypes, respectively and the findings revealed considerable variation in the seed yield characters. In spite of the high economic importance and adaptability across different agro-ecologies of Nigeria, cayenne pepper has not received adequate improvement opportunities. There is need to evaluate the level of variation among available germplasm for crop improvement for the benefit of the teaming population of pepper farmers. This study was carried out to evaluate variations in fruit and seed yield components of cayenne pepper (*Capsicum frutescens*) genotypes through multivariate analysis.

MATERIALS AND METHODS

The study was carried out at the Teaching and Research Farms of the Federal University of Agriculture, Abeokuta (Lat 7^{o}N 37'N, long. 3^{o} 89'E and altitude 210m), under rain-fed field conditions in 2010 and 2011 late cropping seasons. The experiment was conducted in a randomized complete block design with three replicates. Plot size was 3.5m x 1m for each genotype comprising of three rows and a total of 24 plants per replication. Seedlings of 16 cayenne pepper genotypes raised in the nursery using sterilized top soil were transplanted at five weeks after sowing at spacing of 50 cm x 50 cm.

The genotypes were evaluated for -

i. Number of fruits per plant - This was determined as total number of fruits harvested in each plot over the life span of the crop divided by number of plants.
ii. Unit fruit weight - This was determined by weighing 20 fruits from each plot and dividing the value by 20.
iii. Fruit length - Length of 20 fruits sampled randomly from the second harvest were measured with a metre rule and then computed to average.
iv. Fruit yield per plant - This was determined by weighing total number of fruits harvested per plot divided by number of plants per plot.
v. Fruit width - Width of 20 fruits sampled randomly from the second harvest were measured using a vernier calliper and then computed to average.
vi. Number of seeds per fruit - This was determined as average number of seeds of 20 fruits sampled randomly from the second harvest in each plot.
vii. 100 seed weight - 100 seeds in three replications were counted from (vi) above and weighed in gram.
viii. Seed weight per fruit - Seeds obtained from (vi) above was weighed and recorded.
ix. Seed yield per plant – this was determined by multiplying number of seeds per fruit (obtained previously above) by number of fruit per plant.
x. Fruit yield per hectare - This was estimated by multiplying fruit yield per plant by number of plants per hectare.
xi. Seed yield per hectare – This was estimated by multiplying seed yield per plant by number of plants per hectare.
xii. Seed production efficiency (SPE) was determined according to Ogunbodede and Ogunremi (1986) using –

$$SPE = \frac{Seed\ Weight}{Fruit\ Weight} \times 100$$

Data Analysis

Analysis of genetic diversity was done using multivariate techniques. Principal component analysis (PCA) was used to evaluate the contribution of each character to the genetic divergence and bi-plots between the principal axes were used to describe the genetic diversity. Agglomerative single linkage cluster was used to identify similar groups among the genotypes. Also, FASTCLUS analysis was performed for disjoint clusters among the genotypes. Path-coefficient analysis was used to determine the cause-effect relation between fruit yield, seed yield and the other characters (Dewey and Liu, 1959).

RESULTS

The total variance of the first three principal component (PC) axes accounted for 86.25% for the fruit and seed yield characters of the genetic variation among the pepper genotypes (Table 1). Principal component axis one (PC1) explained 50.16% and PC2 25.23 % while PC3 accounted for 10.86 %of the total variation. Unit fruit weight, fruit yield per plant, fruit length, fruit yield per hectare, number of seeds per fruit, seed weight per fruit and seed production efficiency were with high score under PC1. Number of fruits per plant, seed yield per plant and seed yield per hectare were with high

scores in PC2, while 100-seed weight was with high score in PC3.

The PCA biplot between PC1 and PC2 (Figure 1) revealed that Chilli 14 and *Ata wewe* had the farthest distance from the other genotypes. However, BG 3 was the most distinct in the biplot between PC2 and PC3.

The dendrogram obtained from single linkage cluster analysis revealed that there was no linkage among the pepper genotypes at 100% similarity level (Figure 1). There was a link between IA108-3 and Sakarho at 95.1 % similarity level. Three groups were revealed at 40.3% similarity coefficient, group I with Cayenne slim and Ata wewe and group III with Chilli 14. Group II with 81% of the total genotypes consisted of three sub-groups. Sub-group 1 is composed of two genotypes (IA108-3 and Sakarho) while in sub-group 2 are nine genotypes. Sub-group 3 consisted of two genotypes too (PP9955-15 and PP0337-7562).

The means and standard deviation of four cluster groups obtained from FASTCLUSS analysis for fruit and seed yield characters are presented in Table 2. Cluster 1 is composed of five genotypes (Cayenne slim, Ata wewe, NHV 1B, ICPN 19 - 07, BG 4) which were characterised by highest number of fruits per plant (101.93) and seed production efficiency (9.6 %). Chilli 14 is the only genotype in cluster 2 and associated with the highest number of seeds per fruit, seed yield per plant, seed weight per fruit and seed yield per hectare. The third group (cluster 3) exhibited the lowest performance in most of the characters and composed of eight genotypes (IA108-3, Ikire, Sakarho, NHV1A, PP0437-7509, Bird eye 11, PP0438 – 8543 and BG 3). The last cluster (Group IV) is made up of genotypes PP9955-15 and PP0337-7562 which exhibited highest performance in most of the characters.

Table 1: Principal component based on correlation co-efficient matrix of major seed yield traits

Fruit and Seed Yield Traits	PC 1	PC 2	PC 3
Unit fruit weight (g)	0.384	-0.037	0.195
Fruit yield/plant (g)	0.355	0.166	0.194
Number of fruits per plant	0.263	0.369	0.271
Fruit length (cm)	0.321	0.084	0.313
Fruit width (cm)	0.230	0.024	0.218
Fruit yield per hectare (T)	0.355	0.166	0.575
Number of seed per fruit	0.312	0.222	0.312
100 seed weight (g)	0.221	0.161	0.426
Seed yield per plant (g)	0.012	0.569	0.106
Seed weight per fruit (g)	0.341	0.161	0.006
Seed production efficiency	0.335	0.228	0.314
Seed yield Kg/ha	0.012	0.569	0.159
Eigen Value (EV)	6.02	3.03	1.30
Proportion of variation (%)	50.16	25.23	10.86
Cumulative Variation (%)	50.16	75.40	86.25

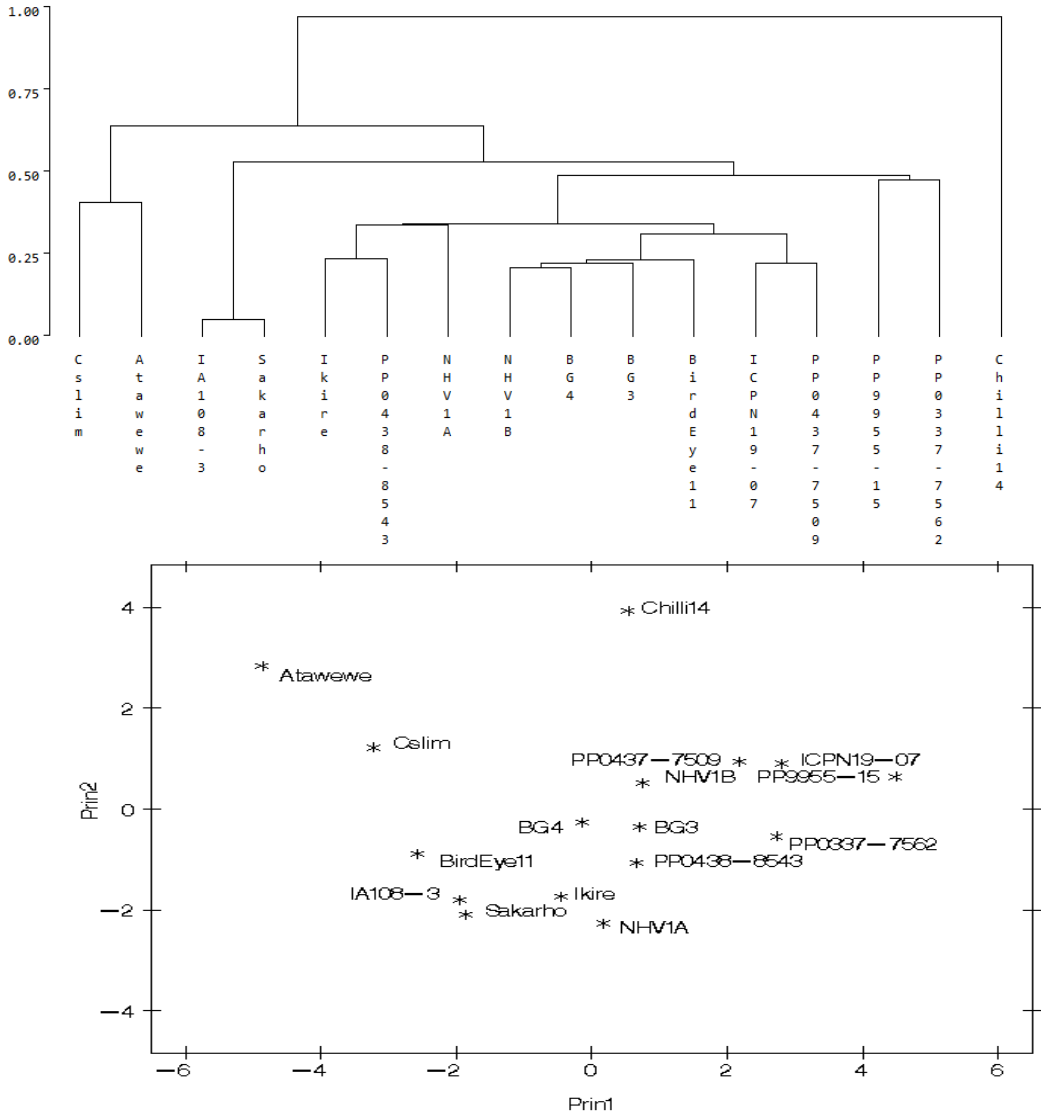


Figure 1: Bi-plots of PC1/PC2 and PC2/PC3 displaying genetic diversity among 16 genotypes of cayenne pepper based on fruit and seed yield characters

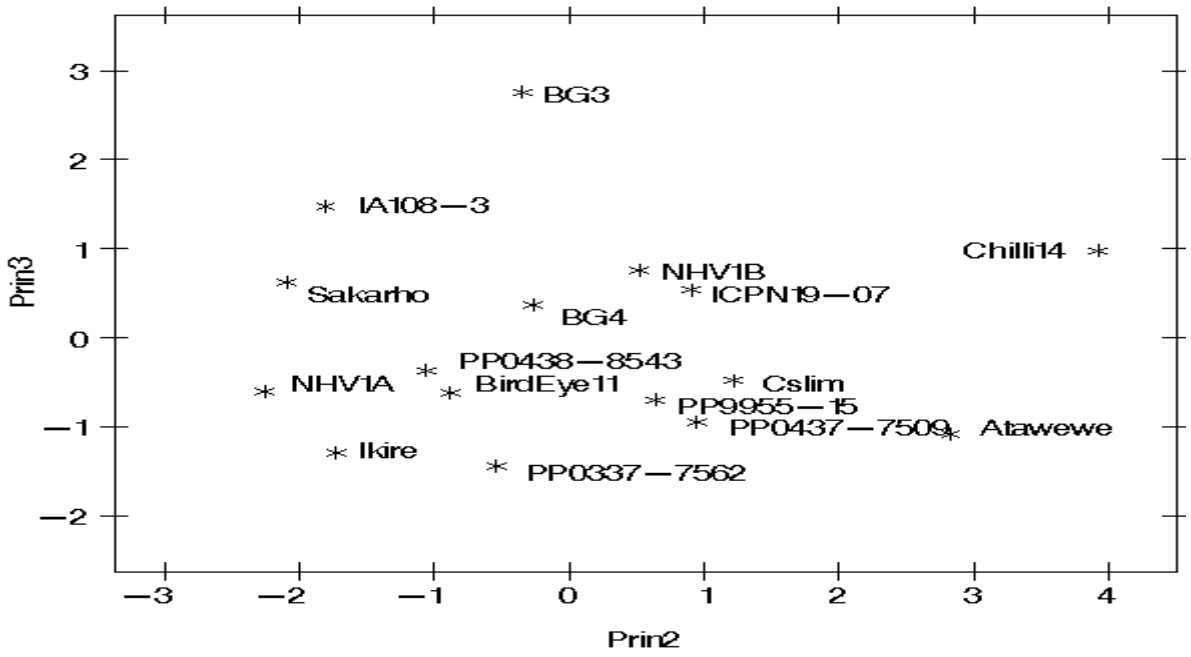


Figure 2: Dendrogram generated from the single linkage cluster analysis showing the relationship among seed yield characters in 16 genotypes of cayenne pepper

Table 2: Variation in fruit and seed yield characters in 16 genotypes of cayenne pepper by FASTCLUS procedure

Seed Yield Characters	Cluster I Cayenne slim, Ata wewe, ICPN 19 - 07, NHV 1B, BG 4	Cluster II Chilli 14	Cluster III IA108-3, Ikire, Sakarho, BG 3, NHV1A, PP0437-7509, Bird eye 11, PP0438-8543	Cluster IV PP9955–15, PP0337–7562
Number of fruits per plant	101.93 (59.16)	98.17	62.00 (14.98)	62.61 (10.98
Unit fruit weight (g)	3.28 (2.09)	3.62	3.54 (1.09)	7.51 (2.10)
Fruit yield per plant (g)	251.67 (78.99)	355.63	215.31 (79.61)	469.41 (73.73)
Fruit length (cm)	7.67 (3.73)	5.46	8.12 (2.19)	12.54 (0.98)
Fruit width (cm)	1.38 (0.5)	1.71	1.69 (0.83)	1.75 (0.47)
Fruit yield per hectare (T)	10.07 (3.16)	14.23	8.61 (3.19)	18.78 (2.99)
Number of seed per fruit	59.43 (17.74)	82.83	53.40 (10.57)	67.94 (8.28)
100 seed weight (g)	0.396 (0.05)	0.380	0.394 (0.02)	0.427 (0.03)
Seed yield per plant (g)	20.76 (2.75)	30.60	12.76 (2.35)	18.60 (2.70)
Seed weight per fruit (g)	0.24 (0.09)	0.31	0.21 (0.04)	0.29 (0.02)
Seed production efficiency	9.60 (0.04)	9.00	7.01 (0.03)	4.30 (0.02)
Seed yield per hectare (Kg)	830.12 (110.04)	1224.11	510.48 (94.01)	743.76 (107.85)

Values in parentheses are cluster standard deviations.

The correlations between seed yield and number of fruits per plant, number of seeds per fruit, seed weight per fruit, SPE and fruit yield revealed significant association (Table 3). Similarly, fruit yield had significant correlations with unit fruit weight, fruit length, number of seeds per fruit, 100-seed weight, seed weight per fruit and SPE. The correlation coefficients between number of seeds and fruit yield were lower than their direct effects on seed yield. This was due mainly to their decreasing effects through unit fruit weight. Unit fruit weight and seed weight had negative direct effect on seed yield. However, unit fruit weight, number of seeds per fruit and 100-seed weight had positive correlation coefficients and direct effects on fruit yield. Number of fruits per plant had reducing effect on seed yield through unit fruit weight and number of seeds. Most of the characters had reducing effect on seed yield through number of fruits per plant and seed weight and increasing effect via unit fruit weight. SPE was negatively associated with seed yield.

DISCUSSION

As a result of the variations observed in terms of fruit and seed yield characters in the 16 cayenne pepper genotypes, the use of multivariate techniques has aided better understanding of the performance of these pepper genotypes by classifying them into clusters or groups based on similarities and diversity observed. Idowu-Agida *et al.* (2012) have reported wide diversity in *C. frutescens* genotypes cultivated in Southwest, Nigeria. Considerable amount of variation has equally been reported by Usman *et al.* (2014) in terms of morphophysiological and yield characters studied in 36 genotypes of chilli. They concluded that such wide variation indicated the scope for possible selection and hybridization among the genotypes.

Table 3: Path-coefficients showing the cause-effect relation between fruit yield, seed yield and the other characters in 16 Cayenne pepper genotypes

Character	Direct effect	Indirect effect									Correlation coefficient
		No of fruit/plant	Unit fruit weight	Fruit length	Fruit width	Number of seeds/fruit	100-seed weight	Seed weight/fruit	SPE	Fruit yield/ plant	
Seed yield											
No of fruit/plant	0.56		0.25	0.01	0.00	-0.25	-0.10	0.08	0.07	-0.03	0.58**
Unit fruit weight	-0.56	-0.24		-0.02	0.00	0.37	0.12	-0.13	-0.10	0.62	0.06
Fruit length	-0.03	-0.22	-0.40		0.00	0.27	0.12	-0.10	-0.07	0.42	-0.01
Fruit width	0.01	-0.10	-0.13	-0.01		0.22	0.01	-0.06	-0.02	0.14	0.06
Number of seed/fruit	0.79	-0.18	-0.26	-0.01	0.00		0.05	-0.21	-0.01	0.33	0.49**
100 seed weight	0.25	-0.23	-0.26	-0.01	0.00	0.14		-0.10	-0.05	0.31	0.06
Seed weight/fruit	-0.22	-0.21	-0.32	-0.01	0.00	0.75	0.11		-0.03	0.40	0.48**
SPE	0.14	0.29	0.42	0.02	0.00	-0.09	-0.09	0.04		-0.42	0.31**
Fruit yield/plant	0.73	-0.02	-0.48	-0.02	0.00	0.36	0.11	-0.12	-0.08		0.47**
Fruit yield											
No of fruit/plant	0.59		-0.35	0.01	0.00	-0.26	-0.17	0.25	-0.11		-0.04
Unit fruit weight	0.78	-0.26		-0.01	0.00	0.38	0.19	-0.39	0.16		0.86**
Fruit length	-0.02	-0.24	0.56		0.00	0.28	0.19	-0.30	0.11		0.58**
Fruit width	0.02	-0.11	0.18	0.00		0.23	0.01	-0.18	0.04		0.19
Number of seeds/fruit	0.82	-0.19	0.37	-0.01	0.00		0.07	-0.64	0.02		0.45**
100-seed weight	0.40	-0.24	0.36	-0.01	0.00	0.15		-0.30	0.07		0.43**
Seed weight/fruit	-0.68	-0.22	0.45	-0.01	0.00	0.78	0.18		0.04		0.55**
SPE	-0.21	0.31	-0.59	0.01	0.00	-0.09	-0.14	0.14			-0.58**

Seed production efficiency (SPE); Residual effect: 0.17 (Seed yield), 0.25 (fruit yield).

The principal component analysis provided essential information by identifying major characters contributing to the observed variation. All the fruit and seed yield characters except the fruit width were identified to be important and could be majorly observed in breeding programme for cayenne pepper. The result obtained by Maga *et al.* (2012) identified total fruit weight per plant, fruit girth, single fruit weight, number of fruits per plant as major traits contributing to variability observed in the yield of Nsukka yellow pepper (*C . annuum*) accessions. They further added that higher percentage loadings recorded among these traits indicate that selection based on them could bring about improvement in the mean value of desirable characters.

The single linkage cluster analysis indicates that hybridization across the groups could be possible resulting in better hybrid. The FASTCLUSS analysis also grouped the genotypes into four and provided further information by associating quantitative values to the parameters under each grouping. This gives a better understanding of the potentials of each group. Therefore, identification of desirable characters for possible hybridization was made easier. Chilli 14 exhibited the highest distinctness according to the biplot followed by BG 3. The dendrogram also showcased the level of relatedness of 13 genotypes which later converged with two other genotypes as Chilli 14 remains the most distinct.

The relationship between the fruit and seed yield and other characters indicated either increasing or reducing effects which can be considered for selection in cayenne pepper fruit and seed yield improvement.

CONCLUSION

Fruit and seed yield characters are the ultimate goal of cayenne pepper producers. The evaluation of diversed

genotypes of cayenne pepper resulting in outstanding fruit and seed yield characters is a significant step towards cayenne pepper improvement and development of hybrids. This study revealed significant diversity among the evaluated cayenne pepper genotypes. The cayenne peppers have been classified into different groups/ clusters for identification of peculiar fruit and seed yield characters for rational selection of parental stocks for hybridization or otherwise in cayenne pepper research and improvement programs. However, the following genotypes have been identified as top fruit yielding PP9955–15 and PP0337–7562 while IA108-3, Ikire, Sakarho, BG 3, NHV1A, PP0437-7509, Bird eye 11, PP0438-8543 were the least yielding. Chilli 14 was the most distinct with averagely high fruit yield and highest seed yield. These cayenne peppers are therefore suitable for breeding program. Direct selection for number of fruits per plant and number of seeds per fruit can be considered for simultaneous improvement of fruit yield and seed yield in pepper.

REFERENCES

Abdul-Rafiu, A. M. 2015. Effect of plant population, processing method and storage duration on seed yield, quality and longevity in pepper (*Capsicum frutescens L.*) genotypes. *Unpublished Ph.D. Thesis,* Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta. Ogun State, Nigeria. Pp. 208.

Adebisi, M. A. 2004. Variation, stability and correlation studies in seed quality and yield characters of sesame. *Unpublished Ph.D. Thesis,* Department of Plant Breeding and Seed Technology, University of Agriculture University of Agriculture, Abeokuta. Pp 122.

Adekoya, M. A., Adebisi, M. A., Abdul-Rafiu, A. M., Ariyo, O. J. and Ayo-Vaughan, M. A. 2011. Multivariate analysis of genetic variability in pod and seed yield characters of okra (*Abelmoschus esculentus* L. (Moench)) grown under different cropping seasons. *Nigerian Journal of Genetics.* 25: 30 - 46

Beiragi, M. A., Sar, B. A. S., Geive, H. S., Alhossini, M. N., Rahmani, A. and Gharibdoosti, A. B. 2012. Application of the multivariate analysis method for some traits in maize. African Journal of Agricultural Research 7(10): 1524 – 1533.

Dewey, J. R. and Lu, K. H. 1959. A correlation and path-coefficient analysis of components of crested wheat seed production. *Agron. J.*51:515-518.

Idowu-agida, O. O., Ogunniyan, D. J. and Ajayi, E. O. 2012. Flowering and fruiting behaviour of long cayenne pepper (*Capsicum frutescens* L.). *International Journal of Plant Breeding and genetics* 6: 228 – 237

Luitel, B. P., Yoon, C. S., Kang, W. H. 2013. Correlation and path coefficient analysis for fruit yield and quality characters in segregating population of mini-paprika (*Capsicum annuum* L.). *Journal of Agricultural, Life and Environmental Sciences 25: 1-7.*

Maga, T. J., Uguru, M. I. and Ogbonna, P. E. 2012. Variability and correlation studies in agro-morphological traits of aboriginal Nsukka yellow pepper (*Capsicum annuum* L.) genotypes. *Nigerian journal of Horticultural Science 17: 88 – 102.*

Mohammadi, S. A. and Prasanna, B.M. 2003. Analysis of genetic diversity in crop plants - salient statistical tools and considerations. *Crop Sci., 43: 1235- 1248.*

Moreira, S. O., Gonçalves, L. S. A., Rodrigues, R. R., Sudré, C. P., Júnior, A. T. A., Medeiros, A. M. 2013. Correlações e análise de trilha sob multicolinearidade em linhas recombinadas de pimenta (*Capsicum annuum* L.). *Revista Brasileira de Ciências Agrárias 8: 15-20.*

Oduwaye, A. O. 2013. Field performance and genetic diversity in germplasm of amaranth (Amaranthus spp.) using morphological and PCR-Based molecular markers. *Unpublished Ph.D. Thesis.* Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta. Pp 154.

Ogunbodede, B. A. and Ogunremi, E. A. 1986. Estimation of sesame (*Sesamum indicum L.)* yield from yield parameters. Nigerian Journal of Agronomy 1(1): 9 – 13.

Rohini, N. and Lakshmanan, V. 2015. Correlation and path coefficient analysis in chilli for yield and yield attributing traits. *Journal of Applied and Natural Sciences 4: 25-32.*

Smith, S. E., AlDoss, A. and Warburton, M .1991. Morphological and agronomic variation in North African and Arabian Alfalfas. *Crop Sci., 31: 1159-1163.*

Usman, M. G., Rafii, M. Y., Ismail, M. R., Malek, M.A. and Abdul-Latif, M. 2014. Heritability and genetic advance among Chili pepper genotypes for heat tolerance and morphophysiological characteristics. *The Scientific World Journal Volume 2014*, *1 – 14.*

CORRELATION AND PATH ANALYSIS OF QUANTITATIVE TRAITS IN WEST AFRICAN OKRA [*Abelmoschus caillei* (A. Chev.) Stevels] GENOTYPES

Jonah, P. M.[1], Fakuta, N. M.[2], Vange, T.[3] and Iyongo, T.T.[3]

[1]Department of Crop Science, Adamawa State University, PMB 25, Mubi, Nigeria.
[2]Rubber Research Institute of Nigeria, Gum Arabic Sub-station, Gashua, Yobe State
[3]Department of Plant Breeding and Seed Science,
University of Agriculture, PMB 2373, Makurdi, Benue State, Nigeria.
Correspondence Email: peterjonah2005@yahoo.com

Abstract: The study was carried at the Teaching and Research Farm, Adamawa State University, Mubi in 2012 and 2013 under rain fed conditions. The investigation was conducted to find out the correlation among ten traits and their direct and indirect effects on pod yield in West African okra (*Abelmoschus caillei* Stevels) using 36 genotypes. The experiment was laid out in a randomized complete block design with each plot replicated three times. In each replication, each genotype was planted in a five-row plot of 3 m long with each row spaced at 0.60 m apart and intra-row spaced at 0.40 m apart. The genotypic and phenotypic correlation values for most pairs of traits were similar in terms of coefficient and direction. Pod length, branches/plant and pods/plant showed significant positive genotypic and phenotypic correlations with pod yield, while the flowering traits and plant height recorded significantly negative correlation with pod yield at genotypic and phenotypic levels. The path coefficient revealed that pods/plant had high positive and significant correlation as well as positive direct effect on pod yield. Apart from the direct influence of pods/plant on pod yield, plant height, pod length and branches/plant recorded positive indirect effect on pod yield. Furthermore, pod length and branches/plant also had significant positive correlation with pod yield as well as a positive direct effect across years of evaluations. In this study both genotypic and phenotypic correlations along with path coefficient analysis have identified branches/plant, pod length and pods/plant to be reliable traits to select for the development of high yielding okra genotypes.

Keywords: Correlation, Path Analysis, Direct Effect, *Abelmoschus caillei* and Pod Yield

INTRODUCTION

Okra, also known as "lady's finger', is one of the most important nutritious vegetables extensively grown throughout the tropical and warm temperate regions. The two most important okra cultivated are *Abelmoschus esculentus* (Common okra) which is a native of Asia and *Abelmoschus caillei* (West African okra) which have 130 and 194 chromosomes respectively [1]. The two species of okra belong to the family *Malvaceae* and order *Malvales* [2]. Pod yield of okra is a polygenic trait, therefore direct selection for yield alone is usually not very effective. Selection based on its contributing traits could be more efficient and reliable [3]. Optimizing pod yield is one of the most important goals for most okra growers and okra breeding programmes. For improving this crop through conventional breeding and selection, adequate knowledge of association that exists between yield and yield related traits is essential for the identification of selection procedure.

Correlation study including path coefficient analysis is a powerful tool for finding out reliable association among traits for aiding selection and development of superior genotypes of a crop [4, 5]. This study was therefore designed to identify agronomic traits that influence the pod yield of West African okra (*Abelmoschus caillei*) using phenotypic and genotypic correlations and to separate correlation coefficient into components of direct and indirect effects.

MATERIALS AND METHODS

Experimental materials comprised of 36 entries of West African okra (8 parents and 28 F_1 hybrids) using Griffing's half diallel mating design [6]. Seven accessions namely Acc 1(NG/AA/SEP/038), Acc 2 (NG/SA/DEC/07/0528), Acc 3 (NG/TO/JUN/09/007), Acc 4 (NG/SA/DEC/07/498), Acc 5 (NG/SA/DEC/07/0475), Acc 6 (NG/SA/JAN/109), Acc 8 (NG/SA/DEC/07/0445) were obtained from National Centre for Genetic Resource and Biotechnology, Ibadan while Acc 7

(Syria/Mubi cultivar) was collected in Mubi. The accessions were used as parents for diallel mating design to generate 28 F_1 hybrids through hand pollination. The 36 entries were evaluated in a randomized complete block design with three replications at the Teaching and Research Farm, Adamawa State University, Mubi during 2012 and 2013 cropping seasons. The experimental field was ploughed and harrowed in order to bury plant residues and to break soil clods before ridging. In each replication, each entry was planted in a five-row plot of 3 m long, with rows spaced at 0.60 m apart and intra-row spaced at 0.40 m apart. Three seeds of each entry were planted per hill and later thinned to one plant per stand at two weeks after sowing to give a total plant population of 41,667 plants/ha. To control flea beetles and other insects associated with okra, Cymbush (Cypermetrin): 10% EC was sprayed at a rate of 50 mL/10 liters of water at vegetative growth stage of plants and repeated at flowering stage as recommended by Katung [7]. Weed control was carried out manually with hoe at 3 and 6 weeks after sowing.

Data were collected on days to first and 50 % flowering, plant height, pod length, pod width, number of leaves/plant, number of branches/plant, number of pods/plant, 100 seed weight and pod yield. In each plot, five plants were randomly selected, tagged and used as representative sample. Data obtained were analyzed using SAS statistical package [8]. Genotypic and phenotypic correlation coefficients were calculated as described by Miller *et al*. [9] and the path analysis to estimate the direct and indirect contributions of the traits to pod yield was conducted using matrix procedure as described by Singh and Chaudhary [10].

RESULTS AND DISCUSSION

Table 1: Mean squares for quantitative traits in West African okra genotypes evaluated across two years in Mubi

SV	Df	DF	D50F	PLHT	PL	PW	NOL	NOB	NOP	SW100	PY
Year (Y)	1	1129.80**	380.67**	50553.04**	235.77**	26.92**	27135.38**	123.00**	3978.38**	1.69	340.13**
Rep(Year)	4	6.35	13.61**	540.76**	0.46	0.08*	188.48**	1.63*	196.44**	0.30	12.16**
Genotype (G)	35	194.17**	394.11**	2544.72**	2.92**	0.07**	104.54**	2.22**	79.09**	0.67	7.62**
Y x G	35	116.28**	234.81**	1750.24**	1.03**	0.05**	104.87**	1.42**	59.83**	0.58	6.66**
Error	140	8.03	5.78	107.09	0.41	0.03	37.09	0.49	8.78	0.50	1.24

*and **: Significant at $p \leq 0.05$ and $p \leq 0.01$ respectively, DF = Days to first flowering, D50F = Days to 50% flowering, PLTH= Plant height, PL = Pod length, PW = Pod width, NOL = Number of leaves/plant, NOB = Number of branches/plant, NOP = Number of pods/plant, SW100 = Hundred seed weight and PY= Pod yield.

Table 2: Genotypic (r_g) and Phenotypic (r_p) correlation for Mubi across years (2012 - 2013 combined)

Traits		DF	D50F	PLTH	PL	PW	NOL	NOB	NOP	SW100	PY
DF	r_g		0.97**	0.71**	-0.55**	0.34	0.61**	-0.58**	-0.72**	0.32	-0.76**
	r_p		0.95**	0.67**	-0.52**	0.25	0.45*	-0.52**	-0.68**	0.17	-0.69**
D50F	r_g			0.75**	-0.58	0.33	0.39*	-0.65**	-0.75**	0.38*	-0.78**
	r_p			0.73**	-0.54**	0.27	0.31	-0.57**	-0.70**	0.18	-0.72**
PLTH	r_g				-0.15	0.42*	0.04	-0.69**	-0.61**	0.29	-0.57**
	r_p				-0.13	0.35*	0.04	-0.60**	-0.57**	0.17	-0.51**
PL	r_g					-0.36*	-0.41*	0.29	0.62**	-0.39*	0.63**
	r_p					-0.20	-0.29	0.28	0.55**	-0.19	0.53**
PW	r_g						0.04	-0.50*	-0.48*	0.63**	-0.28
	r_p						0.07	-0.31	-0.36*	0.19	-0.002
NOL	r_g							0.24	-0.12	0.40*	-0.26
	r_p							0.21	-0.05	0.10	-0.13
NOB	r_g								0.71**	-0.50*	0.63**
	r_p								0.59**	-0.24	0.51**
NOP	r_g									-0.11	0.77**
	r_p									-0.11	0.75**
SW100	r_g										-0.21
	r_p										-0.11

DF= Days to first flowering, D50F= Days to 50% flowering, PLTH= Plant height, PL= Pod length, PW= Pod width, NOL= Number of leaves/plant, NOB= Number of branches/plant, NOP= Number of pods/plant, SW100= Hundred seed weight, PY= Pod yield, *, **: Significant at $p \leq 5\%$ and $p \leq 1\%$ respectively.

Table 3: Path analysis for combined years showing direct (diagonal in bold) and indirect contributions of different traits on fresh okra pod yield

Traits	DF	D50F	PLTH	PL	PW	NOL	NOB	NOP	SW100	Genotypic Correlation
DF	**0.07**	0.33	-0.32	-0.27	0.26	-0.16	-0.29	-0.33	-0.04	-0.76
D50F	0.06	**0.34**	-0.34	-0.28	0.26	-0.10	-0.32	-0.35	-0.05	-0.78
PLTH	0.05	0.25	**-0.45**	-0.07	0.32	-0.01	-0.34	-0.28	-0.04	-0.57
PL	-0.04	-0.20	0.07	**0.49**	-0.28	0.11	0.14	0.29	0.05	0.63
PW	-0.02	-0.11	-0.19	-0.18	**0.78**	-0.01	-0.25	-0.22	-0.08	-0.28
NOL	0.04	0.13	-0.02	-0.20	0.03	**-0.26**	0.12	-0.06	-0.05	-0.26
NOB	-0.04	-0.22	0.31	0.14	-0.39	-0.06	**0.50**	0.33	0.06	0.63
NOP	-0.05	-0.25	0.27	0.30	-0.37	0.03	0.35	**0.46**	0.01	0.77
SW100	0.02	0.13	-0.13	-0.19	0.49	-0.10	-0.25	-0.05	**-0.13**	-0.21

DF= Days to first flowering, D50F= Days to 50% flowering, PLTH= Plant height, PL= Pod length, PW= Pod width, NOL= Number of leaves/plant, NOB= Number of branches/plant, NOP = Number of pods/plant, SW100= Hundred seed weight, PY= Pod yield.

In the two years, the mean squares of the ten characters in the 36 genotypes evaluated are presented in Table 1. The characters showed significant differences ($p \leq 0.01$) in the entries, indicating that an appreciable amount of genetic variability exists among entries as earlier reported by Jonah *et al.* [11].

The correlation coefficients among ten okra traits across the two years of evaluation presented in Table 2 showed days to first flowering had significant positive genotypic and phenotypic correlation with days to 50% flowering, plant height and number of leaves/plant at $p \leq 0.01$. In contrast, days to first flowering recorded significant negative phenotypic and genotypic correlation with pod length, branches/plant, pods/plants and pod yield at both genotypic and phenotypic levels at $p \leq 0.01$. Days to 50% flowering had significant positive genotypic and phenotypic correlation with plant height, but a negative estimate with pod length, branches/plant, pods/plant and pod yield. Also, days to 50% flowering showed positive genotypic associations with number of leaves/plant and hundred seed weight at $p \leq 0.05$. Plant height recorded high significant negative genotypic and

phenotypic correlation with branches/plant, pods/plant, pod yield and significant positive correlations with pod width at $p \leq 0.01$. At phenotypic and genotypic levels, pod length had high significant positive correlation with pods/plant and pod yield, while the genotypic association with pod width, number of leaves and hundred seed weight were significantly negative. Pod width recorded a highly significant positive genotypic correlation with hundred seed weight and a significant negative genotypic association with branches/plant and pods/plants. The genotypic and phenotypic correlation between pod width and pods/plant was significantly negative. Number of leaves/plant had significant positive genotypic correlation with hundred seed weight. Significant phenotypic and genotypic positive correlation was recorded between branches/plant with pods/plant and pod yield at genotypic and phenotypic levels, and a negative genotypic significant association with hundred seed weight. Pods/plant recorded significant positive correlations with pod yield at both genotypic and phenotypic levels.

Generally, the genotypic correlation coefficients were higher in magnitude than their corresponding phenotypic correlation coefficients for all the pair of traits studied. This could be attributed to environmental influence inherent in the phenotypic correlation as earlier reported by Nwangburuka *et al.* [12] and Das *et al.* [13]. The highly significant negative genotypic and phenotypic correlations exhibited by days to 50% flowering with pod length, branches/plant, pods/plant and pod yield implies that any increase in days to 50% flowering would lead to decrease in pod length, branches/plant and number of pods/plant, thereby leading to low pod yield in okra as previously reported [14, 15, 16, 17 and 18]. The highly significant negative correlation between days to 50% flowering and pod yield implies that increase in pod yield could cause decrease in days to 50% flowering, which is desirable by the breeder. The highly significant positive genotypic and phenotypic correlations between pod yield with pod length, branches/plant and number of pods/plant indicated that increase in one of the traits will lead to high yield of okra, which is in agreement of the reports of Rashwan, Simon *et al.*, Nirosha *et al.*, Kumar and Medagam [15, 19, 20 and 21]. On the other hand, the significant negative genotypic correlations observed between pod length with pod width, leaves/plant and hundred seed weight, suggests that increase in pod length will lead to decrease in pod width, leaves/plant and hundred seed weight. Pod length and width are important yield characters, such that simultaneous improvement in the two characters will require painstaking breeding effort to strike a balance between pod length and width.

The path coefficient analysis for ten quantitative traits studied is presented in Table 3. The path analysis revealed high direct effects of number of pods/plant, pod length and number of branches/plant on yield. The emphasis on these characters lies with their relatively high direct effects and same direction for both direct effect and correlation. This is in contrast with high direct effect of 0.78 but low and negative correlation at -0.28 for pod width. The direct effect of branches/plant at 0.50 was aided by positive indirect effects of plant height (0.31) and pods/plant (0.33). Direct effect of pod length (0.49) was aided mainly by pods/plant 0.29. For direct effect of pods/plant at 0.46, it was supported by plant height (0.27), pod length (0.30) and branches/plant (0.35) indirect effects. This study corroborates with the findings of Aminu et al. [18]. In this regard, characters with positive correlation with yield and corresponding positive direct effects have great potential for indirect selection for yield. Such indirect selection will be boosted by the 'aiding' factors. Notwithstanding, the positive role of plant height may be applied with caution because of its negative direct effect (-0.45) and also negative correlation (-0.57) with yield.

The negative direct effect of plant height (-0.45) and corresponding negative correlation with yield (-0.57) perhaps explains why many recommended high yielding okra varieties have low height [22]. The indirect effect of pods/plant at -0.28 was noted. The dual role of pods/plant to aid both positive and negative direct effects on yield suggests that

breeders can apply pods/plant for the desired direction [23].

Days to 50% flowering, though having direct effect of 0.34 recorded negative correlation at -0.78 with yield, being aided by negative indirect effects of plant height, pod length, branches/plant and pods/plant at -0.34, -0.28, -0.32 and -0.35 respectively. Shorter flowering period is desirable in order to allow several plantings in a year. Similarly, days to first flowering recorded low direct effect at 0.07 but high correlation at -0.76. Negative correlation between flowering characters and yield is desirable, but the change of direction between the direct effects and correlation may be a set-back. This may be resolved by boosting the germplasm to possibly get the same and desired direction between direct effects and correlation.

CONCLUSION

In conclusion, significant and positive correlations were observed between growth characters, pod yield and yield related characters of West African okra. When the correlation coefficients were partitioned into direct and indirect effects, branches/plant had the highest contribution to fresh pod yield, followed by pod length and pods/plant. Selection for any of these traits could therefore serve as basis for yield improvement in West African okra.

REFERENCES

[1] Adeniji, O. T., Kehinde, O. B. (2003) Genetic variability of seed yield and components of West African Okra *(Abelmoschus caillei* (A. Chev.) Stevels]. *ASSET series* A, 3 (4):81 – 90.

[2] Shippers, R. R. (2000) African indigenous vegetables. In: An overview of the cultivated species. Natural Resources Institute/ACP-EU Technical Centre for Agricultural and Rural Cooperation, Chatham, UK; Pp 103 – 118.

[3] Kumar, N., Joshi, V. N., Dagla, M. C. (2013) Multivariate analysis for yield and its component traits in maize (*Zea mays* L.) under high and low N levels. *The Bioscan*, 8(3): 959 – 964.

[4] Ado, S. G., Tanimu, B., Echekwu, C. A., Alabi, S. O. (1988) Correlation and path analysis between yield and other characters in sunflower. *Nigerian Journal of Agronomy*, 3:1 – 4.

[5] Dwivedi, M., Sharma, D. P. (2017) Correlation and path coefficient analysis studies in okra (*Abelmoschus esculentus* (L.) Moench) under Japalpur condition. *International Journal of Agriculture Sciences*, 9(34): 4504 – 4509.

[6] Griffing, J. B. (1956) Concept of general and specific combining ability in relation to diallel crossing system. *Australian Journal of biological sciences*. 9:463- 493.

[7] Katung, M. D. (2007) Productivity of okra varieties as influenced by seasonal changes in Northern Nigeria, *Not. Bot. Hort. Agrobot*. Cluj., 35(1):65-71.

[8] Statistical Analysis System (SAS) (1999) *Statistical methods*. SAS Institute, Inc. Cary North Carolina.

[9] Miller, P. A., Williams, J. C., Robinson, H. P., Comstock, R. E. (1958) Estimates of Phenotypic variance and covariance in upland cotton, *Agron J.*, 50: 124 – 131.

[10] Singh, R. K., Chaudhary, B. D. (1985) *Biometrical methods in quantitative genetic analysis*. Kalyani Publishers. New Delhi, India, pp. 46-67.

[11] Jonah, P. M., Bello, L. L., Kalu, B. A., Omoigui, L. O. (2015) Agronomic diversity among accessions of West African okra (*Abelmoschus caillei* [A. Chev. Stevels]). *Adamawa State University Journal of Agricultural Sciences*, 3 (1):124 – 134.

[12] Nwangburuka, C. C., Denton, O. A., Kehinde, O. B., Ojo, D. K., Popoola, A. R. (2012) Genetic variability and heritability in cultivated okra (*Abelmoschus esculentus* (L.) Moench). *Spanish Journal of Agricultural Research,* 10(1): 123 – 129.

[13] Das, S., Arup, C., Sankhendu, B. C., Subrata, D., Pranab, H. (2012) Genetic parameters and path analysis of yield and its components in okra at different sowing dates in the gangetic plains of eastern India. *African Journal of Biotechnology*, 11(95):16132 – 16141.

[14] Mehta, D. R., Dhaduk, L. K., Patel, K. D. (2006) Genetic variability, correlation and path analysis studies in okra [*Abelmoschus*

esculentus (L.) Moench]. *Agricultural Science Digest,* 26(1): 15 – 18.

[15] Rashwan, A. M. A. (2011) Study of genotype and phenotypic correlation for some agro -economic traits in okra [*Abelmoschus esculentus* (L.) Moench]. *Asian Journal of Crop Science,* 3(2): 85 – 91.

[16] Medagam, T. R., Kadiyala, H. B., Mutyala, G., Reddy, K. C., Begum, H. Reddy, R. S. K., Jampala, D. B. (2013) Correlation and path coefficient analysis of quantitative characters in okra [*Abelmoschus esculentus* (L.) Moench]. *Songklanakarin Journal of Science Technology,* 35(3): 243 – 250.

[17] Simon, S. Y., Gashua, I. B., Musa, I. (2013) Genetic Variability and trait correlation studies in okra [*Abelmoschus esculentus* (L.) Moench]. *Agriculture and Biology Journal of North America*, 4(5):532 – 538.

[18] Aminu, D., Bello, O. B., Gambo, B. A., Azeez, A. H., Agbolade, O. J., Iliya, A, Abdulhamid, U. A. (2016) Varietal performance and correlation of okra pod yield and yield components. *Agriculture and environment*; 8: 112 – 125.

[19] Simon, S. Y., Musa, I., Nangere, M. G. (2013) Correlation and path coefficient analyses of seed yield and yield components in okra [*Abelmoschus esculentus* (L.) Moench], *International Journal of Advanced Research*, 1(3): 45 – 51.

[20] Nirosha, K., Irene, P. V., Sathiyamurthy, V. A. (2014) Correlation and path analysis studies in okra [*Abelmoschus esculentus* (L.) Moench]. *Agricultural Science Digest*, 34(4): 313 – 315.

[21] Kumar, S., Medagam, T. R. (2016) Correlation and path coefficient analysis for yield and its components in okra [*Abelmoschus esculentus* (L.) Moench]. *Agriculture and Nature Resources*, 2(6): 01 – 12.

[22] Mihretu, Y., Weyessa, G., Adugna, D. (2014) Variability and association of quantitative character among okra [*Abelmoschus esculentus* (L.) Moench] collection in south western Ethiopia. *Journal of Biological Sciences*, 14(5):336-342.

[23] Abd-allah, S. (2015) Path coefficient analysis for some character on fruit and seed yield of okra. *Journal of Horticulture*, 2(2):140-144.

CHARACTERIZATION OF GROUNDNUT SEEDLINGS USING VIGOUR AND PERFORMANCE INDICES

Olalekan J. Olasan[1], Celestine U. Aguoru[1], Lucky O. Omoigui[2] and Nater Iyorkaa[2]
[1]Department of Biological Sciences, Plant Science Unit,
University of Agriculture, Makurdi, Nigeria.
[2]Department of Plant Breeding and Seed Science, University of Agriculture, Makurdi, Nigeria.
Corresponding Author E-mail: celeaguoru@yahoo.com

Abstract: The present study was designed to measure genetic variability among 100 groundnut cultivars using nineteen seedlings vigour and growth performance indices under screen house conditions. Data collected were subjected to principal component, regression analysis, correlation analysis and trend analysis of the SPSS (20.0 version) and Minitab (16.0 version) softwares. A total of 19 principal components were extracted out of which four explained a cumulative variance of 72.5% in the seedlings. Principal component 1 to 4 contributed 48.4%, 9.7%, 8.2% and 6.2% (respectively) to the total variation. Extraction cummunalities from PCA identified 13 traits that made significant contributions to the observed variation. The top five traits on the list were: Vigour index I, Seedling length, Vigour Index II, Percentage moisture content and Root length. Traits associated with seedling vigour and performances were highly correlated. Predictive modeling of vigour estimation is fit as it gave significant and large F value (139.58, $p<0.05$). The regression value was very high (0.924) with coefficient of determination (R^2) of 85.5 %. This study has shown that prediction of seedling vigour can be made with high level of accuracy in groundnut using mathematical model. Groundnut cultivars which gave vigorous seedlings have been recommended in this study. They are: ICGV-IS-13914, ICGV-IS-13967, Dh-86, ICGV-IS-13938, ICGV-IS-13881, ICGV-IS-13955, ICGV-IS 13952, ICGV-IS-141071, ICGX-13011, ICGV-IS-13856, ICGV-IS-14867, ICGV-02189, ICGV-IS-13940 and ICGV-IS-13853. This approach will enhance the global groundnut productivity and improvement programme.

Key words: Groundnut, Performance, Physiology, Seedling, Variability, Vigour

INTRODUCTION

Seedling characterization is a conventional approach of studying the genetic diversity of crops (Aguoru *et al*., 2015a). It is also an assessment of the quality of stored germplasm (Mathews *et al*., 2012; Bishi *et al*., 2013; OSU, 2017). Physiological characters play major roles in the elucidation of diversity in groundnut (Abdul-Rahman, 2014). *Arachis hypogaea* L. is a valuable seed crop for fighting malnutrition and poverty in Africa (ICRISAT, 2015; FAO, 2017). Breeders often maintain groundnut seeds in the seed bank as a germplasm that constitutes an assemblage of varying genetic resources. This helps in the conservation, and maintenance of genetic resources (Janila *et al*., 2013; Jambunathan, 2015; Aguoru *et al*., 2015c). The assembled seeds belonging to different cultivars or species are expected to express different phenotypic behavior in the field (Filho, 2015). Thus, seedling characterization may be used as a test of seed viability (germination potential) and a measure of genetic competence or variability among the entities (Mathews *et al*., 2012; OSU, 2017). A basic test of seedling performance is vigour evaluation (OSU, 2017). Vigorous seedlings have the intrinsic competence for excellent performances under favourable or unfavourable conditions. These are seedlings that are likely to flourish in growth and yield, in the field (OSU, 2017).

Since laboratory seedling performances usually determine field performances in most crops, groundnut germplasm needs to be evaluated to determine its overall health (Mathews *et al*., 2012). It will also help to select quality seeds for the breeders/growers before field trials, especially when commercial agriculture is involved. Seedling evaluation thus prevents avoidable wastage of resources, labour and time in the field and guarantees quality output (Filho, 2015). It also checks the functionality of seed storage conditions (Aguoru *et al*., 2015b). When seedlings of different cultivars are subjected to the same treatments or given the same conditions, they are expected to have the same performance. Any variation in the performances and vigour should be attributed to genetic responses of the

cultivars (Aguoru *et al*., 2015a). The present study was designed to characterize 100 groundnut cultivars using seedling vigour and performance indices under a controlled environment.

MATERIALS AND METHODS

100 groundnut cultivars (Table 1) were randomly selected from 118 germplasm. Ten seeds of each cultivar were planted in plastic containers of equal diameter and depth (11cm in diameter, 10cm in depth). Each experimental unit received equal treatment of water, soil type and quantity, orientation to sun light and equal agronomic care. Evaluation was done in a screen house (Plate 1). 19 traits (Table 2) were evaluated from the first to fifteenth day of planting. Seedlings were scored for performances on a scale of 0-5 (0= Very poor, 1= Poor, 2= below average, 3=Average, 4=Good, 5=Excellent). Abortiveness and weediness were also assessed qualitatively on ordinal scale. Length, height, plant spread and root collar diameter were measured with metre rules. Weight was determined using a digital weighing balance. Percentage germination was calculated as the number of emerging seedlings out of the total seeds planted expressed in percentage. Seedling length was calculated as the sum of root length and plant height. Percentage moisture was calculated as the difference between seedling wet and dry weight divided by the wet weight and expressed in percentage. Vigour Index I was calculated as percentage germination multiplied by seedling length. A cut off point of 2000 was adopted. Vigour Index II was calculated as percentage germination multiplied by seedling dry weight. Plant slenderness was calculated as seedling height divided by the root collar diameter. Data was subjected to principal component analysis, regression analysis, correlation analysis and trend analysis using the SPSS (20.0 version) and Minitab (16.0 version) softwares.

RESULTS AND DISCUSSION

A total of 19 components were extracted out of which four explained a cumulative seedling variance of 72.5% (Table 3). Component 1 to 4 contributed 48.4%, 9.7%, 8.2% and 6.2% (respectively) to the total variation. The observed cumulative variability was quite high among the seedlings, which suggests differential morphological and physiological expression of the genetic make-up of the cultivars (Aguoru *et al*., 2015a). Extraction cummunalities (Table 4) showed the traits in the order of genetic contribution to the observed variation. 13 out of the 19 evaluated characters made significant contributions. The top five traits on the list were Vigour index I, Seedling length, Vigour Index II, Percentage moisture content and Root length.

The present study confirms previous reports on the need to test germplasm for vigour as a measure of genetic variability (OSU, 2017) and quality control of stored seeds (Mathews *et al*., 2012; Filho, 2015). The huge variation in the seedling vigour is attributed to the intrinsic competences among the cultivars since equal treatments were received by the experimental units (Aguoru *et al*., 2015a). Correlation matrix of seedling traits is shown in Table 5. High positive correlations existed between Percentage germination and Vigour index I (0.870), Percentage germination and Vigour index II (0.782), Seedling length and Seedling wet biomass (0.857), Seedling length and Vigour index 1 (0.894), seedling wet biomass and Vigour index 1 (0.852). Vigour index I was moderately correlated with Plant spread (0.543), Plant slenderness (0.560) and Vigour index II (0.605). The correlation coefficient distance (Figure 1) thus confirms the wide distance of traits such as Root collar diameter and Leaflet length to width ratio (LWR) from other traits. It also confirms the close relationships of traits such as germination, seedling length, vigour index I, wet biomass and performances. These closely related traits may likely be useful as morphological or physiological markers to monitor inheritances (Holbrook and Stalker, 2003; Bayat *et al*., 2017; Olasan *et al*., 2018). Prediction of vigour in groundnut seedlings was siginifcant ($p<0.05$) using Seedling length, Plant spread and L/W ratio (Table 6). The multiple regression equation connecting seedling vigour and other determinants is given as: V= 117.744 + 105.2(SL) + 48.9 (PSP) – 641.2 (LWR) + 0.4 (MC). (V= vigour index, SL= seedling length, PSP= plant spread, LWR= leaflet length to width ratio, MC= percentage moisture content). The model is considered fit as it gave a large significant F value (139.58, $p<0.05$) and significant result. The coefficient (R) value being quite high (0.924). The coefficient of determination of 85.5% has further confirmed the power of the analysed vigour model.

In performance rating, seedlings with best performances scoring the maximum 5 points were genotypes: G49, G42, G3, G30, G46, G97, G102, G47,

G75, G13, G94, G45, G99, G71, G116, G117, G118, G74, G64, G101, G103, G96, G115, G98, G111, G91, G104 and G80 (Figure 2). These are seedling accessions that are more likely to thrive well in the field with high germination rate.

Representative cultivars as given in Table 1 include: ICGX-13011, ICG-4750, ICGV-IS-13854, ICGV-IS-13953, ICGX-11010, SAMNUT-25, ICGV-IS-09996, ICGS-11060, ICGV-IS-141063, ICGV-IS-13877, G-2-52, ICGS-44, ICGV-IS-09828 and ICGV-IS-141178. It is not advisable to select poorly performing seeds for large scale field projects; though performances may be improved using agricultural practices if genetic factors are ruled out (Abdul-Rahman, 2014). Challenges of genetic competence may be overcome through genetic hybridization with those known to have excellent performances through crop improvement (Janila *et al*., 2013; Jake *et al*., 2015).

Vigour index I ranged from 198 to 2460 among the groundnut seedlings of different genotypes, with a mean value and standard deviation of 1351 and 594 respectively. Seedlings showed wide variability in their vigour (Figure 3). Using vigour cut off point of 2000, the genotypes with high vigour values (v) in descending order are: G23 (2460), G79 (2350), G93 (2340), G26 (2330), G15 (2230), G32 (2180), G29 (2170), G62 (2160), G49 (2140), G4 (2140), G61 (2120), G54 (2110), G27 (2050) and G2 (2020). They represent the following cultivars: ICGV-IS-13914, ICGV-IS-13967, Dh-86, ICGV-IS-13938, ICGV-IS-13881, ICGV-IS-13955, ICGV-IS-13952, ICGV-IS-141071, ICGX-13011, ICGV-IS-13856, ICGV-IS-14867; ICGV-02189, ICGV-IS-13940 and ICGV-IS-13853.

Table 1: Groundnut cultivars and codes

Genotype	Code	Genotype	Code	Genotype	Code	Genotype	Code
ICGV-IS 13846	G116	ICGV-IS 13887	G41	ICGV-IS 141151	G59	ICGV-IS 13046	G40
G-2-52	G94	ICGS 44	G45	ICGV-02189	G54	ICGV-IS 13911	G22
ICGV 94379	G81	ICGV 86024	G80	ICGV-IS-13967	G79	ICGV-IS 13897	G20
ICGV-IS 13011	G101	ICGV-IS 13863	G100	ICGV 02022	G58	ICGV-IS 13862	G8
ICGV-IS 09932	G111	JL-24	G91	ICGX 11010	G46	ICGV-IS 13839	G39
ICGX-IS11003-F2-B1-B1	G104	ICGV-IS 09926	G110	ICGV-IS 141193	G69	ICGV-IS 13867	G10
ICGV IS 141198	G76	ICGV-IS 09992	G112	ICGV-IS-14898	G72	ICGV-IS 13940	G27
ICGV-IS 07803	G103	SAMNUT 24	G87	ICGV-IS 141176	G67	ICGV-IS 13907	G21
ICGV-IS 13854	G3	ICGV 91317	G83	ICGV-IS 141144	G73	ICGV-IS 13955	G32
SAMNUT 23	G96	TG-39	G92	ICGV-IS 141063	G75	ICGV-IS 13914	G23
ICGV-IS 13811	G114	ICGV-IS 13828	G115	ICGV 99241	G51	ICGV-IS 13926	G24
ICGV-IS 13851	G118	ICGV-SM 01721	G90	ICGV-IS 13861	G7	ICGV-IS 13874	G11
ICGV-IS 13810	G113	ICGV-IS-89767	G84	MOSSTIGA	G77	ICGV-IS 13857	G5
SAMNUT 25	G97	ICGV-IS 13858	G6	ICGV-IS 141214	G64	ICGV-IS 13891	G16
ICG 2106	G44	ICGV-IS 07965	G106	ICGV-IS 141091	G63	ICG 4750	G42
ICGV-IS 141156	G60	J-11	G78	ICGV-IS 141071	G62	ICGV-IS 13853	G2
ICGV 97182	G56	ICGV-IS 14906	G74	ICGV 00308	G57	ICGV-IS 13938	G26
ICGX 11057	G48	ICGV-IS 141145	G66	ICGV-IS 141199	G68	ICGV-IS 13865	G9
ICGV-IS 141198	G76	ICGV-IS 13943	G28	ICGV-IS 13927	G25	ICGV-IS 13881	G15
Dh-86	G93	ICG 4729	G43	ICGV-IS 13953	G30	ICGV-IS 09828	G99
HAUSA KANO	G98	ICGV 87378	G53	ICGV-IS 13896	G19	ICGV-IS 13952	G29
ICGV-IS 13850	G117	ICGV-IS 07831	G109	ICGV-IS 13856	G4	ICGV-IS 13878	G14
ICGV-IS 09996	G102	TAG-24	G95	ICGV-IS 13875	G12	ICGV 02271	G50
EX-DAKAR	G107	ICGV-IS 14867	G61	ICGS 11060	G47	ICGV-IS 13893	G18
ICGV-IS 141178	G71	ICGX 13011	G49	ICGV-IS 13877	G13	ICGV-IS 13971	G36

Table 2: Seedling characters

S/N	Seedling traits	Abbreviation
1	Percentage moisture content	%MC
2	Branch (leaf) length	BL
3	Cases of abortiveness	CA
4	Leaflet length	LL
5	Leaflet length to width ratio	L/W ratio
6	Number of branches	NB
7	Overall plant performance	PPF
8	Percentage germination	% GM
9	Plant height	PH
10	Plant slenderness	PSL
11	Plant spread	PSP
12	Root length	RL
13	Root collar diameter	RCD
14	Seedling dry biomass	SDB
15	Seedling length	SL
16	Seedling wet biomass	SWB
17	Vigour Index I	V1 I
18	Vigour Index II	VI II
19	Weediness	WD

Table 3: Total variability explained by the principal components

1st Ten Component	Initial Eigenvalues		
	Total	% of Variance	Cumulative %
1	9.204	48.444	48.444
2	1.843	9.702	58.146
3	1.557	8.196	66.341
4	1.178	6.201	72.543
5	.934	4.914	77.457
6	.814	4.286	81.742
7	.660	3.472	85.215
8	.567	2.982	88.197
9	.524	2.756	90.953
10	.483	2.543	93.496

Table 4: Extraction cummunalities of first ten traits

Seedling Characters	Extraction Cummunalities	Variability Rating
Vigour Index I	.944	1st
Seedling length	.927	2nd
Vigour Index II	.908	3rd
% Moisture content	.901	4th
Root length	.867	5th
Seedling dry weight	.866	6th
Plant height	.843	7th
Seedling wet biomass	.833	8th
Percentage germination	.819	9th
Overall plant performance	.819	10th

Table 5: Correlation matrix of seedling traits

Correlations												
		%GM	L/Wratio	PSP	SL	RCD	SWB	SDB	%MC	VI 1	VI II	PSL
%GM	R	1	-.512	.522	.586	.039	.651*	-.297	.135	.870*	.782*	.518
L/Wratio	R	-.512	1	-.165	-.296	-.123	-.297	.024	.184	-.426	-.471	-.198
PSP	R	.522	-.165	1	.445	.013	.437	-.322	.180	.543	.312	.419
SL	R	.586	-.296	.445	1	.105	.857	-.328	.237	.894	.344	.504
RCD	R	.039	-.123	.013	.105	1	.045	-.086	-.107	.089	-.005	-.574
SWB	R	.651*	-.297	.437	.857*	.045	1	-.328	.239	.852*	.413	.528
SDB	R	-.297	.024	-.322	-.328	-.086	-.328	1	-.250	-.363	.332	-.245
%MC	R	.135	.184	.180	.237	-.107	.239	-.250	1	.230	-.059	.226
VI 1	R	.870*	-.426	.543	.894	.089	.852	-.363	.230	1	.605*	.560
VI II	R	.782*	-.471	.312	.344	-.005	.413	.332	-.059	.605	1	.324
PSL	R	.518	-.198	.419	.504	-.574	.528	-.245	.226	.560	.324	1

Legend: Trait abbreviations given in Table 2

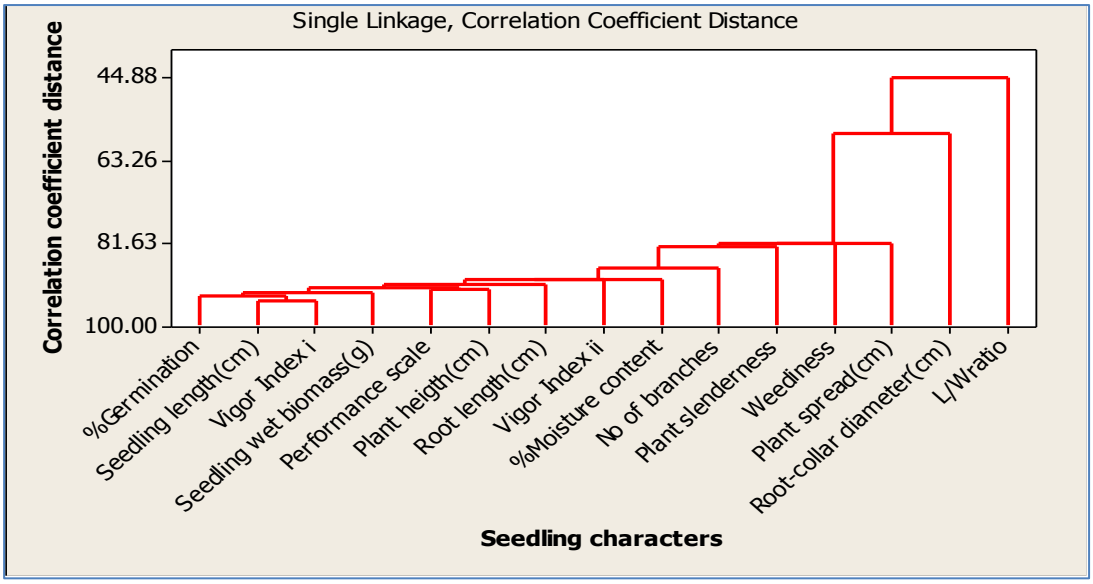


Figure 1: Correlation coefficient distance in seedling traits

Table 6: Model summary for seedling vigour determination

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
(Constant)	117.744	260.560		.452	.652
Seedling length	105.154	6.528	.751	16.108	.000
Plant spread	48.920	12.836	.167	3.811	.000
L/Wratio	-641.187	146.014	-.187	-4.391	.000
% Moisture content	.400	.300	.056	1.336	.185
Dependent Variable: Vigour Index 1		F=139.580, P=0.000;	R=0.924, R^2=0 .855		

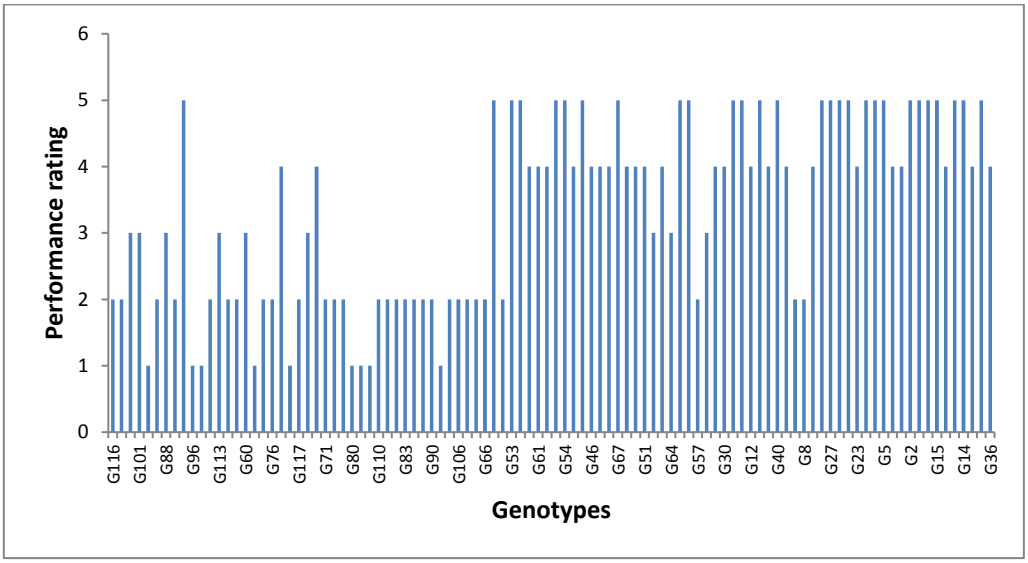


Figure 2: Comparative seedling performance rating in groundnut

Legend: Genotype codes and representative cultivars given in Table 1

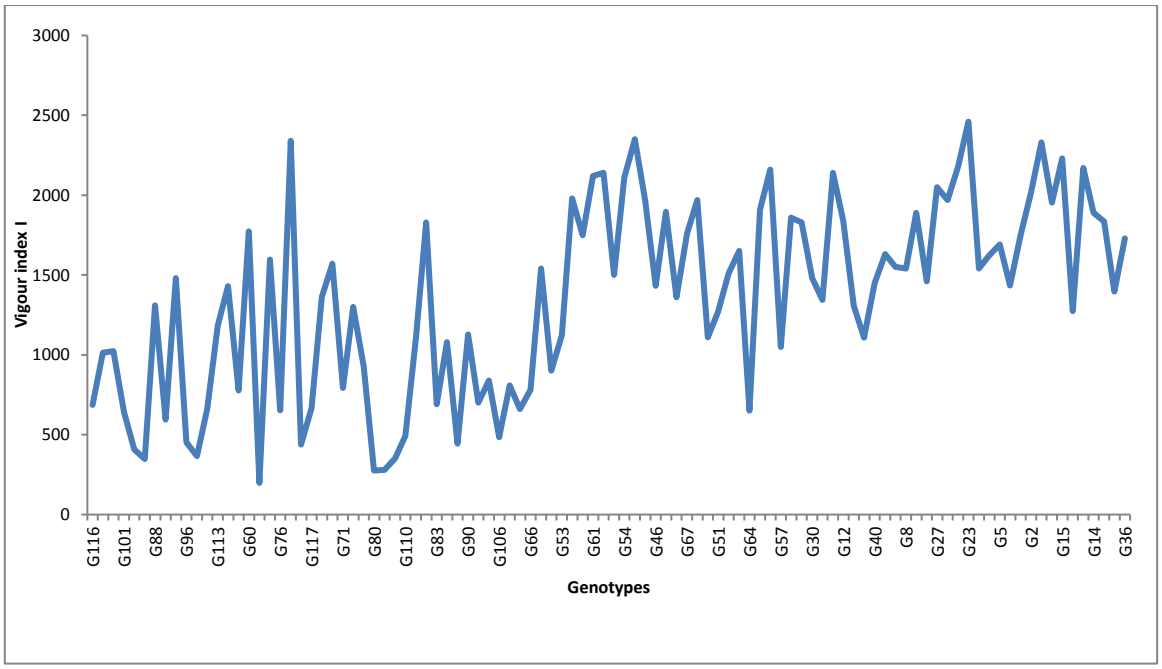


Figure 3: Diversity in seedling vigour indices

Legend: Genotype codes and representative cultivars given in Table 1

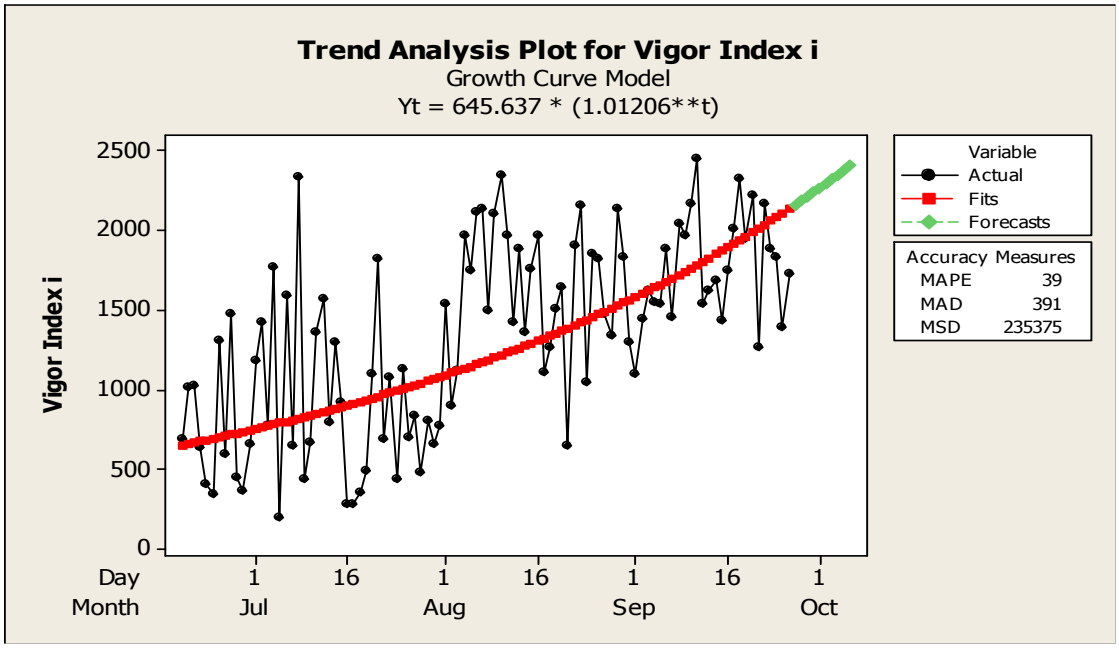


Figure 4: Trend Analysis Plot for Vigour Index I

Legend: MAPE= mean absolute percentage error; MAD= mean absolute deviation and MSD= mean square deviation

The close correlation distance between seedling performance and vigour shows that seeds/seedlings need to be vigorous to display excellent physiological qualities. This supports fundamental knowledge of seed biochemistry and physiology (Mathews *et al*., 2012; Mustapha *et al*., 2015; OSU, 2017). Fungal pathogens and viral diseases are known to reduce seedling vigour and seed germination potential (Ahmed *et al*., 2017). This factor cannot be totally excluded from the present findings cases of abortiveness were recorded among diseased seedlings thus limiting their growth and performances. Though there are fluctuations in the vigour indices across the genotypes (Figure 4), vigour increases exponentially

with time along the growth cycle. This is expected because the parameters which define vigour such as plant spread, number of branches, biomass, moisture content and root collar diameter increased with age of the seedlings.

Plate 1: Screen house pots and seedling evaluation

CONCLUSION

The 100 cultivars of groundnut have a cumulative genetic variability of 72.5% in four components. Vigour indices contributed most to the observed variation with cummunality value of 0.944. Associated seedling traits affecting vigour have been determined. They include seedling length, plant spread, leaflet length to width ratio and moisture content. Prediction of vigour can be made with high level of accuracy in groundnut. High performing and vigorous seedlings coming from different groundnut cultivars have been recommended in this study. They are ICGV-IS-13914, ICGV-IS-13967, Dh-86, ICGV-IS-13938, ICGV-IS-13881, ICGV-IS-13955, ICGV-IS 13952, ICGV-IS-141071, ICGX-13011, ICGV-IS-13856, ICGV-IS-14867, ICGV-02189, ICGV-IS-13940, ICGV-IS-13853, ICG-4750, ICGV-IS-13854, ICGV-IS-13953, ICGX-11010, SAMNUT-25, ICGV-IS-09996, ICGS-11060, ICGV-IS-141063, ICGV-IS-13877, G-2-52, ICGS-44, ICGV-IS-09828 and ICGV-IS-141178. This approach will enhance groundnut productivity and improvement programme.

REFERENCES

Abdul-Rahman, T. (2014). Responses of groundnut (*A.hypogaea* L.) varieties to phosphorus in three Agroecologies in Sierra Leone. *International Journal of Agriculture and Forestry*, 4(2): 106-111.

Aguoru, C.U., Kombur, D.S and Olasan, J.O. (2015b). Comparative Efficacy of Different Species of Pepper (*Capsicum spp*) in the Control of Stored Groundnut (*Arachis hypogea L*) Damage by Pest of Groundnut amongst the TIV Speaking People of the North Central Nigeria. *International Journal of Current Microbiology and Applied Sciences,* 4 (3): 1018-1023.

Aguoru, C.U., Omoigui, L.O and Olasan, O.J. (2015a). Characterization of eggplant seedlings using quantitative trait analysis. *Scholars Journal of Agriculture and Veterinary Sciences*, 2(1B):54-60.

Aguoru, C U., Azua E. T and Olasan J. O. (2015c) Approaches to minimizing and overcoming current biodiversity loss. *British Journal of Environmental Sciences*, 3(3): 12-26.

Bayat, M., Amirnia, R., Rahimi, M. (2017). Phenotypic and genotypic relationships between traits in *Crocus sativus* L. As revealed by path analysis. *Elsevier Journal of Applied Research on Medicinal and Aromatic Plants*, 5: 33-40.

Bishi, S.K., Lokesh, K., Dagla, M.C., Mahatma, M.K., Rathnakumar, A.L., Lalwani, B., and Misra, J.B. (2013). Characterization of Spanish peanut germplasm (*Arachis Hypogea L)* for sugar profiling and oil quality. *Indus products*, 51:46-50

Filho, J.M. (2015). Seed Vigour Testing: An Overview of the past, present and future perspective. *Science and Agriculture*, 72(4).

Food and Agricultural Organization (FAO) (2017). Available at: http://faostat.org/peanut. Retrieved on 12/04/2017, 15:10 GMT.

Holbrook, C.C. and Stalker, H.T. (2003), Peanut breeding and genetic resources. *Plant Breeding Reviews*, 22: 297-356.

ICRISAT (2015). International Crops Research Institute for the Semi-Arid Tropics Annual Report of 2015. Retrieved from: www.icrisat.org. On 15/12/2015, 11:30 GMT.

Jake, C. F., Pawan, K., Liming, Y., Spurthi, N., Nayak, B. T. and Scully, R.D. (2015). Resistance to *Aspergillus flavus* in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives. *The Crop Journal*, 3: 229-337.

Jambunathan, R. V. (2015). IBPGR and groundnut genetic resources. A publication of the International Board of Plant Genetic Resources, Pp 311-315.

Janila, P., Nigam, S. N., Manish, K. P, Pandey, Nagesh, P. and Ranjeev, K. V. (2013). Groundnut improvement: use of genetic and genomic tools. *Frontier in Plant Science*, 4 (23): 1-33.

Mathews, S., Noli, E., Demir, I., Khajeh-Hosseini, M., Wagner, M. H. (2012). Evaluation of Seed Quality from Physiology to International Standardization. *Seed Science Research*, 51: 569-573.

Mustapha, S., Muhammed, U. M., Adeosun, N. O., Mohammed T.J., Mohammed, S. S. and Ibn-Aliyu, A. (2015). Nutritional and functional characterization of

undecoticated groundnut (*A.hypogaea* L.) seeds from Bosso Market, Minna, Nigeria. *American Journal of Food Science and Technology*, 3(5): 126-131.

Olasan, J. O., Aguoru, C. U., Omoigui, L. O. and Ekefan, E. J. (2018). Character Association Studies in Groundnut (*Arachis hypogaea* L.) *American Journal of Plant Sciences*, 9(7):1531-154. https://doi.org/10.4236/ajps.2018.97112

OSU (2017). Importance of Seed Vigour Testing. A publication of yhe Oregon State University Seed Laboratory. Retrieved from: www.seedlab.oregonstate.edu 14:07: 2017, 14:27 GMT.

GENETIC VARIABILITY AND PATH ANALYSIS FOR IMPORTANT TRAITS IN ORANGE FLESHED SWEET POTATO

[1]J.C. Harriman, [2]C.E. Eze, [3]G.C. Onyishi, [1]A.E. Agbo, [4]N. K.Ndulue, [1]C.A. Nwadinobi, [1]C. R. Anunobi.

[1]Department of Agronomy, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[2] International Programme in Climate Change and Agriculture,
Universite des Sciences, des Technique et des Technologie de Bamako, Mali.
[3]Department of Crop Science and Technology, Federal University of Technology, Owerri, Nigeria.
[4]Department of Agricultural Technology, Anambra State College of Agriculture, Mgbakwu, Awka.
Correspondence: chukwuharriman@gmail.com; +2348060690357.

Abstract: Estimation of genetic variability, genotypic and phenotypic correlation and inheritance of among agronomic traits is fundamental to improvement of any crop. Ten (10) OFSP genotypes were evaluated at the National Root Crop Research Institute, Umudike, Abia State , Nigeria, during 2014, 2015 and 2016 cropping seasons.The experiment was laid out in Randomized Complete Block Design with four replications to estimate correlations, genetic variability, heritability and genetic advance of root yield and its component characters in Orange-fleshed sweetpotato. There were significant differences among the genotypes ($p < 0.05$) and in genotype by year interaction for total number of roots per plant, total root weight per plant and root yield. Correlation analysis revealed that significant positive correlation ($p < 0.05$) coefficient (r) values involving root yield were 0.71 ($p < 0.05$), 0.70 ($p < 0.05$), and 0.50 ($p < 0.05$), for root girth, total number of root per plant and root weight per plant respectively. Correlations between root yield and and root girth and between root yield and total number of root per plant were very high (r = 0.71 and 0.70 respectively). Path coefficient analysis for root yield (RY) showed that traits like, root girth (RG), total root weight per plant (TRWPP) and starch (SCH) had high positive direct contribution on RY (4.26, 2.65, and 2.25 respectively). Phenotypic (PCV) and genotypic coefficient of variations (GCV) were highest for beta carotene (98 and 97% respectively) and vine length at 18WAP (248 and 38.96% respectively) across the years. High heritability accompanied by low genetic advance for B-Carotene and vine length at 18WAP was indicative of predominance of non-additive gene actions which could be exploited through crossbreeding to take advantage of heterosis for these traits. Highest heritability was obtained for B-Carotene, dry matter and Starch with each having 1. This was followed by vine length at 18wap (0.93) and internode length at 18WAP (0.81) across the years. This implied the presence of more additive gene effect and a potential for improvement in orange-fleshed sweet potato through selection. The genetic advance for dry matter (128.7), Starch(161.84), day to 50% flowering(22842.3), vine length at 18wap (175993.8) and root yield (665.08) were also high.

Keywords: Correlations, Genetic variability, heritability, OFSP, Path Analysis.

INTRODUCTION

Vitamin A deficiency (VAD) is a serious public health problem in many developing countries, including most countries of Africa (WHO, 1995). It mainly affects poor, young children (6 months to six years of age) and pregnant women. The clinical form of VAD (or xerophthalmia), results when the eye is adversely affected, and is expressed as night blindness or, at its most severe, as total, irreversible blindness. Xerophthalmia affected an estimated 3.1 million children world-wide in 2009 (IVACG, 2009). Sub-clinical VAD affects many more people – an estimated 227.6 million in 2009 (IVACG, 2009) – and results in increased sickness and death rates due to diseases such as diarrhea and measles among those affected. Public health efforts aimed at combating VAD call for a combined approach, including dietary diversification and food fortification. Dietary diversification involves consumption of β- carotene-rich crops like orange fleshed sweetpotato (OFSP). The β- carotene pigment (a dietary precursor of vitamin A) is known to be responsible for the yellow to orange colouration of the flesh of tuberous roots of

some sweetpotato varieties (Degras, 2003; Rodriguez-Amaya and Kimura, 2004).

Sweetpotato (*Ipomoea batatas* [L.] Lam.); 2n=6x=90) is one of the valuable crops producing the highest root dry matter content for human consumption. It provides comparatively high calorie at 152 MJ ha^{-1} day^{-1} and regarded as an important staple food crop in Nigeria (Ukpabi, 2009). These genotypes, especially those of orange fleshed sweet potato (OFSP), were bred as a tool for the global fight against VAD in areas that lack vitamin A rich food materials (Degras, 2003). Thus, it can be used to develop composite flour and added to several products viz., bread, cake, doughnuts, etc thereby increasing the β-carotene intake among the populace. One way to increase the consumption of β-carotene in OFSP is through improvement in in root yield as well as quality of the root produced(Tyagi and Khan, 2008).

Variability plays an important role in crop breeding. An insight into the magnitude of variability present in crop species is of utmost importance as it provides the basis for selection. Yield improvements have been achieved through directional selections for yield and yield contributing traits (Akbar and Kamran, 2006) using correlation and other statistical tools. Correlation is the degree to which two or more variables are related and change together. According to Hallauer and Miranda report (1988) correlation measures the degree of association between two or more characters and is measured by a correlation coefficient. However, under a complex situation, the estimates of correlation alone may be often misleading due to mutual cancellation of component traits, so it becomes necessary to study the path coefficient analysis simultaneously which takes in to account the casual relationship in addition to the degree of relationship. Path coefficient analysis also suggests the selection criterion, and reduces the time taken by a breeder during the selection process (Qaizar *et al.*, 1991). For example, the breeder focuses only on the traits with a large direct effect on a dependent trait such as maturity and yield, thus selection is only restricted to a few essential traits (Vijayabharathi *et al.*, 2009). Path coefficient analysis gives information about the direct and indirect effects of different traits on a complex trait. Genetic variability for agronomic characters is a key component of breeding programmes for broadening the gene pool of crops (Ahmad *et al.,* 2005). However, the success of any crop improvement programme is not only dependent on the amount of genetic variability present in the population but also on the extent to which it is heritable, which sets the limit of progress that can be achieved through selection (Sumathi *et al*., 2005; Wang *et al*., 2011). Again, the assessment of performance of parental lines based on the yield components could aid in the selection of superior parents for the production of better yielding hybrids (Bocanski *et al*., 2009). This can successfully be achieved if the genetic parameters which govern inheritance of important agronomic traits are established (Mahiboobsa *et al.,* 2012).

Heritability is a measure of the phenotypic variance attributable to genetic causes and has predictive function in plant breeding. It provides information on the extent to which a particular morphogenetic character can be transmitted to successive generations. Knowledge of heritability influences the choice of selection procedures used by the plant breeder to decide which selection methods would be most useful to improve the character, to predict gain from selection and to determine the relative importance of genetic effects (Waqar *et al*., 2008; Laghari *et al.,* 2010). The most important function of heritability in genetic studies of quantitative characters is its predictive role to indicate the reliability of phenotypic value as a guide to breeding value (Falconer and Mackay 1996). Characters with high heritability can easily be fixed with simple selection resulting in quick progress. Apart from heritability, genotypic and phenotypic variances, genetic advance and correlations are some of the key parameters which determine the efficiency of a breeding programme. The genotypic variance explains the proportion of phenotypic variance attributable to the failure of homogeneity among genotypes in different environments (Sujiprihati *et al.,* 2003; Bello *et al.*, 2012) while the phenotypic variance explicates the total variance among phenotypes tested in different environments of interest to the plant breeder. Genetic advance is the difference between the mean of the selected plants in

the original population and the mean of the progeny raised from the selected plants in the next generation. The correlations give reliable and useful information on nature, extent, and direction of selection (Zeeshan *et al*., 2 013). Considering the importance of OFSP and its potential for future Nigerian economy, it is imperative to increase its productivity and other important traits through genetic manipulation. Therefore; the present study was proposed to estimate the extent of variability, heritability and genetic advance in OFSP genotypes and to study the associations among yields and yield related traits in OFSP.

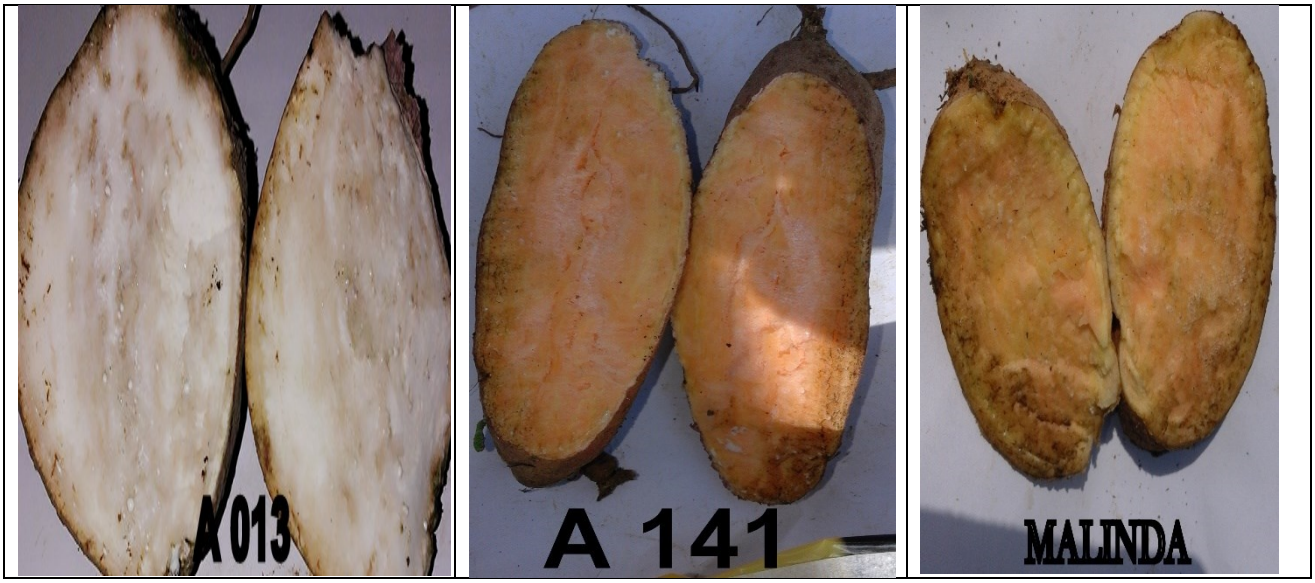
MATERIALS AND METHODS

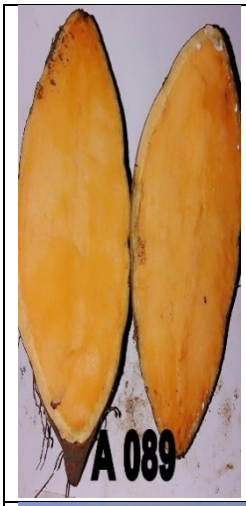
Experimental Site: The experiment was conducted in the National Root Crops Research Institute (NRCRI), Umudike which is situated at Latitude 05°29' N, Longitude 07°33'E and at an altitude of 122m above sea level. Umudike has a total rainfall of about 2000-2500mm per annum with annual average temperature of about 26^{0}C.

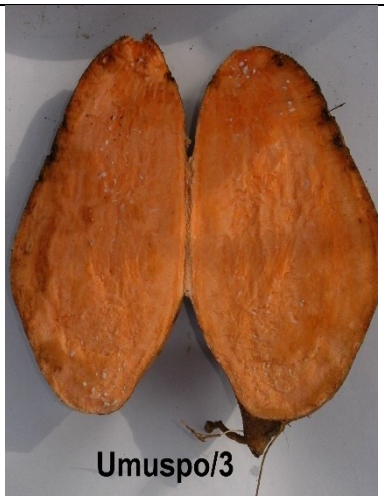
Table 1. Meteorological data of the experimental area at Umudike in 2014, 2015 and 2016

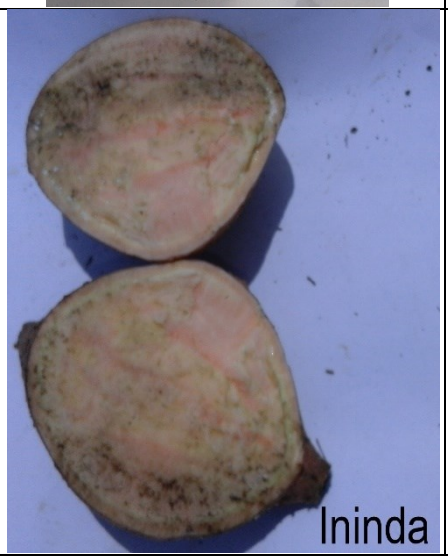
Meteorological Factor	Jan	Feb	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean
2014													
Min. Temp. (^{0}C)	25	25	24	23	23	22	22	23	22	23	23	24	23.3
Max. Temp. (^{0}C)	32	32	31	29	29	28	28	30	28	30	31	31	29.9
Sunshine (Hrs)	6.6	6.7	6.6	4.8	5.8	2.7	2.5	6.3	2.6	6.2	6.4	6.5	5.3
Solar Radiation(ml)	5.5	5.6	4.9	5.0	5.2	2.6	1.8	2.9	1.9	3.0	5.3	5.4	4.1
Monthly Rainfall(mm)	74.8	0.0	13.0	89.7	310.9	361.2	302.7	176.3	361.6	206.1	0.6	15.9	159.4
Number of rainy days	1	0	4	6	18	20	24	16	22	14	3	2	10.8
Relative Humidity (%)	58	56	67	70	72	74	78	68	76	66	62	60	67.3
2015													
Min. Temp. (^{0}C)	23	23	23	22	22	20	20	21	21	20	21	22	258.0
Max. Temp. (^{0}C)	35	35	33	32	32	32	31	33	31	32	33	34	393.0
Sunshine (Hrs)	6.7	6.8	6.5	4.9	5.9	2.8	2.6	6.2	2.7	6.3	6.5	6.6	64.5
Solar Radiation(ml)	5.3	5.4	5.0	4.8	4.6	3.2	1.6	3.8	1.8	2.8	5.2	5.1	48.6
Monthly Rainfall(mm)	18.2	0.0	88.3	169.9	202.8	164.2	232.1	282.5	304.0	205.8	150.2	5.4	1823.4
Number of rainy days	1	0	5	8	20	22	26	18	24	16	4	2	146.0
Relative Humidity (%)	60	58	67	70	76	78	80	68	79	66	64	62	828.0
2016													
Min. Temp. (^{0}C)	24	24	23	22	22	21	21	23	21	22	22	23	268.0
Max. Temp. (^{0}C)	33	33	32	31	30	30	29	31	29	30	31	32	371.0
Sunshine (Hrs)	6.8	6.9	6.7	4.9	6.0	2.9	2.7	3.0	2.8	6.3	6.5	6.6	62.1
Solar Radiation(ml)	5.1	5.2	5.0	4.8	4.9	1.6	1.4	2.8	1.5	3.1	4.7	4.6	44.7
Monthly Rainfall(mm)	4.1	0.0	12.2	88.8	316.8	368.0	402.6	264.1	392.4	277.0	62.1	11.2	2199.3
Number of rainy days	1	0	5	8	19	22	25	16	23	17	5	1	142.0
Relative Humidity (%)	62	59	69	70	71	80	80	85	82	70	65	63	856.0

Source: Meteorological station of the National Root Crops Research Institute (NRCRI), Umudike.









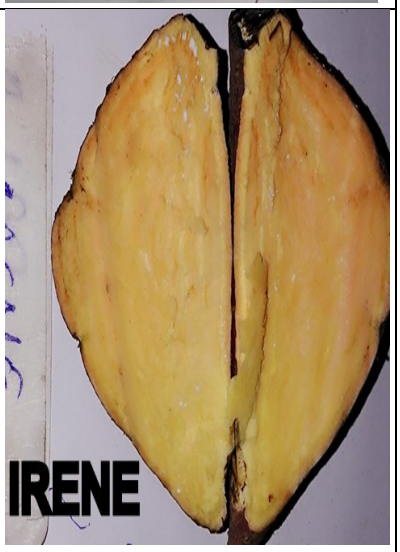


Plate 1. Flesh characteristics of the 10 evaluated OFSP genotypes.

The predominant vegetative type is rain forest (NEST, 1991). However, the soil type is classified as sandy loam ultisol (Agboola, 1979). The ten (10) genotypes (Plate 1) obtained from the (NRCRI) Umudike were grown in a plot size of 1 × 3m ($3m^2$) with a spacing of 1 seedling per stand, with inter and intra row spacing of 1.0 and 0.3m respectively giving a plant density of 33,333 plants per hectare. The experiment was replicated 4 times using a randomized complete block design.

Data collection and analyses: Data was collected on vine length (cm),vine internode length (cm), vine internode diameter (mm), number of secondary stem per plant, number of days to 50% flowering, weight of saleable roots per plant (kg), storage root length (cm), storage root diameter (cm), weight of roots per plant(kg), storage root fresh yield per hectare (t/ha), storage root dry matter content (%),total starch content (mg/100g) and beta-carotene content(mg/100g). The data collected were subjected to uni- and multi-variate analyses; including analysis of variance and Spearman Correlation Coefficient (Ofori, 1996; Hailegebrial, *et al*.,2015). GenStat (2012) and Statistical Package for Social Sciences (SPSS) were the software used for the analysis. Significant means were separated by Fisher's Least Significant Difference Test (LSD), at 5% level of significance. Percentage of each of β-carotene, dry matter, starch and moisture were determined according to standard methods described by FAO (1980).

The genotypic and phenotypic coefficient of variation were calculated by Kwon and Torrie (1964) technique. The genetic advance in percentage of mean was calculated by using Falconer (1989) formula.

Genetic Variance (V_g)

$$= \frac{Genotype\ Mean\ Square - Error\ Mean\ Square\ ((\text{MSG} - \text{MSE}/\text{r})}{Number\ of\ replications\ (r)}$$

Environmental Variance = Error Mean Square (EMS)

Phenotypic Variance (Vp) = Vg + Vg/ r OR (MSG / r)

Genotypic and Phenotypic coefficient of Variation was calculated as

$GCV(\%) = \{(\sqrt{\delta^2_p}/X) \times 100/1\}$,

$PCV = \{(\sqrt{\delta^2_p}/\overline{X}) \times 100/1\}$.

Where, δ_p = phenotypic standard deviation, δ_g = genotypic standard deviation, and X = Grand mean for the characteristic x; PCV and GCV= phenotypic and genotypic coefficient of variation, respectively.

Heritability (H^2) on Entry Mean Basis was calculated as

$$H^2 = \frac{Vp}{Vg}$$

The expected Genetic Advance for each trait was calculated as

$$GA = K\sqrt{VP}\ H^2$$

Where, K = 1.40 at 20% selection intensity for trait;
Vp = Phenotypic variance for trait;
H^2 = Broad Sense Heritability of the trait;

Genetic Advance as percentage of mean is calculated as,

$$GA = \frac{GA}{X} \times 100\%$$

RESULTS AND DISCUSSION

Combined analysis of variance indicated that the effect of year was significant (P< 0.05) for all the characters (Table 2). Rainfall amount and distribution that was higher and favourable in the year 2016 compared to 2014 and 2015 may be responsible for the differences observed between the years for these traits (Table 1). Mean squares due to genotypes, year and genotype x year interaction were significant (P< 0.05) for total number of root per plant, total root weight per plant, dry matter and root yield indicating presence of genetic variability for these traits in the germplasm material studied and which also varied with the differences in yearly conditions. This offers way for further improvement through simple selection. However, the interaction of the year with genotypes is very important in this study. The significant effect of genotype by year interaction indicates the diversity of the genotypes and their differences in environmental responses across the three years for these traits. The mean performances across the three years for culinary qualities, root yield and yield related characters of the OFSP are presented in Tables 2 and 3. The results showed significant differences among the genotypes for culinary qualities, root yield and yield components. The most outstanding genotypes for root yield are Umuspo/3, A

089, and Delvia in descending order with yield ranging from 24.23 to 21.11t/ha, while Malinda had the lowest value for root yield (10.64 t/ha) over the three years. Total root weight per plant varied from 0.47 to 3.38 in the three years with A 089 having the highest (3.38).

The range observed for total number of root per plant was between 3.26 and 6.11. A 089, Umuspo/3 and A 141- recorded the highest number of root per plant with the values of 6.11, 6.09 and 4.58 respectively. With respect to year, 2016 and 2015 had the higher root yield per hectare compared to 2014. The variation was not significant ($p > 0.05$) across the years with respect to culinary characteristics (Table 3). However, highest beta-carotene content (11.031Mg/100gFW) was recorded for A 089, followed by Umuspo/3 (10.233Mg/100gFW) while Delvia had the lowest beta-carotene content (0.141Mg/100gFW). Dry matter content and starch estimate were highest for Delvia (44.4 and 34.0 % respectively). This was followed by Malawi/II that had dry matter content and starch estimate of 43.1 and 31.7% respectively. Umuspo/3 had the least quantity of dry matter and starch (26.0 and 15.7 % respectively). The present results suggest that the available genetic variation observed in important agronomic characters could be useful in designing better effective breeding strategies in OFSP. The variation observed in number of roots per plant, root yield per plant and root yield per t/ha may be as a result of different genetic makeup of the genotypes. The current result is in conformity with the finding of Raham *et al*. (2013).

The findings is also in good agreement with Omiat *et al*. (2005), who indicated that the varietal effect had a significant influence on the total tuberous root yield of sweetpotato. Similarly, Kathabwalika *et al*, (2013) observed significant differences in root yield per plant and root yield per t/ha among the sweetpotato varieties in their trial.

Dry matter content varies due to a number of factors such as variety, location, climate, incidence of pests and diseases, cultural practices and soil types (Shumbusha *et al*., 2010; Vimala and Hariprakash, 2011). Similarly, the variation observed in β-carotene content is attributed to their genetic constitution. According to Woolfe (1992) dark orange flesh roots are rich sources of β-carotene, while yellow/orange roots supply moderate amounts of β- carotene. Teow *et al.* (2007) reported significant variations in respect to β-carotene content among sweetpotato genotypes, and orange flesh had higher β-carotene content than white flesh.

The correlation studies revealed significant ($P < 0.05$) to highly significant ($P < 0.01$) level of probability among the traits (Table 4). The correlation coefficients were generally positive between root yield and other characters, except dry matter, starch and number of branches at 18WAP. The significant positive correlation coefficient (r) values involving root yield were 0.71 ($P < 0.05$), 0.70 ($P < 0.05$) and 0.50($P < 0.05$) for root girth, total number of root per plant and root weight per plant respectively. Positive correlation coefficient (r) of 0.19, 0.13, 0.20, 0.14 and 0.07 were found between root yield and beta carotene, days to 50% flowering, internode length at 18WAP, vine length at 18WAP and root length respectively. However, negative correlations were observed between root yield and dry matter ($r = -0.01$), starch ($r = -0.002$) and number of branches at 18 WAP ($r = -0.02$).

This study revealed that root yield was primarily influenced by root girth and total number of root per plant and secondarily by total root weight per plant as direct contribution factor. Hallauer and Miranda (1988) suggested that selection may be exerted on yield components indirectly, but however, such selection would be effective if the character used possesses high heritability compared to the primary one. In addition, the correlation between them has to be substantial. Correlations between root yield were highest between root girth and total root number per plant ($r = 0.71$ and 0.70 respectively). The high correlations indicate that any increase in root girth and total root number per plant would simultaneously increase root yield. Beta-carotene was significant and negatively correlated with dry matter ($r = -0.42$, $P < 0.01$) and starch ($r = -0.79$, $P < 0.01$) indicating that OFSP genotypes with high beta-carotene content had low dry matter and starch contents. This negative association was attributed to a competition between the dry matter, starch and the β-carotene because they are synthesised in plastids.

Table 2: Mean values of three yield traits of orange fleshed sweetpotato genotypes evaluated for three years in Umudike

Genotype	Total No. of root Per plant				Total root Weight per plant (kg)				Root yield (t/ha)			
	2014	**2015**	**2016**	**Mean**	**2014**	**2015**	**2016**	**Mean**	**2014**	**2015**	**2016**	**Mean**
A 013	5.2	5.63	2.33	**4.39**	0.36	0.66	0.38	0.47	11.92	20.33	12.56	**14.94**
A 089	5.6	8.73	4	**6.11**	0.75	8.73	0.66	3.38	25.11	17.89	22	**21.67**
A 097 a	3.77	6.73	3	**4.5**	0.25	4.84	0.48	1.86	8.26	26.22	16.11	**16.86**
A 141	7.8	3.27	2.67	**4.58**	0.51	3.27	0.35	1.38	17.14	5.22	11.67	**11.34**
Delvia	3.33	5.83	4.33	**4.5**	0.25	2.52	0.86	1.21	8.33	26.33	28.66	**21.11**
Ininda	6.87	1.63	1.33	**3.28**	0.72	1.43	0.23	0.79	23.91	10.27	7.78	**13.99**
Irene	5.33	1.63	3	**3.32**	0.38	1.43	0.38	0.73	12.73	10.27	12.56	**11.85**
Malawi II	6.87	2.1	4	**4.32**	0.68	2.1	0.55	1.11	22.51	8.0	18.33	**16.28**
Malinda	3.4	3.37	3	**3.26**	0.29	2.8	0.46	1.18	9.58	7.11	15.22	**10.64**
Umuspo/3	6.27	6.67	5.33	**6.09**	0.57	1.62	0.74	1.31	19.02	28.89	24.78	**24.23**
Mean	**5.44**	**4.56**	**3.3**		**0.48**	**0.5**	**0.51**		**15.85**	**16.05**	**16.97**	
$LSD_{(0.05)}$ for Genotype	1.78				1.153				7.18			
$LSD_{(0.05)}$ for Year	0.98				0.631				NS			
$LSD_{(0.05)}$ for interaction	3.09				1.996				12.43			
CV (%)	5.9				23.5				8.0			

Table 3. Mean values of three culinary traits of orange fleshed sweetpotato genotypes evaluated for three years in Umudike in three years combined

Genotype	B-Carotene (Mg/100g FW)				Dry matter (%)				Starch (%)			
	2014	2015	2016	Mean	2014	2015	2016	Mean	2014	2015	2016	Mean
A 013	0.187	0.22	0.22	**0.209**	40.3	40.2	40	**40.2**	30.5	30.5	29.8	**30.3**
A 089	11.02	11.02	11.053	**11.031**	28.2	27.8	28.5	**28.2**	18.6	18.0	18.1	**18.2**
A 097 a	0.167	0.167	0.157	**0.163**	34.8	34.8	35.3	**35**	26.4	26.4	25.7	**26.1**
A 141	7.167	7.167	7.167	**7.167**	26	26.3	26.3	**26.2**	16.0	16.7	16.7	**16.4**
Delvia	0.123	0.147	0.153	**0.141**	44.5	44.7	44.1	**44.4**	34.7	33.7	33.7	**34.0**
Ininda	4.613	4.63	4.64	**4.628**	33.6	34	33.7	**33.8**	20.0	20.0	19.7	**19.9**
Irene	1.69	1.667	1.667	**1.674**	38.3	38.6	37.9	**38.3**	30.6	30.5	30.5	**30.6**
Malawi II	3.02	3.087	3.03	**3.046**	43.3	43.6	42.8	**43.1**	31.4	31.8	31.8	**31.7**
Malinda	3.653	3.653	3.653	**3.653**	29.3	29.4	29.4	**29.0**	17.7	17.2	17.5	**17.4**
Umuspo/3	10.233	10.233	10.233	**10.233**	15.2	15.3	48.7	**26.0**	15.4	16.1	15.7	**15.7**
Mean	**4.187**	**4.199**	**4.197**		**33.4**	**33.5**	**36.7**		24.1	24.1	23.9	
$LSD_{(0.05)}$ for Genotype	0.132				10.06				0.7			
$LSD_{(0.05)}$ for Year	NS				5.51				NS			
$LSD_{(0.05)}$ for interaction	NS				17.42				NS			
CV	0.74				5.6				0.5			

Table 4: Simple correlation coefficients among traits measured on 10 OFSP genotypes evaluated in three years combined

TRAITS	1	2	3	4	5	6	7	8	9	10	11	12
B-CaroteneMg100gFW	1											
Dry matter	-0.42**	1										
Starch	-0.79**	0.52**	1									
Days to 50% flowering	0.21*	0.05	-0.10	1								
Internodelength@18WAP	0.26*	-0.24*	-0.22*	0.55**	1							
Vinelength@18WAP	0.18	-0.15	-0.18	0.61**	0.66**	1						
No. of braches@18WAP	-0.09	0.04	0.03	0.63**	0.42**	0.62**	1					
Root length (cm)	0.11	-0.04	-0.17	0.05	0.12	0.11	0.074	1				
Root girth (cm)	0.08	-0.13	-0.03	-0.23*	-0.03	-0.27*	-0.40**	0.23*	1			
Total root No. per plant	0.26*	-0.12	-0.12	-0.21	-0.04	-0.12	-0.31**	-0.18	0.35**	1		
Total root Wt per plant(kg)	0.22*	-0.12	-0.15	-0.15	-0.20	-.220*	-0.21**	-0.18	-0.01	0.37**	1	
Root yield(t/ha)	0.19	-0.01	-0.002	0.13	0.20	0.14	-0.02	0.07	0.71**	0.70**	0.50*	1

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

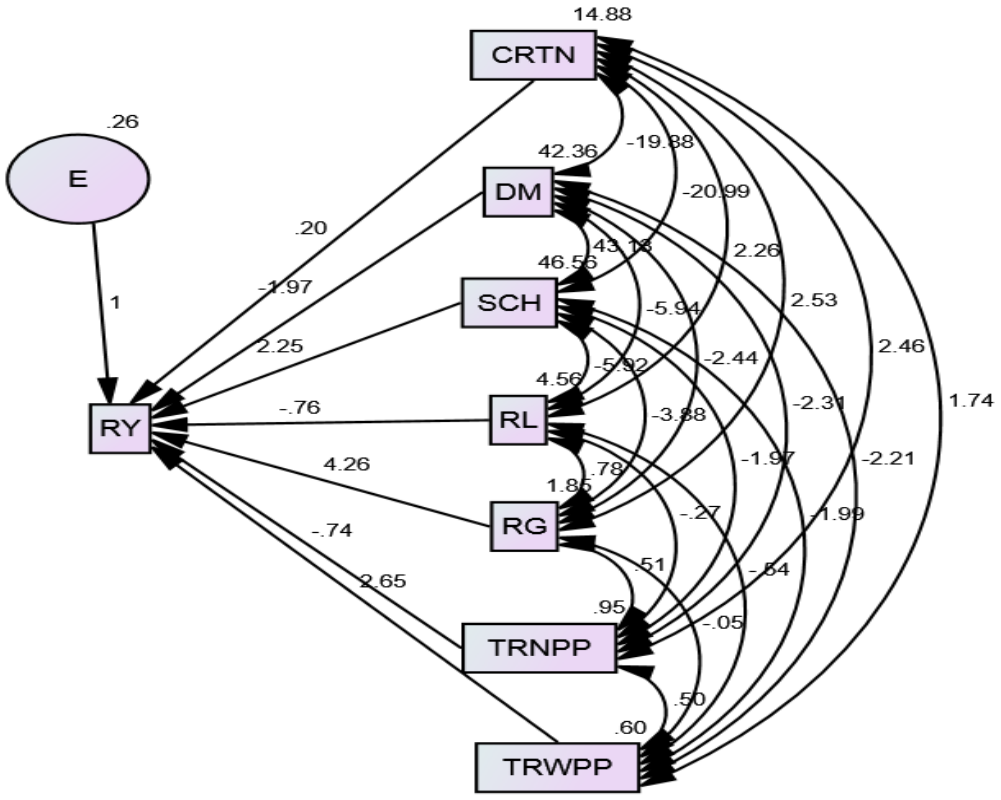


Fig. 1: Path diagram and correlation coefficient of seven characters. Single headed arrow denotes direct effect on root yield, double headed arrow denotes the correlation coefficients between traits

This finding is supported by (Cervantes-Flores *et al.*(2008) who observed negative correlation between dry matter and beta carotene. Knowledge of inter-character relationship is very important in plant breeding for indirect selection of the characters that are not easily measured and for those that exhibit low heritability. CRTN = beta carotene, DM = dry matter, SCH = starch, RL = root length, RG = root girth, TRNPP = total number of root per plant, TRWPP = total root weight per plant, E1 = residual effect.

The phenotypic direct and indirect effects of yield-related traits on root yield are presented in figure 1. Path coefficient analysis for RY showed that traits like, RG, TRWPP and SCH showed highest positive direct effect on RY with 4.26, 2.65, and 2.25 respectively. This means that a slight increase in one of the above traits may directly increase the RY. These traits are therefore, very important components of RY and should be given high weightage in any selection process aimed at improving RY in OFSP. Similar results were reported by Mohanty *et al.*(2016). On the other hand, the maximum negative direct effect was exhibited by dry matter (-1.97) followed by root length (-0.76) and total number of roots per plant (-0.74). Indirect effects often play a more important role than direct effects. For example, the direct effect of TRNPP was negative (-0.74), but its indirect effects through CRTN was high (2.46) explaining why TRNPP was highly positively correlated with RY ($r = 0.70$, $p < 0.01$). The direct effect of RL also was negative (-0.76), but this was confounded by the larger indirect effect through CRTN (2.26) and RG (1.86), explaining why there was a positive correlation between RL and RY ($r = 0.07$). The direct effect of RG and TRWPP on RY were most closely related, indicating that plants producing larger RG and TRWPP produced highest RY in OFSP. These findings are in conformity with Yildirim *et al.*(1997) who stated that root girth and tuber weight/plant had positive and high direct effects on tuber weight/plant. He also reported that main stems/plant; plant height had positive and high direct effects on tuber yield. Conversely, the study is in

disagreement with Maris (1988) who found that number of tuber and average tuber weight had equal effects on total yield. Residual effect (26.0%) measures the role of other possible independent variables not included in the study. This could be attributed to the effect of new environment as reported in cowpea (Afuape *et al*., 2011).

Vine length at 18WAP exhibited the highest genotypic (σ^2g) and phenotypic variance (σ^2p) (2265.22 and 9173.84, respectively -Table 5). This was followed by the dry matter (81.06 and 62.40, respectively). The character with almost equal value of phenotypic and genotypic variances can be considered stable. Lower genotypic and phenotypic variance were obtained for the number of branches at 18WAP (0 and 89.40, respectively) and root length (60.03 for the respectively). A lower value of CV generally depicts low variability among the tested sample; a high proportion GCV to the PCV is desirable in breeding works. The phenotypic variance (σ^2p) and phenotypic coefficient of variation (PCV) was slightly higher than the genotypic variance (σ^2g) and genotypic coefficient of variation (GCV) for most of the traits suggesting the presence of appreciable environmental influence in the expression of these traits. The results for Phenotypic variances (σ^2p) were found to be greater than the corresponding genotypic variances (σ^2g) for most of the characters indicating that the expressions of these characters were influenced by the environmental factors. This agrees with Korkut *et al*. (2001) who obtained similar results in sweet potato. According to Deshmukh *et al.* (1992), PCV and GCV values greater than 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10 and 20% to be medium. With this benchmark, the GCV values obtained for B-Carotene (Mg/100g FW), vine length at 18WAP(cm), Starch (%), internode length at 18WAP(cm) and total root weight per plant (kg) can be classified as high, while medium GCV values were obtained for Root yield (t/ha) and day to 50% flowering. The GCV obtained in B-Carotene (Mg/100g FW), vine length at 18WAP, Starch, internode length at18WAP and total root weight per plant suggests selection can be applied more effectively on these traits to isolate more promising genotypes. These observations are in conformity with the findings of Kashiani *et al.* (2008) and Najeeb *et. al.* (2009).

Though genotypic coefficient of variation measures the amount of variation in character, however, the estimation of heritable variation with the help of genetic coefficient of variation alone may be misleading. Burton (1952) suggested that the genetic coefficient of variation together with heritability estimates gave a better picture of the extent of heritable variation. Similarly, the estimates of genetic advance help in understanding the type of gene action involved in the expression of various polygenic characters. High values of genetic advance are indicative of additive gene action whereas low values are indicative of non-additive gene action (Singh and Narayanan, 1993). Thus the heritability estimates will be reliable if accompanied by a high genetic advance. So, genetic advance should be considered along with heritability in coherent selection breeding program. Dabholker, (1992) classified heritability estimates as low (5 to 10%), medium (10 to 30%) and high (30 to 60%). Considering this benchmark, heritability estimate obtained in this study was high for plant height, B-Carotene, dry matter, starch, vine length at18WAP, internode length at 18WAP, day to 50% flowering and root girth. Similarly, high heritability values coupled with high genetic advance (Table 5) were recorded for dry matter, starch, day to 50% flowering, vine length at18WAP and root yield. This indicated that additive genetic variation was important in the transmission of these traits from the parents to the progeny. Also, these traits can easily be fixed in the genotypes by selection in early generations. This finding is supported by Ahmed *et al*.(2007) and Songsri *et al.* (2008) who reported that better heritability and genetic advance values in important parameters suggest the possibility of improvement in these parameter. However, Hossain *et al*. (2000) and Choudhary *et al*. (1999) reported high estimates of both heritability and genetic advance for number of roots per plant and fresh yield of storage roots per plant in sweet potato. High heritability accompanied by high genetic advance for B-Carotene and vine length at 18WAP is indicative of predominance non-additive gene actions which could be exploited through heterosis breeding. Low heritability with low genetic advance values was

found for number of branches and root length, indicating slow progress through selection for these traits.

CONCLUSION

The mean performance of the character showed substantial amount of variability among the genotypes. Thus the experiment revealed that greater yield response could be obtained through direct selection scheme in OFSP genotypes tested.

REFERENCES

Afuape S.O, Okocha P.I and Njoku D. (2011): Multivariate assessment of the agro morphological variability and yield components among sweet potato (Ipomoea batatas (L.) Lam) landraces. *African Journal of Plant Science*. 5(2):123-132.

Agboola,S.A.(1979):An Agricultural Atlas of Nigeria. Oxford University Press, Great Britain. 153pp.

Ahmad, S.Q., Khan, S., Ghaffar, M. and Ahmad, F. (2011). Genetic diversity analysis for yield and other parameters in sweetpotato genotypes. *Asian J. Agric. Sci*., 3(5): 385-388.

Ahmed NCM, Khaliq IMM (2007). The inheritance of yield and yield components of five wheat hybrid populations under drought conditions. *Indonesian J. Agric. Sci.,* 8(2): 53-59.

Akbar, A.A and Kamran, M. (2006). Relationship among yield components and selection criteria for yield improvement of Safflower. *J. appl. Sci*., 6: 2853-2855.

Bello, O.B, Abdulmaliq, S.Y, Afolabi, M.S. and Ige. S.A (2010). Correlation and Path coefficient analysis of yield and agronomic characters among OFSP varieties and their F1 hybrids. *Afri. J. Biotech*., 9(18): 2633-2639.

Bello, O.B., Ige, S.A., Azeez, M.A., Afolabi, M.S., Abdulmaliq, S.Y. and Mahamood, J. (2012). Heritability and genetic advance for grain yield and its component characters in maize. *International Journal of Plant Research*, 2 (5), 138-145.

Bocanski, J., Sreckov, Z. and Nastasic, A. (2009). Genetic and phenotypic relationship between grain yield and components of grain yield of maize. *Genetika,* 41(2), 145-154.

Burton, G. W. (1952). Quantitative inheritance in grasses. 6th International Grassland Congress 1:277-283.

Cervantes-Flores, J.C., Yencho, G.C., Kriegner, A., Pecota, K.V., Faulk, M.A., Mwanga, R.O.M., Sosinski, B.R. (2008): Development of a genetic linkage map and identification of homologous linkage groups in sweetpotato using multiple dose AFLP markers. M*ol Breeding.* 21: 511-532

Choudhary, S.C., K. Harash, M. Kumar, V.S. Verma, S.K.T. Nasar and H. Kumar,(1999). Genetic variability in sweet potato. J. Applied Biol., 9: 146-148.

Hossain, M.D., M.G. Rabbani and M.L.R. Mollah, (2000). Genetic variability, correlation and path analysis of yield contributing characters in sweet potato (*Ipomoea batatas* Lam.). Pak. J. Sci. Ind. Res., 43: 314-318.

Degras, L. (2003). Sweet Potato. *Macmillan, Oxford*. England.

Deshmukh, S..N, Basu, M.S., Reddy, P.S. (1992). Genetic variability, character associations and path coefficient analysis of quantitative traits in Virginia bunch varieties of ground nut. *Indian J. Agric. Sci.*, 56:515-518.

Falconer, D. S. (1989). *Introduction to Quantitative Genetics* (3rd ed.). Logman Scientific and Technical, Logman House, Burnt Mill, Harlow, Essex, England.

Falconer, D.S and Mackay T.F.C. (1996). Introduction to quantitative genetics. 4th ed. Benjamin Cummings, England, pp. 245-247.

FAO (1980): Agricultural data FAOSTAT. Food and Agriculture Organization of the United Nations. Rome.

Genstat. (2012): *Genstat for Windows, Discovery, 4th edition*. Lawes Agricultural Trust, Rothamsted Experimental Station, UK.

Hailegebrial, K., Getachew, A., Legesse, W. and Yemane, T. (2015): Correlation and Path Coefficient Analysis of Grain Yield and Yield Related Traits in Maize (*Zea mays* L.) Hybrids, at Bako, Ethiopia. *Journal of Biology, Agriculture and Healthcare.*

Hallauer, A.R., Miranda, J.B. (1988): Quantative genetics in maize breeding. 2nd Edn., Iowa State University Press, USA.

IVACG (International Vitamin A Consultative Group). (2009). Two decades of progress: Linking knowledge to action: Report of the XVI International Vitamin A Consultative Group Meeting, Chiang Mai, Thailand, 24-28 October, 1994. *IVACG, Washington*, D.C., USA.

Kashiani, P, Saleh, G., Abdullah, S.N. and Abdullah, N.A.P. (2008). Performance, heritability correlation studies on nine OFSP lines. Proceeding of the 10th Symposium of Malaysian Society of Applied Biology, Nov. 6-8, 2008, Malaysia, pp 48-49.

Kathabwalika, D. M, E. H. C. Chilembwe, V.M. Mwale, D. Kambewa and J.P. Njoloma, (2013). Plant growth and yield stability of orange fleshed sweetpotato (*Ipomea batatas*) genotypes in three agro—ecological zones of Malawi.Int. Res. *J.Agric*. *Sci*. *Soil Sci*.,3: 383-392.

Korkut KZ, Baser I, Bilgin O (2001). Genotypic and phenotypic variability, heritability and phenotypic correlation for yield and yield components in bread wheat varieties. Acta Agron. Hung., 49(3): 237-242.

Kwon, S. H., & Torrie, J. H. (1964). Heritability and interrelationship of two soybean (*Glycine max* L.) populations. *Crop Sci., 4*, 196-198. http://dx.doi.org/10.2135/cropsci1964.011183X00x

Laghari, K.A, Sial MA, Afzal Arain, M.A., Mirbahar, A.A, Pirzada, A.J., Dahot, M.U. and Mangrio, S.M. (2010).

Heritability studies of yield and yield associated traits in bread wheat. Pak. J. Bot., 42(1): 111-115.

Mahiboobsa, M., Dharmaraj, P.S., Muniswamy, S., Sony, Y.T., Avinalapa, H., Patil, R. and Bankar, C. (2012).Genetic variability studies on stable male sterile, maintainer and restore lines of Pigeonpea. *Karnataka J. Agric. Sci*., 25(4), 525-526.

Maris, B., (1988).Correlations within and between characters between and within generations as a measure for the early generation selection in potato breeding. *Euphytica*, 37: 205-224.

Mohanty, P., Ashok, M. K. ,Rout A. and Sasikala, K.(2016): Character association and path analysis of sweet potato [Ipomoea batatas (L.) Lam.] genotypes *P. Journal of Crop and Weed,* 12(1):76-80.

Najeeb, S, Rather, A.G, Parra, G.A, Sheikh, F.A and Razvi, S.M (2009). Studies on genetic variability, genotypic correlation and path coefficient analysis in potato under high altitude temperate ecology of Kashmir. Maize Genetics Cooperation Newsletter. 83: 1-8.

NEST. (1991). Nigeria Environmental Study Action. Nigeria Threatened Environment: A National Profile. Nigeria.

Omiat, E.G, R.E. Kapinga,S. Tumwegamire, T.L. Odong and E. Adipala. (2005). On-farm evaluation of orange-fleshed sweetpotato varieties in Northeastern Uganda. *Afr. Crop Sci. Proc*., 7:603-609.

Qaizar, A., Rana, M.A. and Siddiqui, S.U.H.(1991). Sunflower seed yield as influenced by some agronomic and seed characters. Euphytica, 56: 137-142.

Rodriguez-Amaya DB, Kimura M. (2004). Harvest plus Handbook for Carotenoid Analysis. Harvest plus Technical Monograph 2. IFPRI, Washington D.C.

Shuzbusha, D., G. Tusiime, R. Edema, P. Gibson and R.O.M. Mwanga (2010): Diallel analysis of root dry matter content in sweetpotato. Proceedings of the 2nd RUFORUM Biennial Meeting, September 20-24, 2010, Entebbe, Uganda, pp: 1013.

Singh, P. and S.S. Narayanan, (1993). Biometrical Techniques in Plant Breeding. Kalyani Publishers, New Delhi, India, pp: 74-84.

Songsri P, Joglloy S, Kesmala T, Vorasoot N, Akkasaeng CPA, Holbrook C (2008). Heritability of drought resistance traits and correlation of drought resistance and agronomic traits in peanut. Crop Sci., 48:2245-2253.

Sujiprihati, S., Saleh, G.B. and Ali, E.S. (2003). Heritability, performance and correlation studies of on single cross hybrids of tropical maize. Asian Journal of plant Science. 2(1), 51 – 57.

Sumathi, P. Nirmalakumari, A. and Mohanraj, K. (2005). Genetic variability and traits interrelationship studies in industrially utilized oil rich CIMMYT lines of maize (Zea mays L). *Madras Agric. J*. 92 (10-12): 612-617.

Teow, C.C., V.D. Truong, R.F. McFeeters, R.L. Thompson, K.V. Pecota and G.C. Yencho, (2007): Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colors. *Food Chem*., 103: 829-838.

Tyagi SD, Khan MH (2008). Study on genetic variability and interrelationship among the different traits in lentil. *Indian J. Crop Sci.,* 12:65-78.

Ukpabi, U.J. (2009). Root and Tubers in Nigeria as Sources of Industrial Raw Materials. In: Nigeria Agro Raw Materials Development: Some Industrial Crops and Salient Issues (PA Onwualu, SC Obasi , UJ Ukpabi (Eds), *RMDRC Publications*, Abuja, 1: 1-19.

Vijayabharathi, A., Anandakumar, C.R and Gnanamalar, R.P.(2009). Analysis of correlations and path effects for popping expansion in popcorn (Zea *mays* var. everta Sturt.). Electronic Journal of Plant Breeding, 1: 60-64.

Vimala, B., Hariprakash, B. (2011): Variability of morphological characters and dry matter content in the hybrid progenies of sweetpotato (*Ipomoea batatas* (L) Lam). Gene Conserve. 10: 65-86.

Wang. X, Chang, J., Qin, G., Zhang, S., Cheng, X. and Li C.(2011). Analysis on yield components of root crops variety Xundan 20 with super high yield potential. *Afr. J. Agric. Res*., 6(24): 5490-5495.

Waqar-Ul-Haq M, Malik F, Rashid M, Munir M and Akram Z (2008). Evaluation and estimation of heritability andgenetic advancement for yield related attributes in wheat lines. Pak. J. Bot., 40(4): 1699-1702.

WHO (World Health Organization). (1995). Global prevalence of vitamin A deficiency. Micronutrient Deficiency Information System, Working Paper #2 (Catalog # WHO/NUT/95.3). WHO, Geneva, Switzerland.

Woolfe, J.A., (1992). Sweet potato: An untapped food resource. Cambridge University Press, Cambridge, UK.

Yildirim, M.B., Çali°kan, C.F., Çaylak, Ö. and Budak, N.(1997). Multivariate relationships in potatoes. *Second Turkish Field Crops Symposium*, 22-25 September. Samsun, Turkey. (In Turkish).

Zeeshan, M.., Aseshad, W., Ali, S., Hussain, M. and Khan, M.I. (2013). Estimation of correlated responses for some polygenic parameters in yellow maize hybrids. International Journal of Advance Research, 1(5), 24-29

RELATIONSHIP AMONG YIELD AND YIELD COMPONENT TRAITS IN ACHA AND ITS POTENTIAL FOR SELECTION AS FOOD AND FORAGE CROP

Ojo G. O. S.[1*], Ayongo M. T[2]., Okwori A. I.[2], Ibrahim N. B.[3]

[1]Department of Crop Production, Federal University of Agriculture, Makurdi, Nigeria

[2]Department of Animal Production, Federal University of Agriculture, Makurdi, Nigeria.

[3]Department of Soil Science, Federal University of Agriculture, Makurdi, Nigeria.

Correspondence: gosojo2001@yahoo.com

Abstract: Field evaluations of acha (*Digitaria exilis* and *Digitaria iburua*) were carried out in 2014, 2015 and 2016 at the Federal University of Agriculture Makurdi, with the objective of identifying and selecting suitable accessions for fodder/forage and grain production in the southern Guinea Savanna agro – ecological zone of Nigeria. Differences in means of the accessions were mostly genetic and not the environment. Genetic coefficient of variability (GCV), phenotypic coefficient of variability (PCV) and genetic advance as a percentage of the mean (GA) were very low (< 15%) for plant height, days to panicle emergence and days to maturity, but low to moderate for number of tillers/plant, biomass yield, straw yield and harvest index (20 – 40%). The GCV (>70%), PCV (107%) and GA (178%) for grain yield were however high. Heritability was generally moderate to high (52% - 78%) for all the traits included in this study. Based on 20% selection intensity, three accessions of *D. iburua,* namely, Nibang, Na'an and Nashelleng should be selected for fodder/forage production, while one accession of *D. exilis*, namely Shen, along with two accessions of *D. iburua* (Nibang and Guzuk) should be selected for grain/seed production.

Keywords: Accessions, GA, GCV, Heritability and PCV

INTRODUCTION

Cereal crops are major sources of energy for human and livestock nutrition globally. Hence wheat, rice and maize are ranked as the first, second and third most important world cereal crops (Obi, 1991; Keskin et al., 2005; Wattoo et al., 2009; Haddadi et al, 2012). Other cereals of global importance include cool-season crops like barley, oat and rye, and warm-season cereals such as millet and sorghum (Ayodele and Mohammed, 2011; Ghatge, 2012). However, some cereals, such as acha (*Digitaria exilis* and *Digitaria iburua*) belong to the neglected crops despite its high nutritional value.

Acha is suggested to be the oldest indigenous African cereal whose cultivation dates back to 7,000 years (Cruz, 2004). Acha production in Nigeria is concentrated in the states within the savanna ecology (Kwon-Ndung *et al*., 1998; Kwon-Ndung *et al*., 2001; Maji *et al*., 2005; Nyam *et al*., 2017), except Benue, Kogi and Kwara.The low rating of Acha may be due to low yield and lack of awareness on its agronomic and climatic requirements for production. There is dearth of information on acha production within the states of Benue, Kogi and Kwara in the southern Guinea Savanna ecology of Nigeria. There is therefore the need to evaluate and select acha accessions for production within this southern Guinea Savanna ecology of Nigeria.

The objective of this research was to evaluate some accessions of acha for fodder/forage and grain production in the southern Guinea Savanna ecology of Nigeria.

MATERIALS AND METHODS

The experiment was conducted at the Teaching and Research Farm of the Federal University of Agriculture, Makurdi (latitude 07° 41'N, longitude 08° 37'E and 97m asl), during the rain fed cropping seasons of 2014, 2015 and 2016. Makurdi is located in the southern Guinea Savanna ecological zone of Nigeria. Soil samples were taken and analyzed at the Department of Soil Science of the Federal University of Agriculture, Makurdi, prior to evaluation for each of the years. Meteorological information for Makurdi and the soil physical and chemical characteristics for the experimental site are summarized in Tables 1 and 2, respectively.

A total of 24 accessions of acha comprising of 7 accessions of *Digitaria exilis* and 17 accessions of *Digitaria iburua* were obtained from National Cereals Research Institute (NCRI) Badeggi, Bida, Nigeria and used for a preliminary evaluation in 2014. Based on the results of 2014 preliminary evaluation, 16 accessions (2 accessions of *D. exilis* and 14 accessions of *D. iburua*) were selected for the 2015 and 2016 evaluations.

The experimental design was a 6x4 lattice design for the 2014 and 4x4 lattice design for the 2015 and 2016 evaluations, with three replications for each of the years. Seeds were mixed with soil at a ratio of 2 parts soil to 1 part seeds and broadcasted into already prepared beds of $1.5m^2$ at the rate of 30kg seeds/ha. Each bed constituted a plot. NPK 15-15-15 compound fertilizer was applied at the rate of 60kgN, $60kgP_2O_5$ and $60kgK_2O$ at two weeks after planting (2WAP) while a supplementary dose of 60kgN was applied as urea at 5WAP. Manual weeding was carried out at 2, 5 and 8 WAP, while the grains and straw were harvested at maturity.

Data was taken on plant height at maturity, number of tillers/plant, days to panicle emergence, days to maturity, biomass yield, straw yield, harvest index and grain yield. Data for one accession of *D. iburua* were deleted from the 2015 and 2016 evaluations prior to analysis due incompleteness.

Data generated were subjected to analysis of variance using GLM and ANOVA procedures of SAS (2009). Mean square values were used to estimate variance components according to Bliss *et al*. (1973).

Variance components were obtained by equating the mean square for a source of variation to its expectation and solving for the unknown as given below:

$\delta^2_e = M_3$

$\delta^2_{ge} = M_2 - M_3/r$

$\delta^2_g = M_1 - M_2/rt$

Where: δ^2_e, δ^2_{ge} and δ^2_g are components of variance for error, genotype by year interaction and genotype, respectively. M1, M2, and M3 are the observed values of the mean squares for the genotype, interaction and error, respectively (Fehr, 1987).

Broad sense heritability (H_{BS} **(%)**) was calculated as the ratio of the genotypic variance to phenotypic variance using the formula of Allard (1960):

$\delta^2_g/\delta^2_{ph} \times 100$

Where H_{BS} = broad sense heritability (%), δ^2_g = genotypic variance, δ^2_{ph} = phenotypic variance.

$\delta^2_{ph} = \delta^2_e + \delta^2_{ge} + \delta^2_g$ as defined by Fehr (1987)**.**

PCV (phenotypic coefficient of variation) and GCV (genotypic coefficient of variation) were calculated from the formula of Allard (1960):

PCV = phenotypic standard deviation/mean;

GCV = genotypic standard deviation/mean

Both GCV and PCV were expressed in percentages. Genetic advance as percentage of the mean was calculated at 20% selection intensity (I=1.40) (Allard, 1960). Genotypic means were separated using LSD while correlation coefficient was carried out using the Pearson SPSS version 17.

RESULTS AND DISCUSSION

Variance components, coefficients of variability, heritability and genetic advance as a percentage of the mean for yield and yield components are summarized in Table 3. Genetic variance (δ^2g), was generally higher than the genotype x environment interaction and the error variance for plant height, number of tillers/plant, days to panicle emergence, days to maturity, biomass yield, straw yield, harvest index and grain yield. GCV, PCV and GA were very low (< 15%) for plant height, days to panicle emergence and days to maturity, but low to moderate for number of tillers/plant, biomass yield, straw yield and harvest index (20 – 40%). However, high values were obtained for GCV (>70%), PCV (107%) and GA (178%) of grain yield. Heritability was generally moderate to high (52% - 78%) for all the traits measured in this study.

Means for yield and yield component traits in the different genotypes are summarized in Table 4. Nashelleng had the highest plant height, which was closely followed by Na,an and Shen while Jakah was significantly ($p<0.05$) lower than other accessions. Nibang had the highest number of tillers/plant, although this was not significantly different ($p>0.05$) from Gotit, Abuth and Na,an. Nibang also had the highest number of days to panicle emergence, which was not significantly different ($p>0.05$) from Ja,alak. Shen had the shortest number of days from planting to panicle emergence (73 days). Sapiya (134 days), had the highest number of days to maturity (134 days), while Dinat had the earliest maturity (105 days) compared to all the other accessions evaluated. Nibang, Na,an and Nashelleng were higher in yield of biomass and straw compared with other accessions. Shen, an accession of *D. exilis* had the highest ($p<0.05$) harvest index and grain yield than all the other accessions.

Varying degrees of correlations were observed among the eight traits studied (Table 5). Plant height was positively correlated with all the yield traits (biomass, straw and grain yield), although the correlation values were not significant ($p>0.05$). Similarly, correlations between days to maturity and all other traits were not significant. Number of tillers/plant was highly significantly, positively ($p<0.01$) correlated with biomass yield (0.75) and straw yield (0.79). Days to panicle emergence was however, negatively

significantly ($p<0.05$) correlated with harvest index and grain yield. The highest positive correlation of (0.97) was observed between biomass and straw yield, which was closely followed by the correlation between harvest index and grain yield (0.92).

The range in the number of tillers (3.80 – 29.20) reported by Umar et al. (2017) is consistent with the range observed in the current work. An accession of *D. exilis* (Nyemat) matured in 113 days in both the present and a previous study (Umar *et al*., 2017). However, most of the accessions in the present work (>86%) vary in maturity time compared with the accessions evaluated by Nyam *et al*. (2017) and Umar *et al*., (2017). The plant height observed in the current work far exceeded the height reported by Nyam *et al*. (2017). The longer days to panicle emergence for most of the accessions (>86 days), and the highest grain yield of 3.69 tha^{-1} recorded in the current work far exceeded the values of 82 – 86 days to panicle emergence and the highest grain yield of 1.48 tha^{-1} reported by Dachi *et al*. (2014). These differences could be attributed to differences in agronomic practice, genotypes and location of the experiment. The accessions used in the previous work (Dachi *et al*., 2014; Nyam *et al*., 2017; Umar *et al*., 2017) were generally different from the ones used in the current work. While little or no fertilizer was applied in the previous study (Dachi *et al*., 2014; Nyam *et al*., 2017; Umar *et al*., 2017), the fertilizer application in the present work may have contributed to the vigorous vegetative growth that culminated in the very high straw and grain yield observed.

The higher value observed for genetic variance is an indication that differences in genotypic means were mostly attributable to differences in accessions and not the environment. The highly significant correlations observed between straw yield and biological yield and between each of these two traits and number of tillers/plant, are indications that the three traits are under the same genetic control and could be simultaneously selected for improvement. The high ranking of Nibang in terms of tillers/plant, straw yield, biological yield and grain yield are indications of its suitability for selection as a dual purpose crop for grain and fodder/forage production, while the high ranking of Na'an in plant height, days to panicle emergence, days to maturity, biological yield and straw yield, suggest its suitability as a fodder/forage crop. Based on 20% selection intensity, three accessions of *D. iburua,* (Nibang, Na'an and Nashelleng) should be selected for fodder/forage production, while one accession of *D. exilis*, namely Shen, along with two accessions of *D. iburua* (Nibang and Guzuk) should be selected for grain/seed production. Further studies on the evaluation of Nibang, Na'an, Nashelleng, Shen and Guzuk accessions in different locations within the states of Benue, Kogi and Kwara in the southern Guinea Savanna ecology of Nigeria is required for location specific recommendation.

Table 1: Meteorological data for Makurdi in 2015 and 2016

Year	Month	Total rainfall (mm)	Av. Max Temp. (°C)	Av. Min Temp. (°C)
2015	January	-	36.4	18.7
	February	-	38.3	25.9
	March	12.6	38.6	26.3
	April	31.4	37.3	26.4
	May	134.7	34.0	25.1
	June	119.4	31.4	24.0
	July	192.7	30.1	23.2
	August	178.1	30.3	23.5
	September	305.6	30.5	23.0
	October	116.8	31.7	23.2
	November	24.0	33.7	23.1
	December	-	34.8	16.1
2016	January	-	34.3	16.5
	February	68.8	35.3	24.4
	March	-	37.0	26.2
	April	78.0	35.3	25.0
	May	142.8	33.0	25.1
	June	60.4	31.1	22.6
	July	87.0	30.7	22.0
	August	217.0	29.5	22.4
	September	305.6	30.3	21.9
	October	293.4	30.8	21.6
	November	-	32.4	21.7
	December	-	31.6	20.4

Source: Meteorological Station, Tactical Air Command, Air Force Base, Makurdi, Nigeria

Table 2: Physical and chemical properties of the experimental sites in 2015 and 2016

Soil characteristics	2015	2016
Sand (%)	76.60	76.58
Silt (%)	13.10	13.10
Clay (%)	10.30	10.32
Textural Class	Sandy Loam	Sandy Loam
PH (H_2O)	6.08	6.08
Organic Matter (%)	1.35	1.38
Organic Carbon (%)	0.75	0.77
Total Nitrogen (%)	0.08	0.09
Bray 1 P (ppm)	60	60
Exchangeable Ca ($cmolkg^{-1}$)	2.11	2.20
Exchangeable K ($cmolkg^{-1}$)	0.32	0.36
Exchangeable Mg ($cmolkg^{-1}$)	0.97	0.95
Exchangeable Na ($cmolkg^{-1}$)	0.27	0.26
CEC ($cmolkg^{-1}$)	3.91	3.93

Table 3: Variance components, coefficients of variation, heritability and genetic advance for yield and yield attributes in acha accessions evaluated in Makurdi, Nigeria

Traits	δ^2_g	δ^2_{ge}	δ^2_e	δ^2_{ph}	GCV (%)	PCV (%)	H_{BS} (%)	GA (%)
Plant height at maturity	189.59	24.93	25.04	239.56	10.81	12.15	79.14	13.46
Number of tillers	24.39	4.92	8.80	38.11	28.48	35.60	64.00	31.90
Days to panicle emergence	88.55	13.41	17.46	119.40	10.33	12.00	74.15	12.45
Days to maturity	161.58	32.04	18.23	211.85	10.82	12.39	76.27	13.23
Biomass yield	7.21	1.47	1.92	10.60	27.43	33.26	68.02	31.66
Straw yield	4.03	1.10	1.39	6.52	23.23	29.55	61.81	25.57
Harvest index	8.03	1.99	3.04	13.06	24.39	31.10	61.49	26.77
Grain yield	0.81	0.39	0.33	1.53	78.26	107.56	52.94	178.68

Table 4. Character means and genotypic ranking (superscript) for yield and yield attributes in acha accessions evaluated in Makurdi, Nigeria

Accession	Plant height at maturity	Number of tillers per plant	Days to panicle emergence	Days to maturity	Biomass yield (t/ha)	Straw yield (t/ha)	Harvest index (%)	Grain yield (t/ha)
Jakah	87.43^{15}	8.80^{12}	96.00^{4}	115.00^{8}	6.75^{13}	5.98^{11}	11.41^{6}	0.77^{7}
Guzuk	132.70^{7}	8.00^{13}	91.07^{8}	110.67^{11}	6.84^{12}	4.61^{15}	32.60^{2}	2.23^{3}
Dinat	122.90^{12}	12.33^{9}	88.67^{10}	105.00^{15}	5.89^{14}	5.57^{13}	5.43^{12}	0.32^{12}
Napiya	129.43^{8}	6.47^{15}	87.10^{11}	107.10^{14}	8.64^{9}	7.95^{9}	7.99^{8}	0.69^{9}
Gotit	124.94^{10}	30.67^{2}	84.00^{13}	107.17^{13}	10.68^{6}	10.08^{5}	5.62^{11}	0.60^{10}
Abuth	128.80^{9}	30.50^{3}	85.00^{12}	125.43^{5}	11.68^{5}	11.34^{4}	2.58^{15}	0.30^{14}
Ja,alak	111.33^{14}	14.67^{7}	101.70^{2}	131.20^{2}	5.34^{15}	5.13^{14}	3.93^{13}	0.21^{15}
Nashelleng	143.97^{1}	16.47^{6}	97.03^{3}	120.00^{6}	12.20^{3}	11.88^{3}	2.62^{14}	0.32^{12}
Dampep	136.33^{5}	11.13^{11}	89.10^{9}	110.77^{10}	9.52^{8}	8.65^{7}	9.14^{7}	0.87^{6}
Munsung	136.33^{5}	14.26^{8}	94.67^{6}	118.33^{7}	7.54^{10}	7.07^{10}	6.23^{9}	0.47^{11}
Nibang	113.10^{13}	31.33^{1}	93.00^{7}	126.53^{4}	17.39^{1}	14.91^{1}	14.26^{5}	2.48^{2}
Na,an	141.50^{2}	29.00^{4}	105.77^{1}	128.00^{3}	12.99^{2}	12.23^{2}	5.85^{10}	0.76^{8}
Sapiya	139.47^{4}	18.51^{5}	84.00^{13}	133.73^{1}	11.87^{4}	9.70^{6}	18.28^{3}	2.17^{4}
Shen*	139.50^{3}	7.57^{14}	73.37^{15}	109.87^{12}	7.34^{11}	5.95^{12}	50.27^{1}	3.69^{1}
Nyemat*	123.00^{11}	12.33^{9}	95.67^{5}	113.33^{9}	9.64^{7}	8.61^{8}	14.83^{4}	1.43^{5}
$LSD_{.05}$	8.21	4.87	6.86	7.01	2.27	1.93	2.86	0.94

* *D. exilis*

Table 5: Phenotypic correlation of genotypic means for yield and yield attributes in acha accessions evaluated in Makurdi, Nigeria

Traits	Plant height at maturity	Number of tillers per plant	Days to panicle emergence	Days to maturity	Biomass yield (t/ha)	Straw yield (t/ha)	Harvest index (%)	Grain yield (t/ha)
Plant height at maturity		0.06	-0.23	0.02	0.21	0.22	0.17	0.16
Number of tillers/plant			0.14	0.49	**0.75****	**0.79****	-0.41	-0.15
Days to panicle emergence				0.41	0.08	0.15	**-0.56***	**-0.52***
Days to maturity					0.45	0.44	-0.23	-0.02
Biomass yield						**0.97****	-0.18	0.17
Straw yield							-0.35	-0.02
Harvest index								**0.92****

ACKNOWLEDGEMENT

The contribution of NCRI in the supply of acha accessions for this research is acknowledged.

REFERENCES

Allard R. W. 1960. Principles of Plant Breeding. 1st Edition. John Willey and Sons Inc., New York. Pp. 485.

Ayodele J. T. and Mohammed S.S. (2011). Zinc speciation in maize and soils. *Research Journal of Chemical Sciences* 1 (4), 98-108.

Bliss F.A., Baker L.N., Franckowiak J. D. and Half T. C. (1973). Genetic and environmental variation of seed yield: Yield components and seed protein quantity and quality cowpea. *Crop Science* 13: 656-660.

Cruz, J. F. (2004). Fonio: A Small Grain with Potential. In: Magazine on LEISA (Low External Input and Sustainable Agriculture). 20; 16-17.

Dachi S.N., Mamza W.S., Okeme O. (2014). Yield and yield components of acha (*Digitaria iburua* (Kipp.) Stapf or Black Fonio accession under dibbling method of planting at different seeding rates. *Biological and Environmental Sciences Journal for the Tropics* 11(3) 237 – 240.

Fehr W. R. (1987). Principle of Cultivars Development. Macmillan publishing company. A division of Macmillan Inc. New York. Pp. 1 – 465.

Ghatge N. S. (2012). Food intake pattern of malnourished preschool children after supplementation of soyaladoo. *International Research Journal of Social Sciences* 1(3): 36 – 40.

Kwon – Ndung E.H., Misari S.M. and Dachi S.N. (1998). Collecting Germplasm of Acha, *Digitaria exilis* (Kipp.) Stapf, Accessions in Nigeria. Plant Genetic Resources Newsletter 116, 30 – 31.

Kwon – Ndung E.H., Misari S.M. and Dachi S.N. (2001). Study on the Production Practices of Acha, *Digitaria exilis* (Kipp.) Stapf, in Nigeria. Science Forum 4: 106 – 113.

Haddadi M.H., Eesmaeilof M., Choukan R. and Rameeh V. (2012). Combining ability analysis of days to silking, plant height, yield components and kernel yield in maize breeding lines. *African Journal of Agricultural Research* 7(36): 5153 – 5159.

Nyam D.D., Kwon – Ndung E.H. and Wutep A.P. (2017). Genetic affinity and breeding potential of phenologic traits of acha (Fonio) in Nigeria. *Journal of Scientific and Engineering Research* 4(10): 91 – 101.

Keskin B., Yilmaz I.H. and Arvas O. (2005). Determination of some yield characters of grain corn in Eastern Anatolia Region of Turkey. *Journal of Agronomy*. 4(1):14-17.

Maji A. T., Dachi S. N. and Yisa J. (2005). Improvement of Acha Accessions at NCRI, Badeggi. NCRI Annual Report. 83-93.

Obi, I. U. (1991). Maize, Its Agronomy, Diseases, Pest and Food Values. Optimal Computer Solutions ltd, Nigeria. 206 pp. Umar E, Bright EO, Ishaq MN (2017). Genetic Diversity Studies in Acha Germplasm. *Nigerian Journal of Genetics* 31: 48 – 53.

Wattoo F. M., Saleem M., Ahsan M., Sajjad M. and Ali W. (2009). Genetic analysis for yield potential and quality traits in maize (*Zea mays* L.). *American-Eurasian Journal of Agricultural and Environmental Sciences* 6(6): 723-729

.

DEDUCTION OF STRIGA- SAND RATIO IN RESISTANCE SCREENING OF CEREAL

Timothy Oluwafemi Ajiboye[1, 2*], Ibrahim Paul[2], and Bamiyo Anjorin[2, 3], S.E Aladele[4], A.A.Olosunde[1, 2], Samson Odutem[5]

[1,2]Formally, International Institute of Tropical Agriculture (IITA), P.M.B 5320, Oyo Road, Ibadan, Nigeria; now, National Centre for Genetic Resources and Biotechnology (NACGRAB), Federal Ministry of Science and Technology, Moor Plantation, P.M.B 5382, Ibadan, Oyo state, Nigeria.
[2]International Institute of Tropical Agriculture (IITA), P.M. B 5320, Oyo road, Ibadan, Nigeria.
[2,3]Formerly, International Institute of Tropical Agriculture (IITA), P.M.B 5320, Oyo Road, Ibadan, Nigeria; Now, Monsanto, 85, Kajola Area, Iwo/Ibadan Road, Ejioku, Ibadan, Oyo State, Nigeria.
[4]National Centre for Genetic Resources and Biotechnology (NACGRAB), Federal Ministry of Science and Technology, Moor Plantation, P.M.B 5382, Ibadan, Oyo state, Nigeria.
[5] C/O Department of Microbiology, University of Ibadan, Ibadan, Nigeria.
E mail: ajiboyefemi2002@yahoo.com

Abstract: Direct infestation with *Striga* requires a carrier such as water or sand due to small size of its seed. However, there is dearth of information on the *Striga*-sand ratio for resistance screening of cereals to *Striga* in developing countries. The aim of this study was to determine the average *Striga*-sand ratio for *Striga* resistance in developing countries using *Striga* populations at Mokwa and Abuja environment, Nigeria. The results showed that the *Striga* seed- sand ratio varies between 1:1 and 1:4 depending on germinable seeds per hole and seed purity.

Keywords: Carrier; Germinability; Resistance; Screening; *Striga hermonthica*; *Striga*-sand ratio.

INTRODUCTION

Cereals especially maize are important staple crops in Africa (Ezekwe 1991; Ngwuta *et al.,* 2013). It was ranked third after rice and wheat in terms of importance in the world (Ibikunle, 2002). Other cereals like sorghum and millet are next to maize in importance as staple crops in Africa. The constraints to cereals production in Africa are stemborers and striga. However Striga is the greatest pest and biological threat to cereals production in Africa (Diallo *et al.,* 2005*;* Omogui*,* 2017). In Nigeria, *Striga* is very important in the North of Rivers Niger and Benue. *Striga* belongs to the family Orobanchaeceae (formerly Scrophulariaceae) (Plant scientist, 2013; Spallek, 2015; Omogui, 2017). Members of this genus are obligate annual hemiparasites; they have chlorophyll, but require a host to complete their life cycle (Musselman, 1987; Yallou et al., 2005). Although over 41 species of Striga have been described, only 5 are presently of economic importance in Africa (Ramaiah *et al.,* 1983). These are (in approximately order of economic importance), *Striga hermonthica* (Del.) Benth., *Striga asiatica* (L.) Kuntze, *Striga gesnerioides*(Willd.) Vatke, *Striga aspera* (willd.) Benth., *Striga forbesii* Benth. All are parasites of sorghum, maize, rice and pearl millet except *Striga gesnerioides* (PASCON, 1997; Parker 1991; Johson *et al.,* 1997) which is a parasite of cowpea and other wild legumes. Yield losses due to striga can be of a few percentages up to complete crop failure, depending on soil fertility, prevailing drought condition, crop species, crop varieties and severity of *Striga* infestation (Dogget 1965; Riches and Parker, 1995; Adetimirin *et al.,* 2000). *Striga* problems are more pronounced among resource limited and constrained subsistence farmers (Kroschel 1999; Ransom 2000). A requirement for *Striga* germination is a signal by a specific chemical stimulant (xenognosins) from the proper host root (Saunders, 1933; Vallace 1950; Worsham, 1987). After germination, a series of chemical signal direct the radicle to the host root where it attaches and penetrates. Seeds of striga respond to stimulants called xenognosins in the root exudate of host plants germination (Chang *et al.,* 1986). Before the parasite

becomes a major sink for crop photosynthate, debilitating crop growth and yield, it will first establish a contact with the host. Germinated *Striga* establish a contact with the host root by an organ called haustorium. It is through the established contact that water, mineral salts and organic compounds are withdrawn. However, if the *Striga* seedling does not attach to a host root within 3-5 days, the seedling perishes. The parasite is still growing under the ground. The symptoms of infection include water-soaked leaf lesions chlorosis, and eventual leaf and plant desiccation and necrosis. Severe stunting and drought like symptoms such as leaf margin curling also indicate *Striga* infection. The plants especially the leaves look burnt. This is why *Striga* is called "Uta-Uta" in Hausa language in Northern Nigeria, which literally means "fire-fire" (Parkinson et al., 1985; Vasudeva Rao, 1985).

The need to constantly breed cereals like maize, sorghum, pearl millet and rice for *Striga* resistance due to new strains of *Striga* seeds, mutation of *Striga* seeds, environmental effect on *Striga* seeds and hybridization of *Striga* seeds cannot be overemphasized. This is also due to fact that the best *Striga* control method for resource-constrain African farmers is the use of resistant varieties. The success in breeding for resistance to striga depends on a high and uniform field infestation with the *Striga* seeds (Ibikunle, 2002). However, it is not possible to count striga seeds needed to infest per holes where the cereals to be screened will be planted due to small size of its seed which is estimated as $5X10^{-6}$ (PASCON 1997; Ajiboye *et al.,* 2015). Therefore, direct infestation with *Striga* such as *Striga hermonthica* requires a carrier. The two carriers are water and sand. The used of sand is preferred because of ease of application, availability and because some other consumables will not be required. A major limitation with the use of sand is how to determine the amount of sand to be used as carrier per quantity of *Striga* seeds. The aim of this study is to determine the *Striga*- sand ratio for resistance screening of cereals for *Striga hermothica*.

MATERIALS AND METHODS

Harvesting of *Striga hermonthica* seeds: Large polythene sheets; transparent polythene bags; paper bags; 250 and 150 micron sieves were all materials used for *Striga hermonthica* collection. Heavily infested fields of sorghum and millets were identified in Mokwa and FCT (Abuja) areas. Mature heads (floral heads) with healthy (not shattered or rotten) capsules were harvested. A mature floral head is one on which all florets have completed flowering with no visible flowers or with only uppermost flowers remaining.

Processing of *Striga hermonthica* seeds: The harvested striga heads were transported in polythene transparent bags to IITA offices at Abuja and Ahmadu Bello University, Mokwa station where the floral heads were spread on polythene sheets in a wind sheltered area for sun drying. The *Striga* was mixed daily to facilitate even and thorough drying. After about a week of sun drying, the floral heads were threshed to force the seeds shed. The *Striga* was sieved with 250 and 150 microns opening sieves. The plant trash was removed. Most of the *Striga* were collected on the 150 microns sieve screen. The seeds were then transported to the laboratory in paper bags. Harvesting and processing of Striga seeds were done for two consecutive years.

Germinability: After about five months (dormant period) after each year collection, germination tests were carried out for each location. This involves preconditioning of the seed and use of synthetic germination stimulants (Strigol analog –GR24). Also seed purity of each location was determined. Germination test was carried out using one of the methods packaged by IITA Striga Research Group for the Pan African Striga Control Network (PASCON). Some quantity of *Striga* seeds were disinfected with 1% Sodium hypochlorite. Paper punch was used to punch out small disks of glass fibre filter paper. Two large pieces of regular filter paper were moistened and placed in a sterile petri dish. The small paper disks were arranged on the moist filter paper inside the sterile petri dish. Two petri dishes were then wrapped in Aluminum foil and put in canister (Aluminium container). The

canister was put in incubator in the laboratory. The incubator was set at about 21°C to 30°C for preconditioning of *Striga* seeds for about 11 days.

Fresh petri dishes were prepared like before. Two large pieces of filter paper, moistened were placed in each sterile petri dish. Small punched out small disks of glass filter papers containing preconditioned *Striga* seeds were then arranged in radial pattern of five rows. There were four (4) small paper disks in each row. Two petri dishes of such were prepared for each population. Few drops of germination stimulant GR24 (Strigol analog) were applied to induce germination of the preconditioned Striga seeds. The petri dishes were then stored again for about 72 hours. Germinability of the two *Striga* populations and for the two consecutive years was tested.

Using light microscope, total number of *Striga* seeds in each disk and the number of seeds that germinated per disk were counted. Number of *Striga* seeds that germinated in each disk was expressed as a percentage of the total number of *Striga* seeds in each disk. Average percentage per row was calculated and later average population was calculated.

Calculation of seed purity: Seed purity was calculated by pouring out a small amount (about 5g) of sieved *Striga* seeds onto a white sheet of paper or dry filter paper. The *Striga* seeds were examined under the light microscope (x 200 magnification). The amount of trash and amount of *Striga* seeds in the sample was visually estimated in percentage. The estimates were recorded in percentage.

Formulae and calculation of amount of *Striga* and sand ratio: If we desire to infest a field for screening cereals with first year Abuja population of *Striga* with 60% germinability and 70% seed purity and at the rate of 2000 germinable seeds.

Step 1. Desired infestation=2000 germinable seeds/holes (scoop)

Step 2. Calculate number of seeds to achieve 2000 germinable seeds based on percentage germinability of 60% = 2000/0.6 percent germinability = 3333.33 seeds/hole.

Step 3. The weight of *Striga* needed since striga seeds are too small to be counted.

$3333.33 \times 5 \times 10^{-6}$ =0.0167gram/hole

Step 4 Adjust the weight needed for the amount of seed purity of 70%

0.0167g/hole/0.7= 0.0238g of seeds/hole.

Step 5 Number of hills to be infested. If the row spacing is 75cm and within row plant spacing is 25cm, then each hill will occupy 0.75m x 0.25m= $0.1875m^2$ /hill.

To infest one (1) hectare of land with *Striga* seeds with above spacing, there will be

$10000m^2/0.1875m^2$= 53333.33 hills

Step 6 Total weight of sand needed to infest this amount of hills at one scoop of *Striga*-sand mixture per hill. 0.94g/scoop x 53333.33scoops =50133.33 g of sand

= 50.13kg of sand.

Step 7. Total weight of *Striga* needed.

0.0238g/scoop x 53333.33 scoops= 1269.33g of seeds.

=12.70kg of *Striga* seeds.

RESULTS AND DISCUSSIONS

The percentage percentage germinability of Abuja population of *Striga* was 60% while the seed purity was 70%. The percentage germinability for Abuja population of *Striga* was 51% in the second year while the seed purity was 70%. The percentage germinability of Mokwa population of *Striga* for the year was 70% while the seed purity was 60%. The percentage germinability *Striga* population for the second year for Mokwa was 68% while the seed purity was 60% (Table 1). However average germinability and average seed purity for the first year were both 65%. The average germinability and average seed purity for the second year were 60% and 65% respectively (Table 2). The average germinability for Abuja location for the two years was 56% for Abuja (FCT) while the seed purity was 60% (Table 3). The average germinability for two locations for the two years was 62.5% while the seed purity was 65% (Table 4).

Amount of *Striga* in Kilograms needed for infestation using the same number of germinable seeds vary with *Striga* populations and also with years due to different germination percentage and also percentage purity. However, the average numbers of germinable seeds needed for infestation are relatively stable. The amount of striga needed to achieve various germinable *Striga* per hole is

essentially the same for both Abuja and Mokwa population of *Striga* for first year. Also for both Abuja and Mokwa population for second year, the amount of *Striga* needed to achieve the same germinable seeds are similar.

Application of the findings in Striga research work

Also from the Table 1, to achieve 2000 germinable seeds, the amount of Striga needed in kilogram to infest an hectare varies from 12.70kg to 14.94kg. To infest with 2500 germinable seeds, the average *Striga* needed varies from 15.87kg to 18.67kg of *Striga* seeds. To infest with 3000 germinable seeds the average varies from 19.05kg to 22.41kg of *Striga* seeds. Also, to infest with 5000 germinable seeds the amount of *Striga* varies from 31.75 to 37.35kg of *Striga* seeds. Also, to infest with 10,000 germinable seeds the amount of *Striga* varies from 63.49kg to 74.70kg of *Striga* seeds.

Based on the calculations, it will be easy to develop software that can give amount of *Striga* needed for infestation for resistance screening and also the amount of sand needed as a carrier. Also a meter reading can be manufactured that can give appropriate amount of *Striga* needed to achieve any germinable seeds and amount of sand to be used as carrier for a given area of land to achieve uniform infestation for resistance screening. Software can also be developed based on above data, so that immediately a researcher get *Striga* seeds to be used for resistance screening, he can go ahead with his/her field screening by infestation.

This is shown by various calculations above. Also, many at times, Breeders and Scientists working with *Striga* varies levels of Striga infestations to determine the Striga population pressure at which cereals resistance will break down.

Table 1: Ratio of *Striga* to sand for different populations of *Striga* for two different years

Year	*Striga* population	Germination	Seed purity	Germinable seed per hole	Amount of Striga in Kg	Amount of sand needed in Kg per Ha	Ratio of *Striga* to sand (in weight)
First Year	Abuja	60%	70%	2000	12.70	50.13	1.4
				2500	15.87	50.13	1.3
				3000	19.05	50.13	1.3
				5000	31.75	50.13	1.2
				10000	63.49	50.13	1.1
First Year	Mokwa	70%	60%	2000	12.70	50.13	1.4
				2500	15.87	50.13	1.3
				3000	19.05	50.13	1.3
				5000	31.75	50.13	1.2
				10000	63.49	50.13	1.1
Second Year	Abuja	51%	70%	2000	14.94	50.13	1.3
				2500	18.67	50.13	1.3
				3000	22.41	50.13	1.2
				5000	37.35	50.13	1.1
				10000	74.70	50.13	1.1
Second Year	Mokwa	68%	60%	2000	13.07	50.13	1.4
				2500	16.34	50.13	1.3
				3000	19.61	50.13	1.3
				5000	32.68	50.13	1.2
				10000	65.36	50.13	1.1

Table 2: Average ratio of *Striga* to sand per year

Year	Germination	Seed purity	Germinable seed per hole	Amount of *Striga* in Kg per hectare	Amount of sand needed per hectare	Approximate ratio of *Striga* to sand
First Year	65%	65%	2000	12.70	50.13	1.4
			2500	15.87	50.13	1.3
			3000	19.05	50.13	1.3
			5000	31.75	50.13	1.2
			10000	63.49	50.13	1.1
Second Year	60%	65%	2000	14.01	50.13	1.4
			2500	17.51	50.13	1.3
			3000	21.01	50.13	1.2
			5000	35.02	50.13	1.1
			10000	70.03	50.13	1.1

Table 3: Average ratio of *Striga* to sand per location

Location	Germination	Seed purity	Germinable seed per hole	Amount of *Striga* in Kg per ha	Amount of sand needed per ha	Approximate ratio of *Striga* to sand
Abuja (FCT)	56%	70%	2000	13.82	50.13	1.4
			2500	17.27	50.13	1.3
			3000	20.73	50.13	1.2
			5000	34.55	50.13	1.2
			10000	69.10	50.13	1.1
Mokwa	69%	60%	2000	12.89	50.13	1.4
			2500	16.12	50.13	1.3
			3000	19.33	50.13	1.3
			5000	32.22	50.13	1.2
			10000	64.43	50.13	1.1

Table 4: Total average ratio of *Striga* to sand for all locations and years combined together

Striga	Germination	Seed purity	Germinable Seed per hole	Amount of *Striga* in Kg per ha	Amount of sand needed per ha	Approximate ratio of *Striga* to sand
All locations	62.5%	65%	2000	13.36	50.13	1.4
			2500	16.68	50.13	1.3
			3000	20.03	50.13	1.3
			5000	33.38	50.13	1.2
			10000	66.67	50.13	1.1

Note: *Assumptions*

(1) Weight of each Striga seed = 5×10^{-6}

(2) Between row spacing and within row spacing of area to be planted is 75cm×25cm

(3) 1 hectare is 10,000m^2

(4) 1 scoop of sand is 0.94g

The application of this findings also agreed with the submission of Adetimirin *et al* (2000) and Ibikunle (2002) who submitted that application of Striga seed to every hill or stand of the crop is a good artificial infestation method for screening cereals. Ibikunle (2002) further stressed that inoculation of cereal seed with *Striga* at planting time produced high *Striga* infestation in all environments and that results from the study will help to optimise *Striga* infestation for resistance screening in cereal that will result in efficient progress for tolerance breeding under uniform artificial *Striga* seed infestation.

CONCLUSION

Though, researchers (Ejeta and Butler 1993; Ibikunle 2002) reported that field evaluation of crop germplasm for Striga resistance in artificially or naturally infested experimental plots is cumbersome, unreliable, confounds various factors and therefore inefficient, field screening is still required for the development of tolerant varieties. However, because of spatial variability of *Striga* seed distribution under natural infestation (Berner *et al.,*1996;Ibikunle 2002) and in *Striga*-sick farms or plots (Efron 1993;Ibikunle 2002), efficient progress for resistance or tolerance breeding can only be made under uniform artificial *Striga* seed infestation. Field infestation technique should be continually improved to allow good and efficient discrimination between resistant and susceptible genotypes (Menkir *et al.,* 1999; Ibikunle 2002).

REFERENCES

Adetimirin, V. O., M. E. Aken'Ova and S.K. Kim. 2000. Effect of *Striga hermonthica on* yield components in maize. Journal of Agriculture science 135, 185-191

Ajiboye, T.O., Paul, I., Olosunde A. A., and B. Anjorin. 2015 .Variation in the germination of *Striga* populations collected in different year- case study of striga populations in Nigeria. Conference proceedings of the 39th annual conference of the Genetics Society of Nigeria.

Berner, D.K., F.O.Ikie and E.I Aigbokan. 1996. Methods for soil infestation with Striga hermonthica seeds. Agronomy Journal 88:33-37

Chang, M., Netzly, D.H., Butler, L.G. and Lynn, D.G. 1986. Chemical regulation of distance:

Characterization of the first host germination stimulant for *Striga asiatica*. American Chemical Society 108:7858-7860

Diallo A. O., Kanampiu F., Mugo S., Groote De H. and Mbogo P. 2005. Herbicide resistant maize: a novel method to control Striga in Africa in Demand-driven technologies for Sustainable Maize Production in West and Central Africa. B. Badu-Apraku, M.A.B Fakorede, A.F.Lum, A.Menkir, and M. Ouedraogo

Doggett, H. 1965. *Striga hermonthica* on sorghum in East Africa. Journal of Agriculture science 65, 183-194.

Efron, Y. 1993. Screening maize for tolerance to *Striga hermonthica*. Plant Breeding 110: 192-200

Ejeta, G. and L. G. Butler. 1993. Host plant resistance to Striga. P 561-569. In:D.R buxton (ed). *Int.Crop Science I*, Crop Science Society of America, Madison.

Ezekwe, G. O. 1991. Agricultural Research Policy on Maize in Nigeria. Federal Ministry of Science and Technology, Lagos. 3rd of April, 1991 pp4-6.

Ibikunle, O. A. 2002. Evaluating intra-row spacing to optimize striga infestation for resistance screening in selected maize genotypes. A dissertation in the Department of Crop, Soil and Pest Management in partial fulfilment of Master of Technology in Crop Soil and Pest Management of the Federal University of Technology, Akure.

Johnson, D. E., C.R. Riches, R. Diallo and M. J. Jones. 1997. Striga on rice in West Africa: crop range host and the potential of host resistance. Crop Protection 16, 153-157

Kim, S. K and Adetimirin V. O. 2000. Conditioning effects of *S. hermonthica* seed on field performance of maize. Crop prot.

Kim, S. K. (ed). 1991. Combating Striga in Africa. Proceedings, International Workshop organized by IITA, ICRISAT, and IDRC, 22-24 august 1988. IITA, Ibadan, Nigeria. 151pp.

Kroschel, J. 1999. Analysis of the *Striga* problem, the first step towards further joint actions. In J. Kroschel, H. Mercer-Quarshie and J. Sauerbon (eds). Advance in parasitic weed control at On-farm level. Vol. 1 Joint Action to Control Striga in Africa. Margrave Verlag, Weikersheim, pp 3-26

Lagoke, S.T.O., V. Parkinson, and R.M. Agunbiade. 1991. Parasitic weed control methods in Africa. P3- 14 In: S.K. Kim (ed). Combating *Striga* in Africa. Proc., Int. Workshop 22-24 Aug., IITA, Ibadan, Nigeria.

Menkir, A., J.G. Kling, and J.M Fajemisi. 1999. Breeding stable high yielding maize hybrids for West and Central Africa In: Badu-Apraku, B., M.A.B Fakorede, M. Ouedrago, and F.M Quin.(eds). Strategy for sustainable maize production in West and Central Africa. Proc.,of Regional Workshop, 21-25 Apr., IITA-Cotonou, Benin Republic.

Musselman, L. J. (ed).1987. Taxonomy of witchweed. Pages 3-12. In parasitic weeds in Agriculture Volume 1; Striga. CRC Press, Inc., Boca Raton, Florida. USA 317pp.

Ngwuta A. A., Harriman J. C, Onyishi G. C., Nzee. O, Orji J.O, Okporie E.O and Chukwu S.C 2013. Performance of Maize genotypes for fresh maize consumption in Owerri west, south eastern Nigeria. Nigeria Journal of genetics Volume 27, 2013.

Omoigui L.O, Kamara A.Y., Yonnele D.M. Ogunkanmi L.A and Timko M.P. 2017. Breeding Cowpea for resistance to *Striga gesnerioides* in the Nigerian dry savannas using marker-assisted selection. Plant Breeding Vol. 136, Issue 3.

Parker, C. 1991. Protection of crop against parasitic weeds. Crop Protection 10, 6-22.

Parkinson, V., Kim, S.K., Efron, Y., Bello, L., and Dashiel, K. 1988. Potential of trap crops as a cultural measure of Striga control in Africa. FAO Bulletin No 96.

PASCON, 1997. Striga Research Methods-A manual prepared by IITA Striga Research Group for the Pan Africa Striga control Network (PASCON). Edited by D.K. Beener, M.D. Winslow, A.E., Awad, K.F. Cardwell, D.R. Mohan Raj, and S.K. Kim. International Institute of Tropical Agriculture, P.M.B 5320, Ibadan, Nigeria.

Ramaiah, K.V., Parker, C., Vasudeva Rao, M.J. and Mussselman, L.J. 1983. Striga identification and control handbook. Information Bulletin No 15. Patancheru, A.P., India: International Crops Research Institute for the Semi-Arid Tropics.

Ransom, J.K. 2000. Long term approach for control of striga in cereals; field management options. Crop Protection 19, 759-763.

Riches, C.R. and C. Parker. 1995. Parasitic plants as weeds. In: M.C. press and J.D. Graves (ed). Parasitic plants. Chapman and Hall, London, pp226-255.

Saunders, A.R. 1933. Studies in phaneogamic parasitism, with particular reference to *Striga lutea* (Lour). South Africa Department of Agriculture, Science Bulletin 128, 1-56.

Spallek, T., Mukutu J. M., and Shirasu K. 2015. The genus striga: a witch profile.

Valance, K. 1950. Studies on the germination of seeds of *striga hermonthica*. I. The influence of moisture treatment, stimulant dilution and after-ripening on germination. Annals of Botany 14:347.

Van Mele, P., Van Damme, P., and Berner, D.K. 1992. A new technique to test germination response of Striga seed using host plant root. Med. Fac. Landbouww, Univ. Gent 57/3b:993-999.

Vasudeva Rao, M.J. 1985. Technique for screening sorghums for resistance to Striga. Information Bulletin No 20. Patancheru, A.P. 502324, India: International Crops Research Institute for Semi-Arid Tropics.

Wilson-Jones, K. 1995. Further experiment on witchweed control. II. The existence of physiological strains of *Striga hermonthica* Empire Journal of Experimental Agriculture. Weikersheim, 23:206-213

Worsham, A.D. 1987. Germination of witchweed seeds. Pages 45-62. In Musselman, L.J. (ed). Parasitic weeds in Agriculture. Vol 1. Striga. CRC Press, Inc., Boca Raton, Florida, USA.

Yallou C. G, Menkir A., Adetimirin V.O and Kling J.G. 2005. Combining ability of diverse maize inbred lines under Striga infestation in Nigeria and Benin in Demand-driven technologies for Sustainable Maize Production in West and Central Africa. B. Badu-Apraku, M.A.B Fakorede, A.F.Lum, A.Menkir, and M. Ouedraogo.

CATALOGUE OF THE CUCURBITACEAE FAMILY IN LAFIA, NASARAWA STATE, NIGERIA

Macauley A. Ittah[1*] and Elizabeth M. Ajibodu [2]

[1] Department of Crop Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, PMB 1115, Calabar, Nigeria.

[2]Department of Botany, Faculty of Science, Federal University, Lafia, Nasarawa State, Nigeria.

[*]Corresponding Author: macittah@unical.edu.ng

Abstract: Collection of plant resources in the family Cucurbitaceae was carried out to study the morphological diversity, prevalence and distribution of the species in Lafia, Nasarawa State, Nigeria. The specimens were collected by simple random sampling in the rainy and dry seasons, in 2015. Eleven cultivated and wild species were found in ten regions of Lafia Local Government Area; the species were Cucumeropsis mannii Naudin, Citrullus lanatus (Thunb) Matsum and Nakai, *Cucumis sativus* L, *Cucurbita pepo* L, *Lagenaria abyssinic*a (Hook. f) Jeffrey, Lagenaria breviflora (Benth) Roberty, *Lagenaria rufa* (Gilg) Jeffrey, *Lagenaria siceraria* (Molina) Standl, *Luffa cylindrica* (L.) M. Roem, *Momordica charantia* (L.) Karela and Telfaria occidentalis Hooke. Most of the species in the study area were cultivated vegetables, such as Cucumeropsis manii, Citrullus lanatus, *Cucumis sativus, Cucurbita pep*o and *Telfairia occidentalis. Cucumis sativus, Lagenaria siceraria, Luffa cylindrica* and *Momordica charantis* were of medicinal importance to the people in the region. Lafia is located in the Guinea savannah ecology in north-central Nigeria, which should have supported more species but only five of the eleven species collected were collected from the wild indicating high human activities in the area. This information is available as index of comparison with future studies that monitor species variation.

Keywords: Cucurbitaceae; Biodiversity; Plant Indexing; Plant Genetic Resources; Species Variation.

INTRODUCTION

The Cucurbitaceae family is an important group of plants because of their aesthetics, nutritional and medicinal values. The group commonly called the cucurbit or gourd family contains pumpkin, melon, cucumber and squash (Stafanova *et al.* 1994). Cucurbitaceae family has wide morphologically varied characteristics; there are about 120 genera and more than 800 species distributed mainly in tropical and subtropical agro – ecologies of the world (Jeffery, 1990), a few species are adapted to the arid ecology (Arua *et al.,* 2010). Most parts of the plants are useful, for example, the fruits are very useful in human health, in blood purification, remediation of constipation, beneficial for digestion and as energy booster (Shrivastava and Roy, 2013).

Natural selection has played significant role in the evolution of crop species, while, humans have applied artificial selection pressure to exercise complimentary effects on the expression of fruit size and shape, crop maturity, adaptation to poor soil fertility and resistance to pests including pathogens. Crop improvement by selection of genetically diverse species is a key to reliable and sustainable production of the food crops. IUCN (2004) and FAO (2005) reported that there is increase in biodiversity erosion in the plants kingdom due to natural and human activities; activities such as deforestation engendered by fire and other natural phenomena, farming, unregulated lumbering, rapid population growth with concomitant influence of urbanization are implicated in biodiversity erosion, therefore, there is need to document existing plant types for references and comparison with future studies.

In other continents, several authors, such as Diez *et al*. (2002) and Singh and Sarkar (2014) had carried out extensive research on the Cucurbitaceae family, whereas, in Africa, most species are used as food and for their medicinal properties, as such, species in the family Cucurbitaceae have not benefited from the level of research interest showered on other crop species, such as wheat. Information on the identification and classification of the Nigerian Cucurbitaceae are not widely available in the published writings despite the extensive divergence of the Nigerian genotypes, emphasis should

consequently be focused on filling the knowledge gap, especially, on the genetic diversity of this family. The objective of this study was to evaluate the diversity, distribution and utilisation of species in the family Cucurbitaceae in Lafia, Nasarawa State, Nigeria. The results obtained here are preliminary work to establish a catalogue for this important, but neglected family.

MATERIALS AND METHODS

The survey was carried out in Lafia Local Government Area (LGA), Nasarawa State, Nigeria (Figure 1), Lafia (8º29'N, 8º31'E) is the capital city of Nasarawa State; located in North – central Nigeria; within the Guinea Savannah ecology, it has tropical climate with rainfall of 1311.75cm per annum. The landscape is made up of plain lands and hills measuring up to 300 ft above the sea level (Wikipedia, 2016).

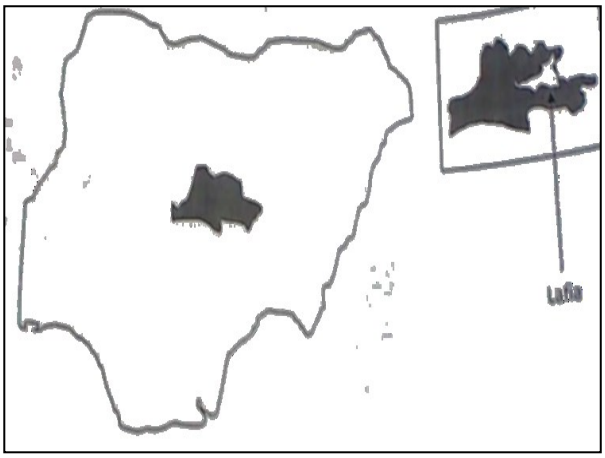


Figure 1. Sketched Map of Nigeria showing Nasarawa State. Insert Sketched Map of Nasarawa State showing Lafia Local Government Area (white).

Specimen collection and morphological evaluation: Plant specimens in the family Cucurbitaceae were collected in Lafia Local Government Area using random sampling method; walking across the town, in fields and along the banks of water - bodies. Morphological characterization was identified according to the descriptor standard of Agro - Botanical Institute (ABI), Tapiószele, Hungary. Utility of the species was conducted by interviews.

RESULTS AND DISCUSSION

The stature and other descriptive traits of species in the family Cucurbitaceae in Lafia, Nasarawa State, Nigeria is presented in Table 1. Of the seven genera in the Cucurbitaceae family, *Lagenaria* had four species, *Cucumeropsis*, *Citrullus*, *Cucumis*, *Cucurbita*, *Luffa*, *Momordica* and *Telfaria* had one species each in the Lafia LGA. The description by Agro – biological feature indicates that of the 11 species, five were cultivated, four were wild species and two were both cultivated and found in the wild. Five species were prostrate, four were trailing and two were climbers.

Citrullus lanatus (Thunb) Matsum and Nakai

Citrullus lanatus fruit is shown in Plate 1a and the seeds in Plate 1b. The common name of *C. lanatus* is watermelon. The fruit colour is generally green but some varieties are mottled or variegated. The number of chromosomes is 2n = 22 (Shrivastava and Roy, 2013). Watermelon is consumed worldwide; it contains 85 - 90 % water and 6 - 10 % fibre; it is a natural diuretic drug to cleanse the kidney and bladder. It is used by women who are exsiccated during menstruation or pregnancy to restore fluid. Fruit has immense health benefits; it amends asthma conditions and cholesterol level, improves digestion, relives symptoms of arthritis, strengthens immunity and heals wounds and reduces skin blemishes. Seeds contain protein, magnesium, calcium and potassium.

Cucumeropsis mannii (L.) Naudin.

The fruit and seeds of *Cucumeropsis mannii* are shown in Plate 2a and 2b respectively. The fruits are greenish and the seed coat is light yellow or gold. *Cucumeropsis mannii* has synonyms such as *Citrullus colocynthis* (L) Shrad. and has many common names, such as colocynth, bitter apple and bitter cucumber. It is also called 'egusi' in West Africa. Soup thickened with ground seed of 'egusi' is choice soup to eat in southern, eastern and western Nigeria. The major ingredients in the seeds are fats and protein. Ogbonna (2013) identified the species as *Colocynthis citrullus* L.

Cucumis sativus L

The fruit and seeds of *Cucumis sativus* are shown in Plate 3a and 3b respectively. The fruits are green and greenish yellow, while the seeds are light yellow. The common name of *C. sativus* is cucumber, the

chromosome number is 2n = 14 (Shrivastava and Roy, 2013). Cucumber promotes good health; it is useful to the skin; cure for sunstrokes, sunburn, swelling under the eye and to softens the skin texture. It remedies eczema. Fruit helps in weight loss (Shrivastava and Roy, 2013).

***Cucurbita pepo* L.**

Cucurbita pepo fruit is presented in Plate 4a and the seeds in Plate 4b. Common names are winter squash and pumpkin, with chromosome number 2n = 40 (Shrivastava and Roy, 2013). The unripe fruits are green but the ripened fruits are orange or yellow. Most parts of pumpkin have medicinal values, for example, the fruits moisten the skin, clear blemishes and pimples. The seeds are used to cure pinworms, tapeworms and other intestinal worms, whereas the leaves reduce fever

***Lagenaria abyssinica* (Hook. f) Jeffrey**

The fruit of *Lagenaria abyssinica* is shown in Plate 5a and the seeds in Plate 5b. The matured fruit is tan while the seeds are light yellow and brown. This species is a climber, the fruits are carved out and inside scraped to make containers and cup. Specimens were collected from the wild.

***Lagenaria breviflora* (Benth) Roberty**

The fruit of *Lagenaria breviflora* is green mottled with white spots (Plate 6a) and the leaves are leathery, the seeds are light yellow or cream (Plate 6b). *L. breviflora* has anti – ulcer and anti – oxidant properties (Onasawo *et al.*, 2011). Specimens were collected from the wild.

***Lagenaria rufa* (Gilg) Jeffrey**

The fruit colour ranges from green to tan and dark brown (Plate 7a) and the seeds were tan and brown (Plate 7b). This plant is of West African origin and not widely studied.

Lagenaria siceraria (Molina) Standl

Lagenaria siceraria fruit and seeds are presented in Plate 8a and 8b respectively, it has the common name, bottle gourd; it has the chromosome Number (2n) = 22 (Shrivastava and Roy, 2013). The fruit and leaves have medicinal values; fruit juice is used in the treatment of urinary tract infections, constipation, indigestion and ulcers, and for the cure of diabetes. Bottle gourd contains 96 per cent water and low in calories, suitable for weight-loss and the leaves prevent tooth decay (Shrivastava and Roy, 2013).

***Luffa cylindrica* (L.) M. Roem**

Luffa cylindrica is probably synonymous with *L. aegyptiaca* (Mill) described by Okoli (1984). The fruit is shown in plate 9a and the seeds in 9b; fruit was green and the seeds black. The common name is sponge gourd and the chromosome number is (2n) = 26 (Shrivastava and Roy, 2013). The seeds, fruit and stem are used to induce nausea and vomiting, and in the treatment of asthma, sinusitis and fever. The stem juice extracted is used in the treatment of respiratory disorder.

***Momordica charantia* (L.) Karela**

The fruit and seeds of *Momordica charantia* are shown in Plate 10a and 10b respectively; the fruits were yellow, orange or red, while seeds were brown. The common name of *M. charantia* is bitter gourd, it has chromosome number 2n = 22 (Shrivastava and Roy, 2013). Fruit juice cures diabetes, by soothing pancreatic cells and increasing secretion of insulin thereby promote the tolerance to sugar. The ripe fruit squashed in olive oil heals wound, burns, itching skin and leprosy (Shrivastava and Roy, 2013). Seeds help to expel intestinal parasitic worms. Extract of leaf juice has antiviral and antibiotic properties. Salaam *et al*. (2011) cautioned against usage of this species as food and as oral medication due to its cytotoxic and genotoxic properties.

***Telfaria occidentalis* Hook. F**

Fruit and seeds of *Telfairia occidentalis* are shown in Plate 11a and 11b respectively. Fruit colour was cream, the seeds were brown. The common names are fluted pumpkin, fluted gourd, "ubong" by Efik and Ibibio people and "*ugwu*" in Igbo land, all in southern Nigeria. The number of chromosomes in *T. occidentalis* is 2n = 22 but polyploids have been reported (Uguru and Onovo, 2011). It is a leafy vegetable with edible seeds grown in West Africa but indigenous to southern Nigeria. The seeds contain fat and protein, leaves contain vitamins and minerals; iron, potassium, tannins and so on. The leaves also contain high amount of antioxidants with hepato –

protective and antimicrobial properties (Aremu and Adewale, 2012).

Distribution of Cucurbitaceae in Lafia, Nasarawa State, Nigeria

The distribution of the species in the family Cucurbitaceae in Lafia, Nasarawa State, Nigeria is presented in Table 2. *Citrullus lanatus*, *Cucumis pepo*, *Luffa cylindrica* and *Telfairia occidentalis* were collected in Lafia North but *L. cylindrica* was more commonly found than the other species. *Cucurbita pepo*, *Luffa cylindrica, Momordica charantia* and *T. occidentalis* were found in Lafia East. *Citrullua lanatus*, *Lagenaria breviflora*, *L. Siceraria*, *Luffa cylindrica*, *Momordica charantia* and *T. occidentalis* were found in Mararaba Akunza. Eight species; *Citrullus lanatus, Cucumeropsis mannii, Cucumis sativus, Lagenaria abyssinica, L. breviflora*, *L. siceraria*, *Luffa cylindrica*, and *T. occidentalis* were collected from the Gandu area. The reasons so many species were found in Gandu are that the area is sparsely developed and has more smallholder farmers. In Wankwa, only two species were found; *Luffa cylindrica and T. occidentalis*. Buka sidi provided five species to the specimen collection; *Cucumeropsis mannii, Lagenaria abyssinica*, *L. Rufa*, *Luffa cylindrica* and *M. charantia*. In Akuruba Osanyan, *Citrullus lanatus*, *L. siceraria*, *Luffa cylindrica* and *T. occidentalis* were found. Buka Kuto had the least number of species collection, only *Luffa cylindrica* was found in the area. *Cucurbita pepo, Lagenaria abyssinica*, *Luffa cylindrica* and *T. occidentalis* were found in Agodu, while in Angwa Madan; *Citrullus lanatus*, *Luffa cylindrica* and *T. occidentalis* were collected.

Luffa cylindrica was found in all the ten areas of Lafia, followed by *T. occidentalis* in eight areas. Lafia is the capital city of Nasarawa State. It is a metropolis and built up, so there is heavy vegetation erosion to accommodate municipal facilities, such as estates, schools, stadium, mosques and churches. There is further natural vegetation destruction to make farms and secondary vegetation.

SUMMARY AND CONCLUSION

The diversity and distribution of plants species in the family Cucurbitaceae were studied in Lafia Local Government Area in Nasarawa State, Nigeria. This family has many valuable species in respect of their nutritional, aesthetics and medicinal properties. Okoli (1984) reported 21 species in the Cucurbitaceae family in Nigeria in an earlier study. The distribution and diversity of this useful species have not been studied in this locality. Eleven species were found dispersed in ten locations of Lafia, these were four species of *Lagenaria* (*L. abyssinaca*, *L. breviflora*, *L. rufa*, and *L. siceraria*), one species of *Cucumis sativa*, *Cucumeropsis mannii*, *Citrullus lanatus*, *Cucurbita pepo*, *Luffa cylindrica*, *Momordica charantia* and *Telfairia occidentalis*. Arua *et al*. (2010) observed that diversity in plants is strongly associated with the agro – ecology and human activities in the environment where the species is found. Lafia is a metropolis, heavily degraded of its vegetation to build businesses, industrial and housing estates, schools, offices and other facilities. The natural environment is also eroded to make farms and plant plantations. This situation is responsible for the very few species collection in the study area. This information is now available for comparison, should similar work be carried out in this environment.

Table 1. Stature, habits and attributes of plants in the family Cucurbitaceae in Lafia, Nasarawa State, Nigeria

	Plants Species	Agro-biology	Stem stature	Plant habit	Leaf shape	Leaf type	Tendril	Seed shape	Seed attributes	Fruit shape
1.	*Cucumeropsis mannii* Naudin.	Cultivated	Trailing	Trailing Herb	Palmate lobed	Compound	Present	Elliptic/ flattened	Multiple, small	Round smooth
2.	*Citrullus lanatus* (Thunb) Matsum and Nakai.	Cultivated	Prostrate	creeper	Palmate	Compound	Present	Oval	Multiple, small	Globose, smooth
3.	*Cucumis sativus* L	Cultivated	Prostrate	Climbers	Palmate lobed	Simple	Present	Emarginated / elliptical	Multiple, small	Cylindrical/ smooth
4.	*Cucurbita pepo* L	Cultivated	Prostrate	Creeping Herb	Palmate lobed	Simple	Present	Elliptic/ flattened	Multiple, small	Oval
5.	*Lagenaria abyssinica* (Hook. f) Jeffrey	Wild	Trailing	Trailing Herb	Palmate	Simple	Present	Elliptic/ flattened	Multiple, small	Spherical
6.	*Lagenaria breviflora* (Benth) Roberty	Wild	Climbing	Trailing Herb	palmate	Compound	Present	Elliptic/ flattened	Multiple, small	Spherical
7.	*Lagenaria rufa* (Gilg) Jeffrey	Wild	Climbing	Trailing Herb	Palmate lobed	Simple	Present	Elliptic/ flattened	Multiple, small	Spherical
8.	*Lagenaria siceraria* (Molina) Standl	Wild/Cultivated	Prostrate	Climbing Herb	palmate	Simple	Present	Flat facial ridges	Multiply, small	Flask shape or globose
9.	*Luffa cylindrica* (L.) M. Roem	Wild	Climbing	Trailing Herb	Palmate lobed	Simple	Present	Elliptical	Multiple, small	Cylindrical, smooth
10.	*Momordica charantia* (L.) Karela	Wild/Cultivated	Climbing	Herb	Palmate lobed	Simple	Present	Flat/ pith	Multiple, small	Oblong
11.	*Telfaria occidentalis* Hooke	Cultivated	Prostrate	Herb	Palmate	Simple	Present	Oval	Multiple, Large	Cylindrical, ridged

Table 2. Distribution and prevalence of the family Cucurbitaceae in different areas of Lafia L.G.A., Nasarawa State, Nigeria.

s/n	Plants Species	LN	LE	MA	G	W	BS	AO	BK	A	AM
1.	*Cucumeropsis mannii* (L) Naudin.	0	0	0	++	0	+++	0	0	0	0
2.	*Citrullus lanatus* (Thunb) Matsum and Nakai	+	0	+	++	0	0	+	0	0	+
3.	*Cucumis sativus* L	0	0	0	++	0	0	0	0	0	0
4.	*Cucurbita pepo* L	++	+	0	0	0	0	0	0	++	0
5.	*Lagenaria abyssinica* (Hook. f) Jeffrey	0	0	0	+	0	++	0	0	++	0
6.	*Lagenaria breviflora* (Benth) Roberty	+	0	+	++	0	0	0	0	0	0
7.	*Lagenaria rufa* (Gilg) Jeffrey	0	0	0	0	0	++	0	0	0	0
8.	*Lagenaria siceraria* (Molina) Standl	0	0	+	+	0	0	+	0	0	0
9.	*Luffa cylindrica* (L.) M. Roem	+++	++	+++	+++	+	++	+	++	+	+
10.	*Momordica charantia* (L.) Karela	0	+	++	0	0	++	0	0	0	0
11.	*Telfaria occidentalis*	+	+	++	++	+	0	++	0	+	+

Key: + = species present; 0 = species absent; LN = Lafia North; LE = Lafia East; MA = Mararaba Akunza, G = Gandu, W = Wankwa, BS = Bukan sidi, AO= Akuruba Osanyan, BK= Buka Kuto, A= Agodu, AM= Angwan Mada

Plate 1a. *Citrullus lanatus*

Plate 2a. *Cucumeropsis mannii*

Plate 3a. *Cucumis sativus*

Plate 4a. *Cucurbita pepo*

Plate 5a. *Lagenaria abyssinica*

Plate 6a. *Lagenaria breviflora*

Plate 7a. *Lagenaria rufa*

Plate 8a. *Lagenaria siceraria*

Plate 9a *Luffa cylindrica*

Plate 10a. *Momordica charantia*

Plate 11a *Telfairia occidentalis* Hooke

Plate 1. Fruit characteristics of the Cucurbitaceae family in Lafia, Nasarawa State, Nigeria

Plate 1b. *Citrullua lanatus*

Plate 2b. *Cucumeropsis mannii*

Plate 3b *Cucumis sativus*

Plate 4b *Cucurbita pepo*

Plate 5b *Lagenaria abyssinica*

Plate 6b *Lagenaria breviflora*

Plate 7b *Lagenaria rufa*

Plate 8b *Lagenaria siceraria*

Plate 9b *Luffa cynlindrica*

Plate 10b *Momordica charantia*

Plate 11b *Telfaria occidentalis* Hooke

Plate 2. Seed characteristics of the species in the Cucurbitaceae family in Lafia,Nasarawa State, Nigeria

REFERENCES

Aremu, C. O. and Adewale, D. B. (2012) Origin and seed position effect on sex ratio of T*elfairia occidentalis* Hook. F. grown in savanna agro – ecology. *Int. J. plant Breeding and Genetics* 6: 32 – 39.

Arua, C. B., Uguru, M. I. and Oyiga, B. C. (2010) Variations among some Nigerian *Cucurbita* landraces. *African Journal of Plant Science* 4: 374 – 386.

Diez, M. J.; Pico, B. and Nuez, F. (2002). Cucurbit genetic resources in Europe. *Ad hoc* meeting 19 January 2002. Adana, Turkey. International Plant Genetic Resources Institute, Rome, Italy. 64pp.

FAO (2005). State of the world's forests, 2005. FAO (Food and Agriculture Organization), Rome, Italy.

IUCN (2004). Red list of threatened species. IUCN (International Union for Conservation of Nature and Natural Resources), Gland, Switzerland.

Jeffery, D. (1990). Appendix: An Outline classification of Cucurbitaceae. In: Biology and Utilization of the Cucurbitaceae. Bates DM, Robinson RW, Jeffery C. (eds). Ithaca and London: Cornell University, pp. 449-463, 485

Ogbonna, P. E. (2013). Floral habits and seed production characteristics in "Egusi" melon (*Colocynthis citrullus* L). *J of Plant Breeding and Crop Science* 5: 137 – 140

Okoli, B.E. (1984) Wild and cultivated cucurbits in Nigeria. *Economic Botany* 38: 350 – 357

Onasawo, S. A., Singh, N., Saba, A.B., Oyegbemi, A. A., Oridupa, O. A. and Palit G (2011) Anti- ulcergenic and *In vitro* antioxidant activities of *Lagenaria breviflora* (LB) whole fruit ethanolic extractin laboratory animals. *Pharmacognosy Res.* 3: 2 – 8

Salaam, T.O.; Adewumi, O. O.; Taiwo, I. A. and Odeigah, P. G. C. (2016) Cytotoxic and genotoxic effects of aqueous extract of *Momordica charantia* L(Cucurbitaeae)leaves on *Allium cepa* roots. *Nigeria Journal of Genetics* 30: 13 – 22

Shrivastava, A. and Roy, S. (2013) Cucurbitaceae: An enthomedicinally important family. *J. Medicinal Plant Studies* 1: 16 – 60

Singh, D. K. and Sarkar, M (2014) Biodiversity of Cucurbitaceous vegetable crops in India pp 64 – 68. In: Cucurbitaceae 2014, M. Havey, Y. Weng, B. Day and R. Grumet (eds). Proceedings of the American Society for Horticultural Science, Alexandria, VA, USA

Stafanova, L., Neykov, S. and Todorova T. (1994) Genetic diversity in the Cucurbit family. *Plant Genetic Resources Newsletter* 99: 3 – 4

Uguru, M. I. and Onovo, J. C. (2011) Evidence of polyploidy in fluted pumpkin (*Telfairia occidentalis* Hook F). *African J. Pl Sci.* 5: 287 – 290.

WIKIPEDIA (2016) Lafia. Wikipedia, the free encyclopedia https//en.m.wikipedia.org/wiki/lafia.

ANALYSIS OF ROSETTE RESISTANCE IN GROUNDNUT (*Arachis hypogaea* L.) CROSSES BY REVERSED TRANSCRIPTASE POLYMERASE REACTION

Usman A[1], Offei, S. K[2], Danquah E[2]. Ofori[2], Mohammed M. S[1], Yahaya M. A.[1], and M. Usman[1]

[1] Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria
[2] West Africa Center for Crop Improvement, School of Agriculture and consumer science, Department of crop Science, University of Ghana, Legon
Corresponding Author Email: almuh2013@yahoo.com; alhassanusman@abu.edu.ng

Abstract: Groundnut Rosette Disease (GRD) caused by a complex of groundnut rosette assistor virus (GRAV), groundnut rosette virus (GRV), and the satellite RNA (sat RNA) of GRV is one of the major factors limiting the production of groundnut (*Arachis hypogaea* L*)* in sub-Saharan Africa. A vector aphid, even though acquires GRAV, GRV, and sat RNA, does not always transmit the three agents together into the inoculated plant, resulting in separation of groundnut rosette disease agents in time and spaceThe objective of this study was to demonstrate the potential of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for the confirmation of field resistance of groundnut genotypes to resette disease. Sixteen groundnut genotypes with field resistance to GRD developed at Institute for Agricultural Research, Samaru Nigeria were tested for the presence of any of the three agents of GRD using one step RT-PCR. Molecular diagnosis showed that none of the genotypes was resistance to all the three components based on RT – PCR assay. Genotypes such as ICGX-SM-000/20/5/P_4/P_1, ICIAR -19-BT and ICGV 07899, which showed negative response to GRAV, could be exploited in breeding programmes to restrict the spread of groundnut rosette disease. The GRV resistant genotypes (ICG-IS-07899 X SAMNUT14 and ICIAR -19-BT X MANIPENTA) could be used as resistant varieties to GRV under commercial production. Pyramiding the two genotypes into a single genotype is suggested to achieve broad-based genetic resistance against groundnut rosette.

Key words: GRAV GRD, Groundnut, sat-RNA, RT-PCR

INTRODUCTION

Groundnut rosette disease is the most destructive viral disease of groundnut in Africa resulting in serious yield losses. Improvement of host plant resistance to this disease provides the most effective control strategy (Olorunju *et al.,* 2001; Herselman *et al.,* 2004). The International Crop Research Institute for Semi-Arid Tropics ICRISAT) and its partners have made significant contributions towards the understanding of the epidemiology of rosette disease. These efforts have contributed to the development of several groundnut genotypes with acceptable levels of field resistance (Subrahmanyam *et al.,* 1998; Olorunju *et al.,* 2001). In an earlier breeding for GRD, rosette resistance was assessed by lack of symptom expression and therefore was largely due to groundnut rosette virus RNA (GRV) and sat RNA resistance (Bock *et al.,* 1990; Subrahmanyam *et al.,* 1998; Olorunju *et al.,* 2001). Although such groundnut rosette disease-resistant materials did not develop symptoms in the field, yield reduction in such plants were observed under artificial and natural disease conditions, presumably due to their susceptibility to GRAV (Subrahmanyam *et al.,* 1998; Olorunju *et al.,* 2001).

Efficient and accurate diagnosis is a key to mitigating the consequences associated with the aphid transmission of viruses in groundnut. Several methods have been used for diagnosing rosette disease in groundnut plants. GRAV were detected in plants and aphids by the triple-antibody sandwich form of enzyme linked immunosorbent assay (TAS-ELISA) using monoclonal antibodies (MAbs) raised on potato leaf-roll virus (Naidu *et al.,* 1998; Rajeshwari *et al.,* 1987; Scott *et al.,* 1996), and GRV and sat RNA were detected in groundnut plants by nucleic acid hybridization (Blok *et al.,* 1995). Due to

their cross reactions with different luteoviruses, a panel of MAbs have to be used in TAS-ELISA to verify that a luteovirus detected in groundnut or in aphids is indeed GRAV (Scott *et al.,* 1996). Moreover, TAS-ELISA cannot provide information on whether the aphids carry particles containing GRAV-RNA and or GRV-RNA and sat-RNA. Additionally, low concentrations of the rosette disease agents in host plant make it essential to develop a reliable and sensitive method for their detection. Studies have shown that viruses can be detected in individual plant and aphid using reverse transcriptase polymerase chain reaction (RT-PCR) (Canning *et al.,* 1996; Singh *et al.,* 1996; Olmos *et al.,* 1997; Stevens *et al.,* 1997, Naidu *et al.,* 1998). The use of RT – PCR could ensure that field resistance genotypes are free from any of GRAV, GRV or sat-RNA viruses. GRD field resistance may be symptomless (Latent) and presence of any of the agents of GRD in the field resistant sources could prevent its use as new sources of planting material in groundnut breeding program. The rapid and accurate confirmation of the new sources of resistance is essential prerequisite for the development of GRD resistant variety. The objective of this study was to confirm the resistance status of promising field GRD-resistant groundnut genotypes by RT – PCR.

Sources of resistance has been previously detected by ELISA. However, as a phloem limited virus, it is present in low concentration and required highly sensitive method for its detection. Nucleic-acid based detection methods are recognized as being very powerful, rapid, specific and highly sensitive techniques for plant virus detection (James *et al.,* 2006; Boonham *et al.,* 2008; Olmos *et al.,* 2008; Vincelli and Tisserat 2008). Use of RT-PCR in plant virus detection was well established, although studies on detection of *Potyviridae* for virus disease of groundnut are rare. Even the few studies that have investigated the detection of *Potyviridae* in plants with low luteovirus titre have relied on the use of species-specific-primers (Naidu *et al.,* 1998; Gibbs *et al.,* 2003). RT-PCR detection of GRAV, GRV and sat-RNA was possible in all plant samples included in this study. The approach of RT-PCR used in this study has facilitated the confirmation of resistance status of identified field GRD resistant groundnut genotypes and subsequent identification of different agents of GRD viruses. The molecular approach employed in this study, whilst not new to modern plant virus disease diagnostics, is unique to the investigation of GRD viruses associated with groundnut germplasm. As such, the findings and experimental methods will be substantially informative, from a phytosanitary perspective, to repositories around the groundnut regional bank which collect, maintain and distribute groundnut germplasm.

MATERIALS AND METHODS

Collection of plant samples

Two seeds of 14 GRD field resistant groundnut genotypes with appreciable sound kernel yield, along with the seeds of susceptible checks (MANIPENTA and KWANKWASO) were planted in the greenhouse during 2012 growing season. Leaf samples were collected randomly from the seedlings when they were two weeks old these were labelled separately at the Institute for Agricultural Research Biotechnology Laboratory, Samaru, ($11^0$10.00″N and $7^0$38.00″ E, 693 m), Ahmadu Bello University Zaria, Nigeria

RNA Extraction and Purification

Extraction was carried out using GeneJET Plant RNA Purification Mini kit #K0801# (Thermo Scientific). About 200 mg of 2 weeks old young leaves obtained from each sample were ground into fine powder under liquid nitrogen with mortar and pestle. It was quickly transferred into RNase, DNase free 1.5ml microcentrifuge tubes containing 500ul of plant RNA Lysis solution and vortexed for 20s, incubated for 3 min at 56^0C and centrifuged for 5 min at 14,000 rpm. The supernatant (550 µl) was transferred into a clean microcentrifuge tube and mixed with 250 µl of 96 % ethanol. The prepared mixture was then transferred into a purification column inserted in a collection tube, the column was centrifuge for 1min at 11,000 rpm, and the flow through solution was discarded while the column and the collection tube was reassembled. 700 µl Wash buffer (WB1) was added to the column and centrifuged for 1min at 11,000 rpm, the flow through and collection tube was discarded and the purification column was placed into a clean

2ml collection tube. 500µl Wash Buffer (WB2) was also added to the purification column and centrifuged for 1min at 11,000 rpm, the flow through was discarded while the column and collection tube was reassembled. Washing with WB2 was repeated but with centrifugation at 14,00rpm. The flow through in a collection tube was discarded. The purification column was inserted into RNase free 1.5ml tube. The RNA was eluted by adding 50µl nucleases free water to the center of purification column and centrifuged for 1min 11,000rpm. This was repeated once to a total of two washes. The purification column was discarded and the RNA was store at -20^{0}C for further downstream analysis.

Complementary DNA (cDNA) Synthesis and Polymerase Chain Reaction

Primers for specific amplification of nucleic acid sequences for each of the three agents of rosette disease were presented in Table 1. RT-PCR reactions were set up separately for GRAV, GRV and sat RNA. cDNA was obtained by using M-MuLV Reverse Transcriptase #EP0352# (ThermoScietific). Total RNA as a template used in RT-PCR reaction was set up separately for GRAV, GRV and Sat-RNA in two different stage protocols: First Strand cDNA synthesis and PCR Reaction. 5 µl RNA template was added into sterile nuclease free tube on Arctic Ice PCR PL* Temp Sensitive. It was followed by adding 20pmol primer (Forward and Revered), the volume was made up to 11.5 µl DEPC-treated water. Subsequently, 4 µl 5X-reaction buffer, 0.5 µl nuclease free water, 2 µl (1mM final concentration) dNTP mix 25mM each and 2ul (40 µl) M-MuLV Reverse Transcriptase were added to a total volume 20ul. It was mixed gently and briefly centrifuged. The reaction was incubated at 37^{0}C for 1hr and terminated by heating at 70^{0}C for 10min.

First Strand cDNA was amplified in PCR machine (PTC 200 MJ Research), using gene specific primers. The PCR reaction mixture 50 µl consists of: 2 µl cDNA, 10 pmol Primer Forward and Reversed each, 25 µl DreamTaq Green PCR Master Mix 2X and 21 µl nuclease free water. (ThermoScientific). PCR amplification was set to run 30 cycles of 90^{0}C for 45s, 55^{0}C for 1min, 72^{0}C for 1min followed by extension cycle of 72^{0}C for 1hr and terminated at 4^{0}C. The PCR product was run on 2% Agarose gel in 0.5X TBE buffer, stained with Ethidium bromide, which was later visualized under UV transilluminator, and the images were captured with the aid of digital camera.

RESULTS

Table 1. Primers used in amplification of various regions of causal agents of groundnut rosette disease complex primers in the 100 series represent internal primers for specified regions

Primers	Sequence	Specific to	Source reference
HRP92	ATGAATACGGTCGTGGTTAGG	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*200; Wangai *et al.,*2001
HRP93	TTTGGGGTTTTGGACTTGGC	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*200; Wangai *et al.,*2001
HRP94	GGAAGCCGGCGAAAGCTACC	GRV ORF3P and 4P	Taliansky *et al.,* 1996
HRP95	GGCACCCAGTGAGGCTCGCC	GRV ORF3P and 4P	Taliansky *et al.,* 1996
HRP96	GGTTTCAATAGGAGAGTTGC	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*200; Scott *et al.,*1996
HRP97	AAATGCCTACTTTGGGCGTG	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*200; Scott *et al.,*1996
HRP110	GGAGGGTCTGGCGAAACATT	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*200; Wangai *et al.,*2001
HRP111	CCCTTGTAAAGGAACCGGAAT	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*200; Wangai *et al.,*2001
HRP104	CGAGGAGACCAAAGGGTGGT	GRV ORF 3P and 4P	Taliansky *et al.,* 1996; Wangai *et al.,*2001
HRP105	AGCTCCGACACAATAGCGAAG	GRV ORF 3P and 4P	Taliansky *et al.,* 1996; Wangai *et al.,*2001
HRP108	GAAAAGGTGAGGGGTGTGT	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*200; Wangai *et al.,*2001
HRP92	ATGAATACGGTCGTGGTTAGG	GRAV-CP	Naidu *et al.,* 1998; Deom *et al.,*200; Wangai *et al.,*2001
HRP109	TAGCTTGATTTCAAGCTCGC	Sat-RNA	Naidu *et al.,* 1998; Deom *et al.,*200; Scott *et al.,*1996; Wangai *et al.,*2001

Source: Wangai *et al.,* 2001.

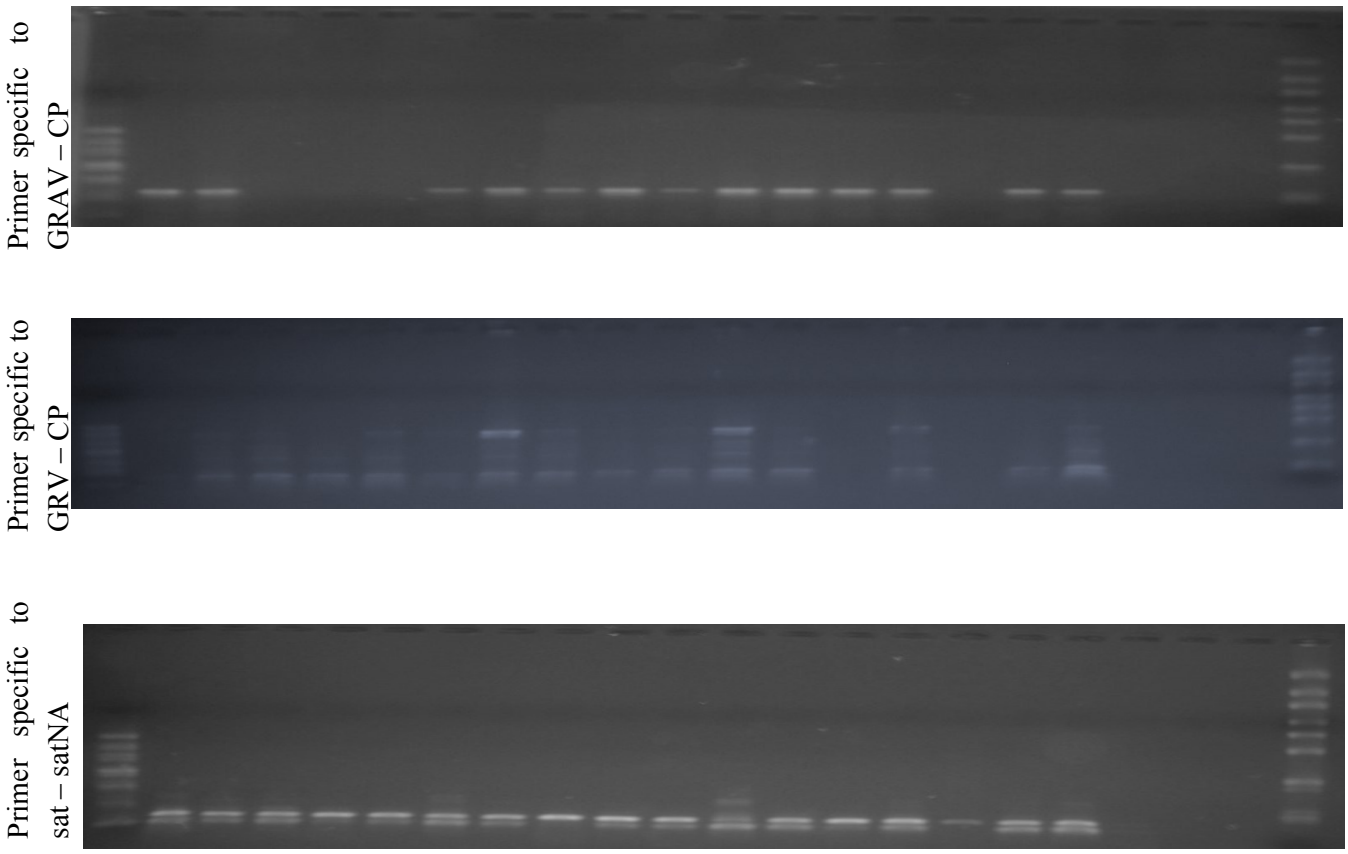


Figure 1: Amplification banding pattern of GRAV – CP (HRP92/93), GRV – CP (OFR3P and 4P) and Sat-RNA markers in 16 groundnut genotypes

Table 2: Field resistance scores and RT – PCR confirmation of GRD-resistance in some groundnut genotypes

Genotypes	SCORES			Field Status	RT – PCR		
	DI	AUDPC	Yield (g)		GRAV	sat-RNA	GRV
ICGX – SM 00020/5/9	17.30	16.59	17.98	Resistance	+	+	–
ICGX – SM 00017/5/P_{10}/P_1	28.41	22.84	38.40	Resistance	+	+	+
ICGX – SM 00020/5/P_4/P_1	27.34	20.09	35.28	Resistance	+	+	+
ICGV IS 07890	18.51	17.28	43.46	Resistance	+	+	+
ICGV IS 07899	33.40	22.16	25.49	Resistance	+	+	+
ICIAR-19BT	29.49	34.16	23.74	Resistance	+	+	+
ICGX – SM 00020/5/9 X ICGX – SM 00017/5/P_{10}/P_1	26.11	22.59	38.72	Resistance	+	+	+
ICGX – SM 00020/5/9 X MANIPENTA	33.69	10.94	11.99	Susceptible	+	+	+
ICGX – SM 00020/5/9 X ICGV IS 07899	20.45	22.84	29.09	Resistance	+	+	+
ICGX – SM 00017/5/P_{10}/P_1 X ICGV IS 07899	35.77	29.84	31.78	Resistance	+	+	+
ICGV IS 07899 X SAMNUT 14	41.28	31.50	22.74	Susceptible	+	+	+
ICIAR-19BT X MANIPENTA	43.21	26.83	20.39	Susceptible	+	–	–
ICIAR-19BT X SAMNUT 14	22.80	45.93	24.25	Susceptible	+	+	+
ICGV IS 07890 X MANIPENTA	46.05	41.35	31.36	Susceptible	+	+	+
MANIPENTA	40.12	34.59	11.03	Susceptible	–	+	+
KWANKWASO	50.02	31.09	9.41	Susceptible	+	+	+

Evaluation of total RNA extraction procedures for RT-PCR

From the phenotypic assessment of the GRD resistant status of the genotypes, 11 genotypes demonstrated resistance with disease severity index ranging from 17.30 for ICGX – SM 00020/5/9 to 35.77 for ICGX – SM 00017/5/P_{10}/P_1 X ICGV IS 07899 as compared with susceptible genotypes with DI ranging from 22.80 for ICIAR-19BT X SAMNUT 14 to 50.02 for KWANKWASO (Table 2). Similar trend s was noticed for Area Under Disease Progress Curve (AUDPC) with resistant genotypes showing lower AUDPC. Following molecular assessment by RT-PCR of both resistant and susceptible genotypes, there was differential amplification of DNA from collected samples. The PCR amplification of cDNA from samples showed various combinations of the GRD agents, some were detected in isolation, or as a combination of two but none was free of the three agents (Table 2 and Fig. 1). With primer GRAV-CP 400 nt fragment amplified 12 of the 16 genotype tested (Fig 1). For PCR fragment specific to GRV, primer also amplified products in 14 out of 16 genotypes. The sat-RNA on the other hand, amplified fragments in all the 16 genotypes, revealing the presence of sat-RNA in all the genotypes (Table 2). ICIAR-19BT X MANIPENTA cross was the only genotype that showed resistance to GRD as no amplification was observed for the two GRAV and GRV specific primers.

DISCUSSION

Leave samples from the seventeen genotypes assayed showed various combinations of the GRD agents. This is attributed to aphid feeding behavior. Naidu et al. (1999) and Deom *et al.* (2000) noted a single aphid vector acquires GRAV, GRV, and sat-RNA. However, it does not always transmit the three disease agents together to a host plant: GRAV or GRV plus sat-RNA can be transmitted separately. However, for the disease to perpetuate in nature, the aphid vector must transmit all three agents to a plant. The most detectable GRD agent was GARV and sat-RNA, with sixteen of seventeen samples testing positive. it should be noted that although plants with symptoms were found to be infected with viruses in most cases, some infected GRD field resistant plant remained symptomless and did not induce any. These plants tested positive for at least one of the viruses (GRV, GRAV and sat-RNA) only after being screened by RT- PCR. Thus, as with other virus–plant interaction systems, some combinations of viruses and groundnut plants can give rise to asymptomatic or possibly latent infection (Odedara *et al.,* 2007; Salem *et al.,* 2010). Because these infected plants are not easily noticeable and can serve as virus reservoirs in the field, this situation could be due to differences in inoculation feeding behavior of the vector. During short inoculation feeding (test probes), vector aphids probing groundnut leaves without reaching the

phloem can transmit GRV and sat-RNA, which multiply in the mesophyll cells. Whereas GRAV, which is phloem limited in infected plants, either does not replicate in mesophyll cells or fails to move from cell to cell (Naidu et al., 1999). Therefore, the success of transmitting all three agents together is high when the inoculation feeding period is longer or when the number of aphids per plant is increased (Misari et al., 1988). Vector aphids fail to acquire or transmit GRV and sat-RNA from diseased plants lacking GRAV and such plants become dead-end sources for the disease. However, if such plants receive GRAV later due to vector feeding, the plants again might serve as source of inoculum (Deom et al., 2000).

These observations also confirmed the notion that symptomatology alone cannot be taken as a reliable basis for resistance to plant virus diseases; especially since symptom induction can be complicated by the occurrence of mixed virus infection, as demonstrated by this study. Gillaspie (2006, 2007) and Salem *et al.* (2010) observed that the use of symptom visualization in field plots improved screening for GRD-resistance in groundnut plants, but failed to improve the speed or accuracy of screening for GRD-resistance, because the GRD symptoms were apparently too mild in many groundnut genotypes for accurate diagnosis. It is important to note that moderation of GRD-induced symptoms can also occur in plants co-infected with satellite RNAs (Salem *et al.,* 2010), as observed for GRD-infected plants showing mild or no GRD symptoms observed in the present study.

The molecular diagnosis in this study clearly demonstrated that at least one of three agents of GRD viruses, GRV, GRAV and sat-RNA, was present in all field resistant genotypes identified in this study. This is consistent with other studies reporting the occurrence of these viruses in groundnut (1993; Naidu *et al.,*1998) and Salem *et al.* (2010) for CABMV and CMV in cowpea and for the occurrence of GRV, GRAV and sat-RNA in groundnut germplasm maintained at the in IAR .

Since none of the genotypes tested has demonstrated resistance to all the three components based on RT – PCR assay, and because GRAV is the main component involved in aphid transmission, genotypes such as ICGX-SM-000/20/5/P_4/P_1, ICIAR -19-BT and ICGV 07899 that showed a negative response to GRAV could be exploited in resistance breeding programme to restrict the spread of groundnut rosette disease. The GRV resistant genotypes ICG-IS-07899 X SAMNUT14 and ICIAR -19-BT X MANIPENTA could be used as sources of resistance to GRV and for commercial production under favourable conditions. Since ICG-IS-07899 X SAMNUT14 and ICIAR -19-BT X MANIPENTA display susceptibility to the aphid but resistant to GRV, while ICGX-SM-000/20/5/P_4/P_1, ICIAR -19-BT and ICGV 07899 were susceptible to rosette disease agents but resistant to feeding by the aphid vector, the two groups of genotypes are good candidates for pyramiding genes for GRV and vector vector resistance into a single genotype to achieve broad-based genetic resistance against groundnut rosette.

CONCLUSION AND RECOMMENDATIONS

This result of the study has shown the sensitivity of RT-PCR to facilitate reliable diagnosis of GRV, GRAV and sat-RNA, and is particularly useful in cases where suspected viral symptoms are mild or absent. Although reverse transcription followed by PCR can be used to detect the presence of GRD in groundnut, the procedure provides little information regarding the relationship between the three agents of GRD. Therefore, information on the relationship of these three agents of GRD is required to establish distribution and epidemiology and for better groundnut rosette disease management.

ACKNOWLEDGEMENTS

Thanks to Alliance for a Green Revolution in Africa (AGRA) for the sponsorship and to the Institute for Agricultural Research, Ahmadu Bello University Zaria, Nigeria for the technical support

REFERENCES

Bock, K., Murant, A., and Rajeshwari. R. (1990). The nature of the resistance in groundnut to rosette disease *Annals of Applied Biology* 117:379–384.

Boonham, N., Glover, R., Tomlinson, J., and Mumford, R. (2008). Exploiting generic platform technologies for the detection and identification of plant pathogens. *European Journal of Plant Pathology*, 121, 355-363

Canning, E. S. G., Penrose, M. J., Barker, I., and Oates. D. (1996). Improved detection of barley yellow dwarf

virus in single aphids using RT-PCR. *Journal of Virology and Methods* 56, 191–197Deom *et al.,*200

Gillaspie Jr, A.G. (2007). Attempts to improve the method for screening cowpea germplasm for resistance to cucumber mosaic virus and blackeye cowpea mosaic virus. *Plant Pathology Journal* 6, 202

Hao, N. B., Albrechtsen, S. E., and Nicolaisen, M. (2003). Detection and identification of the blackeye cowpea mosaic strain of Bean common mosaic virus in seeds of *Vigna unguiculate* species from North Vietnam. *Australasian Plant Pathology* 32, 505–9.

Naidu, RA., & Hughes J.d'A. 2003. Methods for the detection of plant virus diseases. p 233– 260. In: "Plant Virology in Sub-Saharan Africa'' (J.d' A. Hughes, B.O. Odu, eds.). Proceedings of a conference organized by IITA, 4–8 June 2001, Ibadan, Nigeria.

Naidu, RA., Bottenberg, H., Subrahmanyam, P., F. Kimmins., Robinson, DJ., and Thresh, J. M. (1998). Epidemiology of groundnut rosette disease: current status and future research needs. *Annals Applied Biology.* 132, 525–548.

Odedara, O. O., Hughes, J. d'A., and Odu, BO. (2007). Occurrence of latent virus infection in visually-rated cowpea (*Vigna unguiculata* L. Walp) seedlings. *Archives of Phytopathology and Plant Protection* 42, 882–90.

Olmos, A., Bertolini, E., Capote, C. and Cambra, M. (2008). An evidence-based approach to *plum pox virus* detection by DASI-ELISA and RT-PCR in dormant period. *Virology: Research and Treatment*, 1, 1-8.

Olmos, A., Cambra, M., and Dasi, MA. (1997). Simultaneous detection and typing of plum pox potyvirus (PPV) isolates by heminested-PCR and PCR-ELISA. *Journal of Virology Methods* 68, 127–137.

Olorunju, PE., Ntare, BR., Pande, S., and Reddy, SV. (2001). Additional sources of resistance to groundnut rosette disease in groundnut germplasm and breeding lines. *Annals of applied Biology* 139:259-268Rajeshwari *et al.,* 1987

Salem, NM., Ehlersb, J. D., Robertsb PA and Ng, JCK. (2010). Biological and molecular diagnosis of seedborne viruses in cowpea germplasm of geographically diverse sub-Saharan origins. *Plant Pathology.* 59, 773–784. Doi:10.1111/j.1365-3059.2010.02285.x

Scott, K. P., Farmer, M. J., Robinson, DJ., Torrance, L., and Murant, A. F. (1996). Comparison of the coat protein of groundnut rosette assistor virus with those of other luteoviruses. *Annals of Applied Biology* 128, 77–83.

Singh, RP., Kurz, J., and Boiteau, G. (1996). Detection of styletborne and circulative potato viruses in aphids by duplex reverse polymerase chain reaction. *Journal of Virology Methods* 59, 189–196

Stevens, MR., and Smith HG. (1997). Comparison of ELISA and RT-PCR for the detection of beet yellows closterovirus in plants and aphids. *Journal of Virology Methods* 68, 9–16.

Subrahmanyam, P, Hilderbrand, G.L., Naidu, RA., Reddy, L. J., and Singh, AK., (1998) Sources of resistance to groundnut rosette disease in global groundnut germplasm. *Annals of Applied Biology*. 132:473-485.

Taliansky M. E., Robinson, DJ., and Murant, AF. (1996). Complete nucleotide sequence and organization of the RNA genome of groundnut rosette umbravirus. *Journal of General Virology* 77:2335–2345.

Vincelli, P., and Tisserat, N. (2008). Nucleic acid-based pathogen detection in applied plant pathology. *Plant Disease*. 92:660-669.

Wangai, AW., Pappu, SS., Pappu, H.R., Deom, CM., & Naidu, RA. (2001). Distribution and characteristics of groundnut rosette disease in Kenya. *Plant Disease.* 85:470-474.

COMBINING ABILITY OF GROWTH TRAITS, ZINC AND MANGANESE IN GUM ARABIC (*Senegalia senegal* [L.] Britton)

N. M. Fakuta,[1] A. D. Aba,[2] S. G. Ado,[2] I. F. Ojiekpon,[1] P. M. Jonah[3] and A. M. Bichi[2]

[1]Rubber Research Institute of Nigeria, Gum Arabic Sub-Station, Gashua, Nigeria.

[2]Department of Plant Science, Faculty of Agriculture, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.

[3]Department of Crop Science, Adamawa State University, PMB 25, Mubi, Nigeria.

Correspondence Author Email: fakuta1st@gmail.com

Abstract: Gum arabic is produced by *Senegalia senegal* trees in northern Nigeria from different provenances. Gum quality is influenced by provenances such as variation in Zinc and Manganese concentrations and this affects export potentials. Growth traits are important for early maturity as a critical factor to reduce gestation period of tree crops such as *S. senegalia*. The objective of this study was to provide estimates of genetic parameters of five growth and two chemical factors. The growth traits were tree height, canopy diameter, number of primary branches, bark thickness, stem diameter and the chemical factors were concentrations of Zinc and Manganese. The six provenances of *S. senegal* were evaluated in a 6 x 6 half diallel mating scheme. The 15 F_1 hybrids and their provenances were evaluated in a randomized complete block design and data were collected on the five growth traits, shoot tissue Zinc and Manganese concentration. Variation was significant for all traits. General and specific combining ability variances were significant for tree height, bark thickness and shoot tissue Manganese. The GCA effects found Damaturu and Kukareta as the best provenances and could be utilized in hybridization program. On the basis SCA effects, Kasawan Jakana x Kukareta and Damaturu x Dandalmari were identified for the lowest shoot tissue Zinc and Manganese concentration. The findings suggest that considerable progress for height, bark thickness, low gum Zinc and Manganese concentration of gum arabic can be achieved through breeding programs for *S. senegal*.

Key words: Gum Arabic, Provenances, Daillel Analysis, Combining Ability, Gene Action

INTRODUCTION

Gum arabic is dried exudate obtained from stems and branches of *Senegalia senegal* trees, which until lately were known as *Acacia senegal* [L.] Willd [1]. Gum arabic was approved for use as safe food additive by the US Food and Drug Administration [2]. The physical and chemical properties of gum arabic, established as quality parameters include ash content, moisture, optical rotation, viscosity, Nitrogen content, pH and cationic concentration of total ash (Iron, Copper, Manganese and Zinc).

The cationic concentration of ash is used to determine the specific levels of heavy metals in gum arabic [3]. The abundance of heavy metals in acacia gum is a disincentive to the food/export value. This requirement of low Zinc and Manganese concentration is therefore important. *S. senegal* tree from different provenances exhibit divergent growth traits which produce gum with different qualities [4]. Recognizing these variations in the provenances, it is likely that the quality of gum arabic might be improved genetically. The genetic bases of growth and chemical traits may therefore be studied to help in the improvement of earliness and gum quality for export. The objective of this study was to evaluate variation for five growths and two chemical factors in half diallel of six provenances of *S. senegal*.

MATERIALS AND METHODS

Experimental Field: The experiment was conducted at the Rubber Research Institute of Nigeria, Gum Arabic Sub-Station Gashua (Latitude 12^0 45' 52" N and Longitude 11^0 00'45" E). Gashua is at an altitude of 360 meters, above sea level and is located within the Sahel savannah ecological zone of Nigeria. The soil is well drained sandy silt, brown to grey-brown in colour and is strongly acidic with pH value of 5.40. The average annual rainfall is 350mm. The wet season is within the months of June through

September, with heaviest rainfall in August. Average annual temperature ranges between 23^0C (min) and 40^0C (max). Relative humidity ranged from 18.8% in February to71.1% in August [5].

Planting Materials: Six provenances as described in Table 1 were used as parents in a half diallel according to Griffing's method 2, model 1. The provenances were Kasawa Jakana, Kukareta, Damaturu, Gajiram1, Molai and Dandalmari.

Field Evaluation: The 21 genotypes consisting of six provenances and 15 hybrids were transplanted to the field at three months seedling/nursery age in August, 2014. Field dimension was 24 x 36 m in a randomized complete block design replicated eight times as single tree replication. Field spacing was 3m x 3m spacing for (≈1,111trees/ha). Cultural practices such as weed control, supplementary watering and formation of water harvesting pits around the base of each tree were applied as recommended by Ojiekpon [8]. Data on immature tree growth traits were collected in November 2015. **(1)** Tree height (cm) was determined by measuring from the root collar of immature tree to the top of the apical bud using measuring tape. **(2)** Canopy diameter (cm) which was the distance from the widest part of the immature tree canopy in two dimensions parallel to the row and perpendicular to the row was measured using measuring tape to determine the mean. **(3)** Number of primary branches was obtained by counting the number of each branch attached to the main stem of each immature tree in the field. **(4)** Bark thickness (cm) was determined by cutting and removing a portion of the stem bark of each immature tree in the field using budding knife and the thickness measured using digital vernier. **(5)** **S**tem diameter (cm): the stem was measured at the root collar of each immature tree directly above soil line using digital vernier. Two chemical traits viz Zinc (Zn) and Manganese (Mn) were evaluted by taking immature tree shoot tissue samples which were dried, ground to powder and sieved. Samples were digested using triple acid procedure while the concentration of Zn and Mn in the final solution was determined using atomic absorption spectrometer model AA24OF from Varian Company, USA [9].

Statistical Analysis: Analysis of variance (ANOVA) was conducted using SAS statistical program [10]. Ansalysis III of Gardner and Eberhart was used to estimate variances for GCA and SCA and their respective effects using DIALLEL-SAS05 [11]. The variances and standard errors of the estimates and their significance as described by Griffing [12] were included in the program DIALLEL-SAS05 [11]. The provenances used were specifically selected and of limited number, they were considered fixed effects while replication and experimental error were considered random. Conclusions drawn from this study are only limited to the provenances and environment used herein [13]. The model used as recommended by Griffing [14] was $y_{ijk} = \mu + g_i + g_j + s_{ij} + b_k + e_{ijk}$ where y_{ijk} = the phenotypic value of the mating of the ith provenance with the jth provenance in the kth replication, μ= population mean, g_i = GCA effect for the ith provenance, g_j = GCA effect for the jth provenance, s_{ij}=SCA effect for provenances ith and jth, b_k = effect of the kth replication and e_{ijk} = error effects peculiar to the ijk^{th} observation. Estimates of the expectation of mean squares were used to estimate~~s~~ the ratio of GCA to SCA.

Table 1: International specifications of quality parameters for six gum arabic provenances based on FAO/WHO Joint Expert Committee on Food Additive (JECFA) and their origins

	Within JECFA Specification			Outside JECFA specification		
Provenance	**Kasawa Jakana**	**Kukareta**	**Damaturu**	**Gajiram1**	**Molai**	**Dandalmari**
Latitude (^{0}N)	11^0 49′	11^0 43′	11^0 43′	12^0 27′	11^0 43′	11^0 42′
Longitude (^{0}E)	12^0 43′	12^0 09′	12^0 05′	13^0 11′	13^0 04′	12^0 07′
Altitude (m)	410	412	424	319	389	479
Zinc (mg/kg)	73.33	101.00	110.33	117.67	111.05	132.67
Manganese (mg/kg)	38.00	29.00	9.33	176.00	236.33	193.00

Source: Fakuta [6], Aghughu [7].

RESULTS

Mean squares for six provenances and their 15 F_1 hybrids for immature tree growth traits and shoot tissue Zinc and Manganese concentration is presented in Table 2. The mean squares due to genotypes was significant for all the traits. The mean squares for provenance vs hybrid interaction was significant for shoot tissue Manganese. Significant GCA mean square was observed for tree height, bark thickness, stem diameter, shoot tissue Zinc and Manganese while SCA mean squares were significant for all the traits except shoot tissue Zinc. Respective mean squares due to GCA were higher than SCA for tree height, bark thickness, shoot tissue Zinc and Manganese. The ratio of variance due to GCA/SCA in all estimates were less unity (Table 2).

Mean Performance

The mean performance of provenances and their hybrids for immature tree growth traits and shoot tissue concentration of Zinc and Manganese is presented in Table 3. Among the provenances, Damaturu had the tallest trees followed by Kukareta, Kasawa Jakana, Dandalmari, Molai and Gajiram1.

For canopy diameter, provenance Damaturu and Kasawa Jakana were the widest while the narrowest was Gajiram1. Provenances Kukareta and Damaturu had the highest number of primary branches while the lowest was recorded by Dandalmari. The thickest bark was indicated for Kukareta and Damaturu while the thinnest was recorded for Gajiram1 and Molai. For the stem diameter, Damaturu had the largest while Gajiram1 and Molai had the smallest stem sizes. The highest shoot tissue Zinc concentration was indicated for Gajiram1 (13.77 mg/kg) and Molai (12.80 mg/kg) while the rest of the provenances recorded low values (2.57 – 5.9mg/kg). For shoot tissue Manganese, Gajiram1 (59.20 mg/kg) recorded the highest value while Kukareta (12.97 mg/kg) had the lowest value for the trait. Gajiram1 (13.77 mg/kg) and Molai (12.80 mg/kg) while the rest of the provenances recorded low values (2.57– 5.9mg/kg).

Table 3: Mean performance of provenances and their hybrids for five immature tree growth traits, shoot tissue Zinc and Manganese concentrations of *S. senegal*

Genotypes	THT (cm)	CDM (cm)	NPB (no)	BKTN (cm)	STDM (cm)	TZn (mg/kg)	TMn (mg/kg)
Provenances							
Kasawa Jakana	164.50	209.94	48.63	0.48	4.27	4.87	13.83
Kukareta	169.88	195.13	52.63	0.54	4.72	4.10	12.97
Damaturu	189.00	235.75	50.50	0.53	5.11	2.57	16.83
Gajiram1	114.75	163.13	46.25	0.38	3.50	13.77	59.20
Molai	119.88	182.81	43.88	0.44	3.78	12.80	31.47
Dandalmari	138.50	192.88	36.63	0.49	3.95	5.90	40.23
Hybrids							
Kasawa Jakana x Kukareta	149.13	191.44	44.75	0.50	3.86	6.03	27.17
Kasawa Jakana x Damaturu	175.25	208.75	56.38	0.53	4.73	8.90	22.87
Kasawa Jakana x Gajiram1	139.25	185.56	46.38	0.42	3.80	8.00	14.10
Kasawa Jakana x Molai	128.63	174.38	43.13	0.40	3.54	14.83	25.13
Kasawa Jakana x Dandalmari	164.13	208.81	47.75	0.54	4.39	8.57	62.30
Kukareta x Damaturu	173.00	205.63	53.25	0.53	4.72	2.20	18.00
Kukareta x Gajiram1	140.75	179.31	44.75	0.41	4.01	13.33	14.77
Kukareta x Molai	144.63	187.63	39.63	0.42	3.98	10.90	60.87
Kukareta x Dandalmari	178.25	222.19	48.13	0.56	4.73	7.13	66.20
Damaturu x Gajiram1	136.63	182.13	40.75	0.42	3.91	9.63	22.97
Damaturu x Molai	167.88	215.56	50.13	0.48	4.51	2.37	31.80
Damaturu x Dandalmari	126.75	156.38	43.88	0.43	3.25	0.87	17.93
Gajiram1 x Molai	124.38	171.69	46.88	0.43	3.59	10.13	38.07
Gajiram1x Dandalmari	124.00	170.69	48.25	0.42	4.13	8.43	35.33
Molai x Dandalmari	127.00	175.88	37.88	0.41	3.71	10.27	55.77
Mean	147.43	191.22	46.21	0.46	4.10	7.89	32.70
LSD (0.5%)	25.74	35.39	9.41	0.05	0.63	2.91	6.39
CV (%)	17.66	18.72	20.61	9.89	15.45	37.28	19.75

THT = tree height, CDM = canopy diameter, NPB = number of primary branches, BKTN = bark thickness, STDM = stem diameter, TZn = shoot tissue zinc, TMn = shoot tissue manganese.

Table 2: Mean squares of six provenances and their hybrids for immature tree growth traits and shoot tissue Zinc and Manganese concentrations of *S. senegal*

Source of variation	df	THT	CDM	NPB	BTKN	STDM	TZn	TMn
Replications	7	3487.97**	8155.68**	117.13	0.008 ns	1.38**	2.46	134.61*
Genotypes	20	4009.12**	3357.24**	201.39**	0.025**	2.01**	133.14**	2606.24**
Provenances (P)	5	7050.48**	4859.73*	259.93*	0.027**	2.95**	180.18**	2686.91**
Hybrids (H)	14	3190.43**	2921.30*	194.66*	0.025**	1.75**	124.40**	2700.79**
P vs H	1	264.02	1947.91	2.92	0.008	0.93	20.35	879.21**
GCA	5	4768.38**	2571.84	181.54	0.040**	1.35*	192.23**	483.22**
SCA	9	2313.78**	3115.45*	201.95*	0.017**	1.98**	86.71	1520.55**
Error	147	811.91	1609.08	91.95	0.002	0.45	8.34	46.14
σ^2 gca: σ^2 sca		0.14	0.06	0.06	0.16	0.05	0.41	0.42

*and ** = significant at 0.05 and significant at 0.01 levels of probability, respectively. THT = trees height, CDM = canopy diameter, NPB = number of primary branches, BKTN = bark thickness, TZn = shoot tissue zinc, TMn = shoot tissue manganese, STDM = stem diameter.

For canopy diameter, provenance Damaturu and Kasawa Jakana were the widest while the narrowest was Gajiram1. Provenances Kukareta and Damaturu had the highest number of primary branches while the lowest was recorded by Dandalmari. The thickest bark was indicated for Kukareta and Damaturu while the thinnest was recorded for Gajiram1 and Molai. For the stem diameter, Damaturu had the largest while Gajiram1 and Molai had the smallest stem sizes. The highest shoot tissue Zinc concentration was indicated for Gajiram1 (13.77 mg/kg) and Molai (12.80 mg/kg) while the rest of the provenances recorded low values (2.57 – 5.9mg/kg). For shoot tissue Manganese, Gajiram1 (59.20 mg/kg) recorded the highest value while Kukareta (12.97 mg/kg) had the lowest value for the trait. Gajiram1 (13.77 mg/kg) and Molai (12.80 mg/kg) while the rest of the provenances recorded low values (2.57– 5.9mg/kg).

The tallest hybrid was Kukareta x Dandalmari while Gajiram1 x Dandalmari recorded the shortest trees. The hybrids Kukareta x Dandalmari, Damaturu x Molai, Kasawa Jakana x Dandalmari, Kasawa Jakana x Damaturu and Kukareta x Damaturu showed the widest canopies at 205 – 215cm, while Damaturu x Dandalmari and Gajiram1 x Dandalmari had the narrowest canopies. The highest number of primary branches was recorded for Kasawa Jakana x Damaturu, Kukareta x Damaturu and Damaturu x Molai at 50 – 56, whereas hybrids Molai x Dandalmari, Kukareta x Molai and Damaturu x Gajiram1 were the lowest for the trait.

The hybrids Kukareta x Dandalmari, Kasawa Jakana x Dandalmari, Kasawa Jakana x Damaturu and Kukareta x Damaturu at 0.53 – 0.56cm had the thickest tree bark while Kasawa Jakana x Molai, Kukareta x Gajiram1 and Molai x Dandalmari recorded the thinnest bark. Wide stem diameter was obtained in Kasawa Jakana x Damaturu and Kukareta x Dandalmari, followed closely by Kukareta x Damaturu, Damaturu x Molai, Kasawa Jakana x Dandalmari and Gajiram1 x Dandalmari. For shoot tissue Zinc of the immature trees, hybrids Kasawa Jakana x Molai (14.82 mg/kg) recorded the highest concentration while Damaturu x Dandalmari (0.87), Kukareta x Damaturu (2.20) and Damaturu x Molai (2.37) expressed the lowest concentration. The highest shoot tissue Manganese concentration was recorded for Kukareta x Dandalmari (66.20 mg/kg) while Kasawa Jakana x Gajiram1 (14.10 mg/kg), Kukareta x Gajiram1 (14.77 mg/kg) showed lowest concentrations.

The coefficient of variation for provenances and hybrids for immature growth traits and shoot tissue Zinc and Manganese concentrations indicated that the highest estimates was for shoot tissue Zinc (37.28%), number of primary branches (20.61%), shoot tissue Manganese (19.75%), canopy diameter (18.72%), stem diameter (15.45%) and the lowest was recorded for bark thickness (9.89%).

General combining ability effects

The estimates of general combining ability effects (GCA) for six provenances of *S. senegal* for immature tree growth traits and shoot tissue Zinc and

Manganese concentration are presented in Table 4. Two provenances Damaturu and Kukareta had significant positive general combining ability for tree height while Gajiram1 and Molai revealed significant negative GCA effects for the same trait. Provenances Gajiram1 showed significant negative GCA effects for canopy diameter. Damaturu and Molai indicated significant positive and negative GCA effects for number of primary branches, respectively. Kukareta and Dandalmari recorded significant positive GCA effects for bark thickness. Gajiram1 and Molai expressed significant negative GCA effects for bark thickness. Provenances Kukareta and Damaturu recorded significant positive GCA effects for stem diameter, whereas Gajiram1 and Molai showed significant negative GCA effects for the trait. Provenances Damaturu and Dandalmari recorded significant negative GCA effects for shoot tissue Zinc concentration, while Kasawa Jakana, Gajiram1 and Molai expressed significant positive GCA effects for the trait. For shoot tissue Manganese concentration, Kasawa Jakana, Damaturu and Gajiram1 showed significant negative GCA effects while Kukareta, Molai and Dandalmari recorded significant positive GCA effects for the trait.

Specific combining ability effects

The estimates of specific combining ability (SCA) effects for 15 hybrids of *S. senegal* for immature tree growth traits, shoot tissue Zinc and Manganese concentration is presented in Table 5. The SCA estimates revealed Kukareta x Dandalmari as specific combiner with significant positive value for tree height while Damaturu x Molai had significant positive SCA effects for the trait. Significant negative SCA effects were recorded for Damaturu x Dandalmari and Kasawa Jakana x Kukareta for tree height. Hybrids Kukareta x Dandalmari and Damaturu x Molai recorded significant positive SCA effects for canopy diameter while Damaturu x Dandalmari expressed significant negative SCA effects for the trait. For number of primary branches, Damaturu x Gajiram1 showed significant negative SCA effects. Hybrids Kasawa Jakana x Damaturu, Kasawa Jakana x Dandalmari, Kukareta x Damaturu, Kukareta x Dandalmari and Damaturu x Molai indicated significant positive SCA effects for bark thickness while Kasawa Jakana x Kukareta, Kasawa Jakana x Molai, Kukareta x Gajiram1, Kukareta x Molai, Damaturu x Gajiram 1, Damaturu x Dandalmari, Gajiram1 x Molai and Molai x Dandalmari recorded significant negative SCA effects for the trait. Hybrids including Kasawa Jakana x Damaturu, Kukareta x Dandalmari and Damaturu x Molai recorded significant positive SCA effects for stem diameter whereas Damaturu x Dandalmari and Kasawa Jakana x Kukareta showed significant negative SCA effects for stem diameter. For the shoot tissue Zinc concentration, hybrids Kasawa Jakana x Damaturu, Kasawa Jakana x Molai, Kukareta x Gajiram1, Damaturu x Gajiram1, Gajiram1 x Molai exhibited significant positive SCA effects while Kasawa Jakana x Kukareta, Kasawa Jakana x Gajiram1, Damaturu x Molai, Kukareta x Damaturu and Damaturu x Dandalmari showed significant negative SCA effects for the trait. Kasawa Jakana x Damaturu, Kasawa Jakana x Dandalmari, Kukareta x Molai, Kukareta x Dandalmari and Kukareta x Gajiram1 expressed significant positive SCA effects for shoot tissue Manganese concentration. Kasawa Jakana x Kukareta, Kasawa Jakana x Molai, Kukareta x Damaturu, Kukareta x Gajiram1, Damaturu x Dandalmari, Molai x Dandalmari, Gajiram1 x Molai and Gajiram1 x Dandalmari showed significant negative SCA effects for shoot tissue Manganese concentration.

Table 4: Estimates of general combining ability effects of six provenances for five immature trees growth traits, shoot tissue zinc and manganese concentrations of *S. Senegal*

Provenances	THT	CDM	NPB	BKTN	STDM	TZn	TMn
Kasawa Jakana	5.79	5.9	1.94	0.02*	0.01	1.45**	-5.04**
Kukareta	13.14*	10.21	-0.03	0.03**	0.26*	-0.24	4.06**
Damaturu	11.57*	5.78	3.44*	0.02*	0.21*	-4.14**	-14.30**
Gajiram1	-17.05**	-13.99*	-0.91	-0.05**	-0.21*	2.25**	-11.38**
Molai	-10.18*	-5.05	-3.25*	-0.04**	-0.25*	1.99**	9.97**
Dandalmari	-3.27	-2.85	-1.19	0.02*	-0.02	-1.31*	16.69**
SE±(GCA)	4.60	6.47	1.55	0.01	0.11	0.47	1.10

*and** = significant at 0.05 and 0.01 levels of probability, respectively. THT=tree height, CDM=canopy diameter, NPB=number of primary branches, BKTN=bark thickness, STDM=stem diameter. TZn=shoot tissue zinc, TMn= shoot tissue manganese.

Table 5: Estimates of specific combining ability effects for fifteen F1 hybrids for immature tree growth traits, shoot tissue Zinc and Manganese concentrations of *S. senegal*

F_1 hybrids	THT	CDM	NPB	BKTN	STDM	TZn	TMn
Kasawa Jakana x Kukareta	-16.44*	-13.74	-3.28	-0.02*	-0.46*	-3.29**	-5.99**
Kasawa Jakana x Damaturu	11.24	8.01	4.88	0.02*	0.46*	3.49**	8.06**
Kasawa Jakana x Gajiram1	3.87	4.58	-0.78	-0.01	-0.06	-3.80**	-3.62
Kasawa Jakana x Molai	-13.63	-15.54	-1.69	-0.04**	-0.29	3.28**	-14.95**
Kasawa Jakana x Dandalmari	14.96	16.69	0.88	0.04**	0.34	0.33	16.50**
Kukareta x Damaturu	1.65	0.57	3.72	0.02*	0.20	-1.58*	-5.92**
Kukareta x Gajiram1	-1.98	-5.98	-0.44	-0.03**	-0.09	3.21**	-12.07**
Kukareta x Molai	-4.98	-6.60	-3.22	-0.03**	-0.09	1.04	12.69**
Kukareta x Dandalmari	21.74**	25.76*	3.22	0.05**	0.44*	0.58	11.29**
Damaturu x Gajiram1	-4.54	1.27	-7.90**	-0.02*	-0.14	3.42**	14.50**
Damaturu x Molai	19.84*	25.77*	3.81	0.04**	0.48*	-3.59**	1.98
Damaturu x Dandalmari	-28.19**	-35.62**	-4.50	-0.07**	-1.00**	-1.78*	-18.62**
Gajiram1 x Molai	-4.96	-1.66	-4.90	-0.06**	0.01	2.21*	-5.33*
Gajiram1x Dandalmari	-2.32	-1.54	4.22	0.00	0.30	-0.61	-4.14*
Molai x Dandalmari	-6.19	-5.29	-3.81	-0.03**	-0.09	1.49	-5.05**
SE±(SCA)	7.80	10.99	2.63	0.01	0.18	0.79	1.86

*and** = significant at 0.05 and 0.01 levels of probability, respectively. THT = trees height, CDM = canopy diameter, NPB = number of primary branches, BKTN = bark thickness, STDM = stem diameter, TZn = shoot tissue zinc, TMn = shoot tissue manganese.

DISCUSSION

The significant mean squares observed for the trees indicated considerable improvement potential for growth and shoot tissue Zinc and Manganese concentrations of *S. senegal* through selection for the superior genotypes. Similarly, highly significant provenance vs hybrid mean squares for shoot tissue Manganese concentration indicated substantial difference in hybrid performance across provenances. This may be due to variability within a provenance, as expected since a provenance may consist of different lines while in the wild. The significant GCA and SCA variances for tree height, bark thickness, stem diameter and shoot tissue Manganese concentration signify the presence of both additive and non-additive gene action in the inheritance of the traits. This suggests that additive gene effects may be as important as non-additive gene effects in the performance of these immature growth traits of *S. senegal*. Selection progress is a function of heritability and heritability estimates assume additivity. The significant non-additive gene action as revealed by significant SCA may retard selection progress. An exemption in this study is Zinc concentration with significant GCA but insignificant SCA. The significant SCA variance for canopy diameter and number of primary branches indicates the appreciable presence of non-additive gene action controlling these traits. In this case, population management methods may be applied such as evaluation of reciprocals, full diallel or infusion of new provenances. Otherwise, slow selection progress will be achieved with the same population due to

insignificant GCA but significant SCA. The higher magnitude of mean squares due to GCA than SCA for all traits except number of primary branches and shoot tissue Manganese concentration suggests the relative advantage of GCA over SCA for the traits. Similar result was reported by Fakuta *et al*. [15] for seedling height and canopy diameter in *S. senegal*. The estimate of $\sigma^2 gca/\sigma^2 sca$ ratios for immature trees growth traits and shoot tissue Zinc and Manganese concentration however indicated higher variance of non-additive gene action in *S. senegal*

Damaturu provenances expressed good potentials for tree height, canopy diameter and stem diameter while Kukareta expressed superior number of primary branches and bark thickness which is in agreement with Fakuta *et al*. [16]. This suggests that Damaturu and Kukareta provenances are superior for the growth traits, and hence have potential for improvement in these traits. The high shoot tissue Zinc concentration expressed by Gajiram1 and Molai provenances was expected as the two provenances were known for high gum Zinc content [6]. Similarly, the low shoot tissue Zinc concentration observed for Kukareta and Damaturu tree was expected because both provenances were selected for their low gum Zinc concentration [6]. The lower shoot tissue Manganese concentration recorded by Kukareta in addition to its superior growth characters indicated potentials for growth and quality of *S. senegal* at maturity. The superior tree height, canopy diameter and bark thickness recorded for Kukareta x Dandalmari was characteristic of the two parental provenances as reported by Fakuta *et al.* [17]. Trees of Kasawa Jakana x Damaturu recording the highest number of primary branches and stem diameter was noted. The strong growth performance expressed by this hybrid could be attributed to the presence of Damaturu as one of the parents since Damaturu growth supriority has been documented [16]. The low shoot tissue Zinc concentration expressed by Damaturu x Dandalmari and Kukareta x Damaturu was expected because each of the hybrids had parent-provenance with low gum Zinc concentration. Similarly, the low shoot tissue Manganese concentration observed in Kasawa Jakana x Gajiram1 was desirable for the development of genotypes with low shoot tissue Manganese. The sharp decline in the shoot tissue Manganese concentration of Kasawa Jakana x Gajiram1, Kukareta x Gajiram1, Damaturu x Dandalmari and Kukareta x Damaturu hybrids shows potential to breed for low Manganese content among the hybrids. In view of the long gestation period of *S. senegal*, the analysis of immature growth traits was adopted as an important technique to establish the relative importance of different traits as determinants for the improvement in *S. senegal*. In this study, lower shoot tissue Zinc and Manganese concentration coupled with superior tree height, canopy diameter and number of primary branches recorded in Kukareta, Damaturu and Kukareta x Damaturu make these populations potential candidates to breed for desired gum quality and early tapping maturity in *S. senegal*.

The significant GCA effect was observed in Damaturu as the best general combiner for tree height and number of primary branches while Kukareta was the best general combiner for bark thickness and stem diameter. Damaturu was the best general combiner for shoot tissue Zinc and shoot tissue Manganese concentration. This study identified Kukareta and Damaturu as promising provenances to be crossed to obtain F_1 hybrids or as a source population for further selection in *S. senegal* improvement for growth and gum quality. Míguez-Soto *et al.* [18] suggested that most studies related to long term tree improvement programs are based on recurrent selection for general combining ability, in which only additive genetic effect is virtually captured by selection. Traits that determine early tapping maturity (tree height and stem diameter) and low shoot tissue Zinc and Manganese concentration could be improved simultaneously using recurrent selection procedure in *S. senegal*.

The specific combining ability effects were identified in Kukareta x Dandalmari and Damaturu x Molai as desirable specific combiners for tree height, canopy diameter, bark thickness and stem diameter. The significant positive SCA effect expressed by these hybrids was noted since Damaturu and Kukareta were also reported as good general combiners for the trait. The highly negative and significant SCA effects observed in Kasawa Jakana x Gijiram1 and Damaturu x Dandalmari for shoot tissue Zinc and Manganese respectively, implies that the hybrids are good specific combiners desirable for low shoot tissue Zinc and Manganese improvement. This indicated that early

selection for Kasawa Jakana x Gijiram1 and Damaturu x Dandalmari could reduce generation time and maximize gain in the improvement for gum Zinc and Manganese concentration in *S. senegal*. This is because the negative and significant SCA effects suggest a retarding effect which may be desirable for traits such as Zinc and Manganese.

CONCLUSION

This study has identified Kukareta and Damaturu as the promising genotypes to be exploited as a source population for improvement in *S. senegal* for earliness and low gum Zinc and Manganese concentration. The inter-crossing of these provenances and hybrids may produce desirable genetic recombinants for growth, Zinc and Manganese concentration.

ACKNOWLEDGEMENT

The authors are grateful to the Management and staff of RRIN, Benin City and IAR, Zaria for the support and encouragement for this study.

REFERENCES

1. KSP, Kew Species Profile (2017) International plant name index and world checklist of selected plant families. http://creativecommons.org/licenses/by/3.0
2. Dondain, G., Phillips, G. O. (1999) The regulatory journey of gum arabic. *Journal of Foods and Food Ingredients*, 179: 38-56.
3. FAO (1996) A review of production, markets and quality control of gum arabic in Africa. FAO, ROME. 61p.
4. Aghughu, O., Emuedo, O., Fakuta N. M. (2017) Floral biology and identification of the various gum producing *Acacias*: A veritable tool in ensuring good quality gum arabic in Nigeria market. In: Ojiekpon I. F., Abubakar M., Fakuta N. M. (Eds.) Manual on gum arabic value chain training workshop on good tapping techniques/ fabrication of tapping knives for gum arabic farmers and other stakeholders for 12 gum arabic producing states of Nigeria. Rubber Research Institute of Nigeria, Gum Arabic Sub-Station, Km 13, Gashua-Potiskum Road, Tajuwa-Gashua, Yobe State, 31st October- 2nd November, 2017. Pp 1-12
5. Nigerian Meteorological Agency (NiMet) (2014) Agro-meteorological Bulletin No. 1-22.
6. Fakuta, N.M. (2017). Genetic analysis of growth traits, zinc and manganese in gum arabic (*Acacia senegal* [L.] Willd) PhD Thesis, Ahmadu Bello University, Zaria, Nigeria, 146p.
7. Aghughu, O. (2001). Provenance variation of gum arabic (*Acacia senegal* [L.] Willd) in Nigeria. PhD Desertification, Ahmadu Bello University, Zaria, Nigeria, pp. 42-127.
8. Ojiekpon, I. F. (2013). Effect of rainwater harvesting, cow dung and tree density on the establishment and growth of gum arabic (*Acacia senegal* [L.] Willd) in Sahel Savana. Ph.D. Desertification, Ahmadu Bello University, Zaria. Nigeria, pp. 1-137.
9. Robert, O. M. (1998). Tripple acid wet digestion. In: Yash P. Kalra (Ed.) 1998. *Handbook of reference methods for plant analysis*.CRC Press, Boston, London, pp. 25-36.
10. SAS Institute (2002). SAS/STAT *Guide for Personal Computer*: version 9.0. SAS Institute Inc., Cary, NC, USA.
11. Manjit, S. K., Yudong, Z., Kendall, R. L. (2005). DIALLEL-SAS05: A Comprehensive Program for Griffing's and Gardner–Eberhart Analyses. *Agronomy Journal*, 97:1097-1106, doi:10.2134/agronj2004.0260
12. Griffing, B. (1956a). A generalized treatment of the use of diallel crosses in qualitative inheritance. *Heredity*, 10: 31–50.
13. Hallauer, A.R. Carena, M. J., Filho J.B.M. (2010) Heriditary variance: Mating Designs. In: A. R. Hallauer, M. J. Carena and J. B. M. Filho (Eds.) *Quantitative Genetics in Maize Breeding*, Springer, New York, 6: 122-132.
14. Griffing, B. (1956b). Concept of General and Specific combining ability in relation to diallel Crossing system. *Australian Journal of Biological Science*, 9:463- 493.
15. Fakuta, N. M., Kadams, A.M., Ojiekpon, I. F. (2009) Diallel analysis of the combining ability of F_1 hybrids in provenance population of gum arabic (*Acacia senegal* [L.] Willd). *Nigerian Journal of Tropical Agriculture,* 11: 288–295.
16. Fakuta, N.M., Ojiekpon, I.F., Gashua, I .B., Ogunremi, O.C. (2015). Quantitative genetic variation in gum arabic (*Acacia senegal* [L] Willd) Provenances. *American Journal of Plant Sciences*, 6: 2826-2831.http://dx.doi.org/10.4236/ajps.2015.618279
17. Fakuta, N. M., Aba, D. A., Ado, S. G., Usman, I.S., and Ojiekpon, I. F. (2017) Estimation of heterosis for seedling growth traits in gum arabic *Acacia senegal* [L.] Willd. *Greener Journal of Agricultural Sciences*, 7(4): 082-089, Http: //doi. org/10.15580/GJAS.2017.4. 031017034
18. Míguez-Soto, B., López-Villamor, A., Fernández-López, J. (2016). Additive and non-additive genetic parameters for multipurpose traits in a clonally replicated incomplete factorial test of *Castanea spp. Tree Genetics & Genomes*, 12: 47-61 doi 10.1007/s11295-016-1004-0

DIVERSITY STUDY OF A NEGLECTED AND UNDERUTILIZED CROP SPECIES: A CASE STUDY OF ACHA (*DIGITARIA SPP*) (Kippist) (stapf)

Ogunkanmi, L. A.[1], Illoh, A. C.[2], Dachi, S.[3], Onuminya, T. O.[4], Osundinakin, M. I.[4]., Ajikah, L. B.,[4], Rotimi, O. T.[4], Abdul, S. D.[5] and Ogundipe, O. T.[4]

[1]Cell Biology and Genetics, University of Lagos, Nigeria.
[2]Biodiversity and Climate Research Lab, Biotechnology Advanced Research Centre, Sheda Science and Technology Complex Abuja, Nigeria.
[3]National Cereal Research Institute, Badegi, Niger state, Nigeria.
[4]Department of Botany, Faculty of Science, University of Lagos, Nigeria.
[5]Biological Sciences Department, Abubakar Tafawa Balewa University, Bauchi, Nigeria.
Corresponding Author: adebayoogunkanmi@yahoo.com

Abstract: Acha is a crop endowed with wealth of nutritional and medicinal uses, yet the rate at which the crop is being lost is at alarming rate. Hence, the need for germplasm collection, preservation and utilization for future improvement. This study carried out exploration trip on Acha collections (*Digitaria exilis* and *Digitaria iburua*) with a view to unravel the diversity study and investigating its genetic resources for the benefit of mankind. Five Northern states in Nigeria where Acha was cultivated were fully explored for sample collections. Germplasm collections was preceded by initial discussions with farmers on their fields, at market places and homes. At the end of the exploration tour, a total of thirty eight villages were visited out of which seventy five Acha collections were identified. The germplasm collected includes seed saved from previous harvest, purchased from the market, and those shared among colleagues. At the end of the trip, a pre project workshop was organized where farmers from the five states were invited and discussion on the uses, cultivation, processing and benefit of Acha were fully discussed.

Keywords: Acha, Neglected, Genetic, Germplasm, *Digitaria spp.*

INTRODUCTION

Fonio otherwise known as Acha belongs to the family *Poaceae,* sub-family of *Panicoideae,* tribe of *Paniceae* and the genus *Digitaria* (Henrard, 1950; Clayton and Renvoze, 1986). Most people in developing countries depend on special crops as their food source and/or income generation. These crops are mostly known as Orphan crops but also as Understudied, Lost, Disadvantaged-Crop, or Neglected Underutilized Species

Acha popularly known as "Hungry man rice" is probably one of the African oldest cereal (Pulseglove, 1975) majorly cultivated in the Northern states on marginal, mountainous, sandy, poor degraded soil and hilly agro -ecological zones of Nigeria.

According to the FAO (2008) they are regarded as "grain of life" in many communities of West Africa and in Nigeria in particular as it provides food early in the farming seasons when other crops are yet to mature for harvest (Ibrahim, 2001). It is considered as a staple food for millions of people from Senegal to Lake Chad (Vietameyer *et al.,* 1996; Vodouhe *et al*., 2003).

Fonio consumption and uses is in high demand in the Northern region; however, its yields is still very low. In Nigeria, about 70,000 metric tons of the crop is produced annually (CBN, 1998) and that the economic returns of acha when computed showed that it is more profitable to grow the crop compared to other crops like rice, sorghum and cowpea. (Dauda, *et al* 2003, Dachi and Gana, 2008).

Acha is of two different species (*Digitaria exilis* and *Digitaria iburua*). The straggling and free-tillering annual type of 3 to 5cm tall, with linear to lanceolate leaves of up to 15cm length and glaborous surface, terminal digitate panicle of 2-5 slender sessile racemes, up to 15cm long with spikelets which are 2-flowered elliptic – oblong and acute is known as *Digitaria exilis*. *Digitaria iburua* is erect but does not tiller well as *Digitaria exilis.* It grows to a height of about 140cm at maturity. It has 2-11 sub digitate

racemes up to 13cm long (Kwon-Ndung and Ochigbo, 2004). The seed size ranges from 1.5-2.0mm.

It is regarded as a staple food or part of major diet in the Arid and semi- Arid region. It is one of the best tasting cereal with varieties of value added products like jollof acha, gote, acha cake, nakann (a traditional acha drink), acha pap, kunu, kunuzaki, coker oat, acha bread, kuskus, tuwo, acha-soy weaning porridge flour, acha biscuits, acha based candy etc. The grain is highly consumed by diabetes patients (Jideani 2012) and it's highly rich in methionine (a Sulphur containing amino acid) which is absent in most food crops. Acha is reputed to be richer in magnesium, zinc and manganese than other cereals. It is also significantly richer in thiamine (Vitamin B1), Riboflavin (Vitamin B2), calcium and phosphorus than white rice (Kwon-Ndung 2014). The plant straw is used as broom and also burnt into ash that can serve as potash.

Despite its ancient heritage and widespread importance, knowledge of fonio's evolution, origin, distribution, and genetic diversity remains scanty even within West Africa itself. The crop has received but a fraction of the attention accorded to sorghum, pearl millet, and maize, and a mere trifle considering its importance in the rural economy and its potential for increasing the food supply (National research council 1996).

The last published data by (Kwon-Ndung 2014) that recorded 166 *D. exilis* and *43 D. iburua* at National Cereals Research Institute (NCRI) Badegi (Niger state), 29 *D. exilis* and 15 *D.iburua* at Riyom substation (Plateau state) has been lost drastically over the period of 3 years as a result improper storage and preservation, hence no comprehensive checklists for this crop.

The aim of this study is to re unlock all genetic resources of acha across Nigeria cultivating state, carry out proper survey on the history/origin, effect of rainfall, preservation methods and proper conservation at National Centre for Genetic Resource and Biotechnology(NACGRAB) genbank to maintain its viability and availability for future purposes is of paramount importance.

MATERIALS AND METHODS

Description of study area: The study was conducted between 24th April to 10th May, 2017 in Niger state located on longitude 6° 55' E, latitude 9° 35' N, Kaduna state 7° 22' E, 9° 28'N, Plateau state 8° 46' E, 9° 38' N, Bauchi state 9° 36' E 9° 40' N and Nasarawa state 8° 22' E, 8° 54' N representing Acha cultivating zones in Northern Nigeria. Northern Nigeria covers about 79% of Nigeria total landmass, It is a typical tropical region where relatively high temperatures are recorded all year round by its latitudinal location (Chima et. al. 2011) while the regional mean temperature is about 27°C (Oguntoyinbo, 1983).

Acha sampling: A total of 38 villages in 15 local government across these 3 Northern regions where acha are mainly cultivated were visited for collections of germplasm on farms and markets. Collections was preceded by discussions with Village heads, farmers and traders on farms, homes and markets for data collection on historical value, yields, shattering and lodging susceptibility, planting and harvest season, threshing procedure, storage means, native local names, nutritional value, medicinal uses, cost and edible by-products.

Project workshop: At the end of the exploration tour, a workshop was held at the Assembly hall in Nasarawa state University, Keffi with stakeholders and farmers to interact and document vital information on origin of crops, degree of consumption, value added products, planting season, and effects of rainfall, methods of harvesting /storage and factors affecting low cultivation of the crop. Samples have been deposited at the National Centre for Genetic Resources and Biotechnology (NACGRAB) for effective preservation and utilization for future generation.

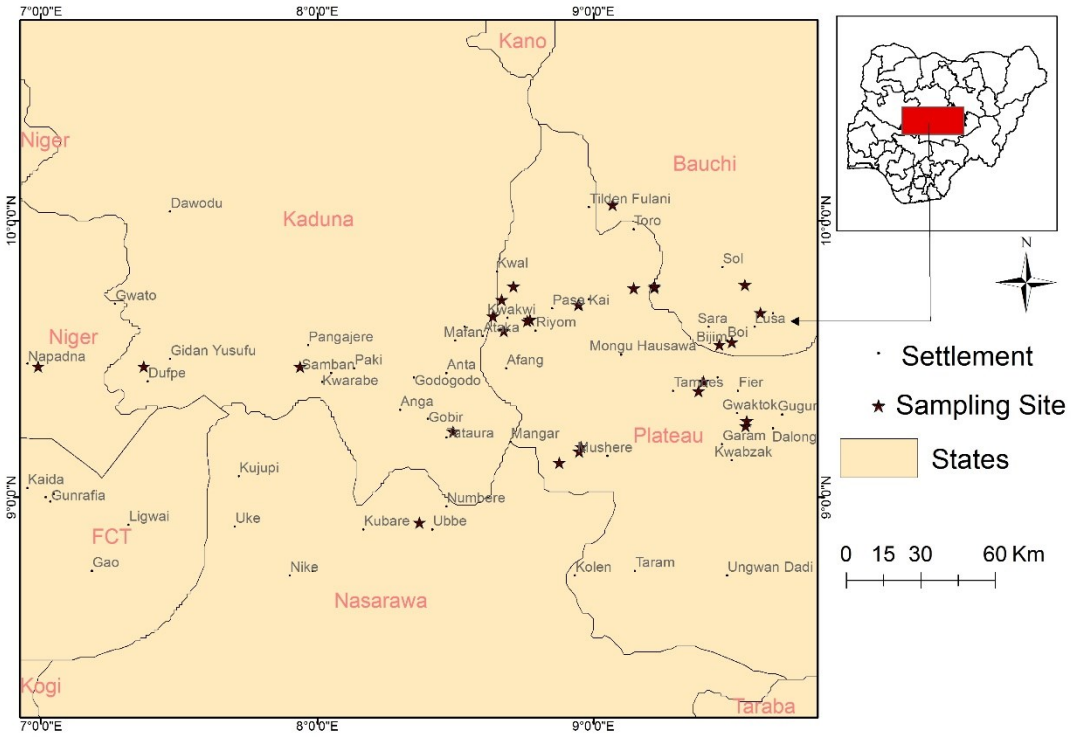


Figure 1: Study map of sampling location

RESULTS AND DISCUSSION

Summary and origin of collections; A total of 75 germplasm collections were made in this study out of which 39 belong to D. exilis and 36 to D. ibura showing diversity in their distribution in the study location. Plateau state recorded the highest collection of (33) samples (Table 1a) followed by Bauchi state (18) (Table 1c), Niger state (17) (Table 1d), Kaduna state (5) and Nasarawa state (2) (Table 1b). The Origin of the crop (Acha) could not be ascertained from village heads, farmers and traders interviewed as they also grew up to know the crop as Acha from their forefathers.

Local names/degree of consumption: Native local names of these species varies within villages but D. exilis cultivars are commonly referred to as "acha" while D. iburua are known as "aburu". Farmer perception towards acha from interviews/interactions which include the focal group discussion method at the workshop revealed that D. iburua is less cultivated and consumed. D. iburua grow taller with less lodging and shattering. It mature late and more expensive than D. exilis despite is commercial use in the production of cake and a special drink called Nakann. Kaduna state cultivate and consumed D. iburua more than D. exilis.

Planting season: Sowing of Acha in Nasarawa, Plateau and Bauchi states is done in late June/early July for D. exilis while D. iburua is planted between Early May and June when there is relatively high rainfall, but in Kaduna State, sowing is done between June – July for D. exilis and July – August for D. iburua.

Effects of rainfall: Generally, high rainfall and temperature affect the yield of acha by making the seeds of D. exilis matured early than expected. Kaduna State farmers stated that higher rainfall is required for D. iburua while late planting of D. exilis with short rainfall will reach maturity stage within September – October. Nasarawa state farmers revealed that planting of D. iburua is usually early because short rainfall affects its yield mostly due to poor maturity of the seeds.

Methods of harvesting/storage: Harvesting of the two species varies within states. The farmers in Niger, Plateau, Kaduna and Nasarawa states make use of the uprooting method or sickle in cutting the straw, after which it is packed in small bunches, transported and beaten on hard rocks for threshing. The straw removed or sacks are used to sweep over the threshed seeds to

remove the bad ones and have the clean seeds left. Bauchi state farmers use their hands in drawing the seed from the straw into a basin leaving behind the straw for animals to feed on. Granary is mostly used for storage while others put them in bags, pots and large plastic container for storage.

Factors affecting low cultivation: Information gathered from the workshop revealed that the low pricing of acha is one of the major reasons why the interest rate on cultivation dropped, younger generation loose interest because of identification problems from other Poaceae family, hence the crop is been weeded out with other poaceae weed family because of their resemblance. It was also recorded that women were no more interested in the threshing /dehulling activities of acha using pistle and mortar because of is intense labour.

CONCLUSION

This study has highlighted a comprehensive checklist and distribution of Acha value chain in the Northern part of Nigeria where it is majorly cultivated. Their local names, degree of consumption, effect of rainfall and temperature on the crop, planting season, lodging and shattering susceptibility, local uses and harvesting method were documented. *Digitaria exilis* is the commonest species of Acha collected. As food production is facing many challenges due to the increased population growth and climate change, indigenous and new plants and plant products that can provide nutritionally rich foods can contribute to food security. Preservation of the genetic diversity in a gene bank that otherwise could be displaced by other crop commodities as well as a research tool for other vital studies can be guaranteed.

There is need to sustainably conserve the genetic resource of this plant as it's been ranked 3rd position on the "lost crops of Africa" (National Research Council, 1996).

ACKNOWLEDGEMENTS

This research was sponsored by Tetfund (Tertiary Education Trust Fund) 2017. The authors would like to thank all Acha farmers in Plateau, Nasarawa and Niger in North central zone, Bauchi in North East zone and Kaduna in North West zone who collaborated and offered necessary supports to the research team on their fields.

Table 1a: List of germplasm collections from Plateau state

S/N	Local Govt	own	Botanical Name	Local Name	Coordinates	Altitude
1	Riyom	NcriAcha Sub Station	*D. exilis*	Gusuk II	N 9.64234 E 8.77021	1223 M
2	Riyom	NcriAcha Sub Station	*D. iburua*	Gotip	N 9.64234 E 8.77021	1223 M
3	Riyom	Kwi	*D. exilis*	Badama	N 9.63763 E 8.76065	1206 M
4	Riyom	Kwi	*D. exilis*	Gindiri	N 9.63763 E 8.76065	1206 M
5	Riyom	Kwaki Area Ganawuri	*D. exilis*	Tsun Wild Acha	N 9.60141 E 8.67631	1107 M
6	Riyom	Danto Area Ganawuri	*D. exilis*	Loma	N 9.65511 E 8.63626	947 M
7	Riyom	Danto Area Ganawuri	*D. exilis*	Kpos	N 9.65511 E 8.63626	947 M
8	Riyom	Danto Area Ganawuri	*D. exilis*	Randon	N 9.65511 E 8.63626	947 M
9	Riyom	Danto Area Ganawuri	*D. exilis*	Sheng	N 9.65511 E 8.63626	947 M
10	Riyom	FinikonDanwal	*D. exilis*	Sun-Sharang	N 9.71508 E 8.66765	1113 M
11	Riyom	FinikonDanwal	*D. exilis*	Nkpes	N 9.71508 E 8.66765	1113 M
12	Bassa	ShekauKwall	*D. iburua*	Gindiri	N 9.76302 E 8.71089	1171 M
13	Bassa	ShekauKwall	*D. iburua*	Sun-Pia	N 9.76302 E 8.71089	1171 M
14	Barinkinladi	Foron	*D. exilis*	Were	N 9.69716 E 8.94516	1250 M
15	Barinkinladi	Foron	*D. iburua*	Ney-Cun	N 9.69749 E 8.94731	1235 M
16	Pankshin	Fier	*D. exilis*	Chisu	N 9.41923 E 9.39877	1191 M
17	Pankshin	Fier	*D. iburua*	Shalak	N 9.41923 E 9.39877	1191 M
18	Pankshin	Fier	*D. exilis*	Chika Rai	N 9.38392 E 9.38120	1050 M
19	Pankshin	Fier	*D. iburua*	Shalak	N 9.38392 E 9.38120	1050 M
20	Kanke	Tigya, Garram District	*D. iburua*	Dampep	N 9.25778 E 9.55120	850 M
21	Kanke	Tigya, Garram District	*D. iburua*	Gotip	N 9.25778 E 9.55120	850 M
22	Kanke	Tigya, Garram District	*D. iburua*	Nashelleng	N 9.25778 E 9.55120	850 M
23	Kanke	Tigya, Garram District	*D. iburua*	Jipel	N 9.25778 E 9.55120	850 M
24	Kanke	Goktok, Langshi District	*D. exilis*	Dampep	N 9.27538 E 9.55510	695 M
25	Bokos	Mangar	*D. exilis*	Nyemat	N 9.18192 E 8.95595	1114 M
26	Bokos	Mangar	*D. exilis*	Diya Mibwal	N 9.16569 E 8.94840	1154 M
27	Bokos	Mangar	*D. iburua*	Diya Mukucok	N 9.16569 E 8.94840	1154 M
28	Bokos	Richa	*D. exilis*	Dapiya	N 9.12530 E 8.87650	1061 M
29	Bokos	Richa	*D. iburua*	Black Jarab	N 9.12530 E 8.87650	1061 M
30	Bokos	Richa	*D. iburua*	White Jarab	N 9.12530 E 8.87650	1061 M
31	Bokos	Richa	*D. iburua*	Red Jarab	N 9.12530 E 8.87650	1061 M
32	Jos East	Maijuju	*D. exilis*	Gadeh	N 9.75598 E 9.14564	943 M
33	Jos East	Maijuju	*D. exilis*	Chele	N 9.75598 E 9.14564	943 M

Table 1b: List of germplasm collections from Kaduna and Nasarwa state

S/N	State	Local govt	Town	Botanical name	Local name	Coordinates	Altitude
1	Kaduna	Kagarko	Gujeni	*D. exilis*	Fulu	N 9.47308 E 7.37181	546 M
2	Kaduna	Kagarko	KurminJibrin	*D. exilis*	Ekpeshegwa	N 9.47267 E 7.93811	765 M
3	Kaduna	Kagarko	KurminJibrin	*D. exilis*	Uwun	N 9.47267 E 7.93811	765 M
4	Kaduna	Sanga	Nandu Ngbok	*D. iburua*	Izayi	N 9.23792 E 8.49196	526 M
5	Kaduna	Sanga	Nandu-Ngbok	*D. exilis*	Andama	N 9.23792 E 8.49196	527 M
6	Nasarawa	Akun Dev. Area/NasarawaEggonLga	Alushi	*D. exilis*	Aneme	N 8.90774 E 8.36988	455 M
7	Nasarawa	Akun Dev. Area/NasarawaEggonLga	Alushi	*D. iburua*	aburu	N 8.90774 E 8.36988	455 M

Table 1c: List of germplasm collections from Bauchi state

S/N	Local Govt	Town	Botanical Name	Local Name	Coordinates	Altitude
1	Bogoro	Bogoro	*D. iburua*	Lhad	N 9.66736 E 9.60542	633 M
2	Bogoro	Bogoro	*D. exilis*	WondatFyali (White)	N 9.66736 E 9.60542	633 M
3	Bogoro	Bogoro	*D. iburua*	Chit Kusung	N 9.66736 E 9.60542	633 M
4	Bogoro	Bogoro	*D. exilis*	Chit Nazaari/Naghali	N 9.66736 E 9.60542	633 M
5	Bogoro	Boi Market	*D. iburua*	Kokum/Chit Kusung	N 9.56142 E 9.50180	774 M
6	Bogoro	Boi Market	*D. iburua*	Wondat	N 9.56142 E 9.50180	774 M
7	Bogoro	GambarLere	*D. iburua*	Dampep	N 9.55239 E 9.45600	741 M
8	Tafawa Balewa	Tafawa Balewa	*D. exilis*	Chit Fiyali (Gadian) Late Variety	N 9.76899 E 9.54898	656 M
9	Tafawa Balewa	Tafawa Balewa	*D. iburua*	Chit Kusungh Early Variety	N 9.76899 E 9.54898	656 M
10	Tafawa Balewa	Tafawa Balewa	*D. exilis*	Chit Wandat Medium Variety	N 9.76899 E 9.54898	656 M
11	Toro	Toro	*D. exilis*	Chali (Very White)	N 10.05832 E 9.06957	961 M
12	Toro	Toro	*D. iburua*	Aburu	N10.05832 E 9.06957	961 M
13	Toro	Toro	*D. iburua*	Abayama	N10.05832 E 9.06957	961 M
14	Toro	Toro	*D. iburua*	Chali (Not Pure White)	N 10.05832 E 9.06957	961 M
15	Tafawa Balewa	B'otoJuwa	*D. iburua*	Aburu	N 9.75918 E 9.22075	910 M
16	Tafawa Balewa	B'otoJuwa	*D. exilis*	Chele	N 9.75918 E 9.22075	910 M
17	Tafawa Balewa	Riseh	*D. exilis*	Chele	N 9.76403 E 9.22146	914 M
18	Tafawa Balewa	Riseh	*D. exilis*	ItsonIkan	N 9.76403 E 9.22146	914 M

Table 1d: List of germplasm collections from Niger state

S/N	Local Govt	Town	Botanical Name	Local Name	Coordinates	Altitude
1	Bida	Badegi/Ncri	*D. exilis*	Kure'ep	N 9.06517 E 6.09830	98 M
2	Bida	Badegi/Ncri	*D. exilis*	Churriwe	N 9.06517 E 6.09830	98 M
3	Bida	Badegi/Ncri	*D. exilis*	Ndat	N 9.06517 E 6.09830	98 M
4	Bida	Badegi/Ncri	*D. exilis*	Loma	N 9.06517 E 6.09830	98 M
5	Bida	Badegi/Ncri	*D. exilis*	Lalaku	N 9.06517 E 6.09830	98 M
6	Bida	Badegi/Ncri	*D. exilis*	Ndai	N 9.06517 E 6.09830	98 M
7	Bida	Badegi/Ncri	*D. exilis*	Nipiya	N 9.06517 E 6.09830	98 M
8	Bida	Badegi/Ncri	*D. burua*	Pelking	N 9.06517 E 6.09830	98 M
9	Bida	Badegi/Ncri	*D. iburua*	Dampep	N 9.06517 E 6.09830	98 M
10	Bida	Badegi/Ncri	*D. iburua*	Zor	N 9.06517 E 6.09830	98 M
11	Bida	Badegi/Ncri	*D. iburua*	Dinat	N 9.06517 E 6.09830	98 M
12	Bida	Badegi/Ncri	*D. iburua*	Nibang	N 9.06517 E 6.09830	98 M
13	Bida	Badegi/Ncri	*D. iburua*	Dipiya	N 9.06517 E 6.09830	98 M
14	Bida	Badegi/Ncri	*D. iburua*	Nashelleng	N 9.06517 E 6.09830	98 M
15	Bida	Badegi/Ncri	*D. iburua*	Suapiya	N 9.06517 E 6.09830	98 M
16	Paiko	Puluko Settlement Chimbi/	*D. exilis*	Fulu	N 9.47360 E 6.98927	189 M
17	Paiko	Puluko Settlement Chimbi/	*D. iburua*	Katakpwo	N 9.47360 E 6.9892	189 M

REFERENCES

Central Bank of Nigeria (1998). Annual report and statement of account pp. 84.

Clayton, W. D. and Renvoize, S. A. (1986). *Genera graminum, Grasses of the World*. Kew Bulletin Additional Series 13:1-389.

Chima, G.N., Ijioma, M.A., Nwagbara, M. O. and Nwaugo, V.O. (2011). Sensitivity of vegetation to decadal variations in temperature and rainfall over Northern Nigeria. *Journal of Soil Science and Environmental Management* 2(**8**): 228-236.

Dachi, S. N. and Gana, A.S. (2008). Adaptability and yield evaluation of some Acha (*Digitaria Exilis* and *Digitaria iburua* Kippis Stapf) accessions at Kusogi – Bida, Niger State, Nigeria. *African Journal of General Agriculture* 4(2): 73

Dauda, A. and Luka, D. (2003). Status of Acha (*Digitaria exilis*) production in Bauchi State, Nigeria. In: Kwon – Ndung, E. H., Bright, E.O. and Vodouhe, R.(eds). *Proceedings of the first National Acha Stakeholders workshop at PADP*, Jos (9-11th March).

FAO (2008). Food and Agriculture Organization. Production year Book, Rome, Italy

Henrard, J. T. (1950). *Monograph of the genus Digitaria*. Leaden University Press, Leaden, Netherlands.

Ibrahim, A. (2001). Hungry rice (Fonio): A neglected cereal crop. NAQAS Newsletter, Vol. No. 4-5.

Jideani, I.A. (2012). *Digitaria exilis* (acha/fonio), *Digitaria iburua*(iburu/fonio) and *Eluesine Coracana* (tamba/finger millet) – Non- conventional cereal grains with potentials. *Scientific /Research and Essays* 7 **(45)**:3834-3843

Kwon-Ndung, E. H. (2014). Acha *Digitaria exilis* stapf.: A small but mighty plant. Fu Lafia senate Internal education 9th April

Kwon-Ndung, E. H. and Ochigbo, A. A. (2004). Acha fonio genetic diversity distribution and Conservation in Nigeria. *Plant genetic resources and food security in west and central Africa.* pp. 18 https://www.researchgate.net/publication/267839335

National Research Council (1996). *Lost crops of Africa.* National Academies press. pp.408. http://www.nap.edu/catalog/2305.html

Oguntoyinbo, J. S. (1983). "Climate". In: Oguntoyinbo, J.S., Areola, O. O. and Filani, M. (Eds.). *A Geography of Nigerian Development.* Heinemann Educational Books. Ibadan: pp.45-70.

Pulseglove, J. W. (1975). Tropical crops. Monocotyledons, vol. I. Longmans publication 531pp

Vietameyer, N. D., Borlaugh, N. E., Axtell, J., Burton, G.N., Harlan, J.R. and Rachie, K.O. (1996). *Fonio in Lost Crops of Africa*.Grains Bostid Publication. National Academy press, New York.Vol.1 pp. 59-75.

Vodouhe, S.R., Zannou, A. and Achigan-Dako, G. E. (2003). West Africa, Guinea Conakry, 4-6 August, 1998. IPGRI; Rome, Italy: Proceedings of the 1st workshop on Genetic Diversity of Fonio *(Digitaria exilis Staph)*.

GENETIC GAIN IN GRAIN YIELD AND AGRONOMIC TRAITS OF RELEASED MAIZE (*Zea mays* L.) CULTIVARS UNDER OPTIMUM GROWING CONDITIONS

M. Oyekunle* and B. Anjorin
Department of Plant Science, Institute for Agricultural Research,
Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
*Corresponding Author: moyekunle@abu.edu.ng

Abstract: Information on genetic gains is crucial for assessing progress made in breeding for improved cultivars over a period of time. A study was conducted to assess genetic gains in grain yield and other agronomic traits of released maize cultivars and to assess the relationship between grain yield and other traits. Twenty-two released cultivars plus two checks were evaluated under optimum growing conditions at Zaria in 2015 and 2016. The 24 cultivars were evaluated using 6 x 4 randomized incomplete block design with three replications. Data collected on grain yield and other traits were subjected to analysis of variance, correlation, and regression using appropriate statistical software. Genotype mean squares were significant ($p < 0.01$) for grain yield and all measured traits except anthesis-silking interval and husk cover. The mean grain yield of the cultivars increased from 2488 kg ha^{-1} for SAMMAZ 13 to 6007 kg ha^{-1} for SAMMAZ 48 with an average of 4065 kg ha^{-1}. The average rate of increase in grain yield was 125.4 kg ha^{-1}yr^{-1} corresponding to a genetic gain of 4.6% yr^{-1}. Grain yield had significant positive correlations with plant height (r = 0.6**) and ear height (r = 0.5**) whereas significant negative correlations with husk cover (r = -0.3**), plant aspect (r = -0.8**), and ear aspect (r = -0.8**). The substantial genetic gain in grain yield was associated with improvement in agronomic traits and this demonstrated progress made in breeding over a period of time.

Key words: Breeding Period, Correlation, Genetic Gain, Maize, Regression

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop in West Africa (WA) and ranked third after rice and wheat in the world [1]. In the sub-region, maize has rapidly gained popularity due to its high potential as a major food for human consumption, animal feed and industrial raw material. It is the most widely growing cereal crop in Nigeria [2] due to its high productivity, wide adaptation and relative ease of cultivation, processing, storage and transportation. It is consumed in large quantities daily in a variety of local food preparations and provides most of the calories, proteins, vitamins, and mineral intake in the diets of the poor household in the sub-region. It is potentially used to fill the hunger gap in the savannas of the sub-region in July when all other food reserves are depleted after the long dry period [3].

Genetic gain studies comparing old and new cultivars have been conducted routinely in the temperate zones in an effort to understand how genetic selection has shaped important traits such as grain yield in maize [4; 5; 6]. Maize breeders in developed countries, especially the United States and Canada, have measured breeding progress by comparing the performance of cultivars developed and released over a long period of time in the same environments [7]. Similar studies have been conducted in other crops such as soybean [*Glycine max* (L.) Merr.] [8], oats (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) [9; 10]. In general, the studies revealed that varieties developed in later breeding eras are superior in terms of grain yield and other agronomic traits. Similar studies have also been conducted in WA, one of which was reported by Kamara et al. [11] who reported a genetic gain of 0.4% per year for late-maturing maize cultivars released from 1970 to 1999 in the Nigerian savannas. In addition, Badu-Apraku et al. [12] reported that average rate of increase in grain yield under optimum growing conditions was 40 kg ha^{-1} yr^{-1} with a genetic gain of 1.3% yr^{-1} for early maize cultivars developed during three breeding eras.

In order to increase maize production in Nigeria, the Institute for Agricultural Research (IAR) Samaru, Zaria, in partnership with the International Institute of Tropical Agriculture (IITA), Ibadan, conducted research to develop several maize varieties for

registration and release for farmers use within the last two decades. Since 2000, several early, extra-early and intermediate/late maturing maize varieties with high yield potential and resistance or tolerant to biotic and/or abiotic stresses have been released in Nigeria. No direct comparisons of grain yield potential and other agronomic traits of the released varieties have been made under optimum growing conditions. It is therefore, important to assess genetic gain in grain yield of maize varieties released in the last two decades. This information is vital in assessing progress made in breeding for improved maize varieties and to identify source of genetic materials for development of inbred lines, synthetic varieties, and populations as well as valuable information for the seed enterprise in Nigeria. In addition, knowledge and understanding of the interrelationships among traits is crucial for designing effective breeding programs for maize improvement. The objectives of the study therefore were (i) to assess genetic gain in grain yield and other agronomic traits of released maize varieties and (ii) to assess the relationship between grain yield and other agronomic traits of released maize varieties.

MATERIALS AND METHODS

Twenty-two released maize cultivars from IAR Samaru plus two checks were used for the study as shown in Table1. Check 1 (SAMMAZ 51) passed through registration process and was released in October 2016. The 24 cultivars were evaluated at Zaria (northern Guinea savanna, 11º11′N, 7º38′ E, 640 m asl, 1200 mm annual rainfall) during 2015 and 2016 rainy seasons using 6 x 4 randomized incomplete block design with three replications. Experimental unit consisted of two-row 5-m-long plots, with inter- and intra-row spacing of 0.75 m and 0.40 m, respectively. Three seeds were planted per hill, and the resulting maize plants were thinned to two per stand about 2 weeks after emergence to give a final plant population density of 66,000 plants ha^{-1}. All trials received 60 kg NPK ha^{-1} in form of NPK 15-15-15 2 weeks after planting (WAP). An additional 60 kg N ha^{-1} top-dressed at 5 WAP. Weeds were controlled with herbicides and/ or manually.

Days to 50% anthesis and days to mid-silk were recorded as the number of days from planting to when 50% of plants shed pollen and had emerged silks, respectively. Anthesis-silking interval (ASI) was calculated as difference between number of days to mid-silk and days to 50% anthesis. Plant and ear heights were measured in centimeters as the distance from the base of the plant to the flag leaf and from the base to the node bearing the upper ear respectively. Plant aspect was rated on scale of 1 to 5 where 1 = true to type plants with minimal reduction in height, ear size, low ear placement, resistance to foliar diseases and lodging and 5 = plants with severally stunted growth, small ears, susceptible to foliar diseases and lodging. Ear aspect was scored on a 1 to 5 scale, where 1= clean, uniform, and large ears and 5= rotten, variable and small ears. Husk cover was rated on a scale of 1 to 5, where 1= husks tightly arranged and extended beyond the ear tip and 5= very loosely arranged husk with ear tip exposed. Numbers of ears per plant (EPP) was calculated as number of ears harvested divided by the number of plants at harvest. Samples of ears harvested from each plot were shelled to determine percent moisture. Grain yield adjusted to 15% moisture was computed from ear weight and grain moisture assuming, a shelling percentage of 80% (Menkir and Kling, 2007).

Combined analysis of variance (ANOVA) across years was performed on plot means for grain yield and other agronomic traits with PROC GLM in statistical analysis System (SAS) version 9.0 using a RANDOM statement with the TEST option [13]. In the combined ANOVA, year, genotypes and replicates were considered as random factors. The linear model for the combined ANOVA is as follows:

$$P_{bklmi} = \mu_i + Y_{ki} + R(Y)_{l(k)i} + B(RY)_{b(kl)i} + G_{mi} + GY_{kmi} + \varepsilon_{bklmi}$$

where P_{bklmi} is the observed measurement of trait i of m genotype within l replicate, in k year, b block within l replicate and k year, μ_i is mean of trait i , Y_{ki} is the random effect of year k on trait i, $R(Y)_{l(k)i}$ is the random effect of replication l within year k on trait i, $B(RY)_{b(kl)i}$ is the random effect of block b within replicate l and year k on trait i, G_{mi} is the random effect of genotype m on trait i, GY_{kmi} is the random effect of the interaction between genotype m and year k on trait i, and ε_{bklmi} is the experimental error effect associated with genotype m and block b within replication l and year k on trait i.

Pearson correlation analysis was carried out to determine the interrelationship among the traits. The relationship between cultivar yield and year of released

(expressed as number of years since 2001) was determined using regression analysis. The mean grain yield of the maize varieties was used as the dependent variable and regressed on the year of released as independent variables to obtain regression coefficient (b-value) using SAS version 9.0. The b-value was then divided by the intercept and multiplied by 100 to obtain the relative genetic gain per year [3].

RESULTS AND DISCUSSION

Table 1: Maize cultivars used for the study, their maturity and year of release in Nigeria

Cultivar	Year of release	Year reference to year of release	Maturity	Grain Colour
SAMMAZ 13	2001	1	Extra-early	White
SAMMAZ 14	2005	5	Early	White
SAMMAZ 15	2008	8	Intermediate	White
SAMMAZ 16	2008	8	Intermediate	White
SAMMAZ 17	2009	9	Intermediate	White
SAMMAZ 18	2009	9	Early	White
SAMMAZ 19	2009	9	Intermediate	White
SAMMAZ 20	2009	9	Early	White
SAMMAZ 26	2009	9	Intermediate	White
SAMMAZ 27	2009	9	Early	White
SAMMAZ 28	2009	9	Extra-early	White
SAMMAZ 29	2009	9	Extra-early	White
SAMMAZ 31	2009	9	Intermediate	White
SAMMAZ 32	2011	11	Extra-early	White
SAMMAZ 33	2011	11	Extra-early	White
SAMMAZ 34	2011	11	Early	White
SAMMAZ 35	2011	11	Early	White
SAMMAZ 37	2011	11	Intermediate	Yellow
SAMMAZ 38	2013	13	Intermediate	Yellow
SAMMAZ 39	2013	13	Intermediate	Yellow
SAMMAZ 40	2013	13	Intermediate	Yellow
SAMMAZ 48	2016	16	Early	White
Check 1 (SAMMAZ 51)	2016	16	Intermediate	White
Check 2 (TZComp1syn-W)		16	Intermediate	White

Table 2: Analysis of variance of grain yield and other agronomic traits of released maize cultivars evaluated at Zaria in 2015 and 2016

Source	df	Grain yield	Days to anthesis	Days to silking	ASI	Plant height	Ear height	Husk cover	Plant aspect	Ear aspect	EPP
Year	1	82490435*	165.0*	336.1*	33.3	38669.2*	14749.0*	0.12	1.2*	20.8*	0.48*
Block (Rep x Year)	18	3138140	1.3	2.3	0.9	567.4	205.6	0.6	0.1	0.1	0.03
Rep (Year)	4	1218477	1.3	5.2	0.8	370.7	385.7	0.4	0.1	0.1	0.95
Cultivar	23	9765704**	18.0**	14.4**	1.8	411.7*	432.4**	0.6*	0.4**	0.4**	0.07**
Cultivar x Year	23	697939*	7.6*	7.0*	0.8	234.6*	83.7**	0.3	0.1*	0.2*	0.20**
Error	384	3015959	1.9	2.6	1.1	227	148.8	0.3	0.1	0.1	0.05

*, ** Significant difference at P< 0.05 and P < 0.01 levels, respectively. ASI, anthesis-silking interval EPP, number of ears per plant

Table 3: Grain yield and other agronomic traits of released maize cultivars evaluated at Zaria in 2015 and 2016

Cultivar	Grain yield, kg ha^{-1}	Days to anthesis	Days to silking	ASI	Plant height, cm	Ear height, cm	Husk cover	Plant aspect	Ear aspect	EPP
SAMMAZ 48	6007	58	59	1.5	159	76	1.9	2.4	2.4	1.0
SAMMAZ 14	5567	54	56	3.5	158	78	1.8	2.5	2.5	0.8
Check 1 (SAMMAZ 51)	5471	57	60	3.0	155	67	2.2	2.6	2.6	1.0
SAMMAZ 29	5249	55	59	3.5	124	47	2.2	3.1	4.1	2.0
SAMMAZ 19	4971	60	61	1.5	170	75	2.4	2.0	2.5	1.0
SAMMAZ 26	4961	58	59	1.5	154	68	2.8	2.7	3.2	1.1
SAMMAZ 17	4767	62	63	1.5	151	71	2.6	2.6	3.1	1.4
SAMMAZ 35	4480	58	60	2.0	148	59	2.6	2.7	3.2	0.8
SAMMAZ 34	4333	54	56	2.5	147	64	3.4	2.9	3.4	1.0
SAMMAZ 20	4186	53	56	3.0	134	54	2.4	2.9	3.9	0.9
SAMMAZ 33	4115	55	58	3.0	144	62	3.5	2.5	3.5	1.1
SAMMAZ 18	4110	56	59	2.5	154	76	2.8	2.9	3.4	1.0
SAMMAZ 16	4093	61	63	2.5	150	67	2.5	2.7	3.2	0.8
SAMMAZ 40	4089	56	58	2.0	163	79	2.7	2.8	2.8	0.9
SAMMAZ 39	3956	59	60	1.5	148	65	1.7	3.0	3.0	1.1
SAMMAZ 38	3865	58	61	3.0	150	71	2.7	2.9	3.4	0.9
Check 2	3858	58	60	2.0	144	63	3.0	3.0	3.5	0.9
SAMMAZ 15	3639	59	61	2.0	158	66	2.7	2.9	3.4	1.1
SAMMAZ 27	3112	57	59	1.5	130	48	3.2	2.9	3.4	1.3
SAMMAZ 28	3028	55	57	1.5	125	46	3.1	2.9	3.9	1.0
SAMMAZ 31	2829	55	57	2.0	138	52	2.6	2.9	3.4	1.0
SAMMAZ 37	2603	55	57	2.0	148	67	2.5	3.0	3.0	0.7
SAMMAZ 32	2536	53	55	2.0	130	50	2.4	3.0	4.0	0.7
SAMMAZ 13	2488	54	56	2.0	141	58	3.3	3.0	4.0	1.0
Mean	4065	56	59	2.2	146.7	64	2.6	2.8	3.3	1.0
SE	1196	1	1	0.1	12.3	10.0	0.5	0.3	0.3	1.1
CV	25	3	3	20.0	8.6	15.0	22.0	14.6	14.6	0.2

ASI, anthesis-silking interval; EPP, number of ears per plant

Table 4: Correlation coefficients of grain yield and other agronomic traits of maize cultivars evaluated at Zaria in 2015 and 2016

	Days to mid-silk	Plant height	Ear height	Husk cover	Plant aspect	Ear aspect	ASI	EPP	Grain yield
Days to anthesis	0.9**	0.1	0.1	-0.1	-0.1	-0.1	-0.4**	0.1	0.0
Days to mid-silk		0.1	0.2	-0.1	-0.1	-0.1	0	0.1	0.1
Plant height			0.8**	-0.2	-0.5**	-0.5**	0.1	-0.1	0.6**
ear height				-0.2	-0.5**	-0.5**	0.3**	-0.2*	0.5**
Husk cover					0.5**	0.5**	0.1	0.1	-0.3**
Plant aspect						0.7**	-0.1	0.2	-0.8**
Ear aspect							-0.1	0.2	-0.8**
ASI								-0.1	0.2
EPP									0.2

*, ** Significant difference at P< 0.05 and P < 0.01 levels, respectively.

ASI, anthesis-silking interval

EPP, number of ears per plant

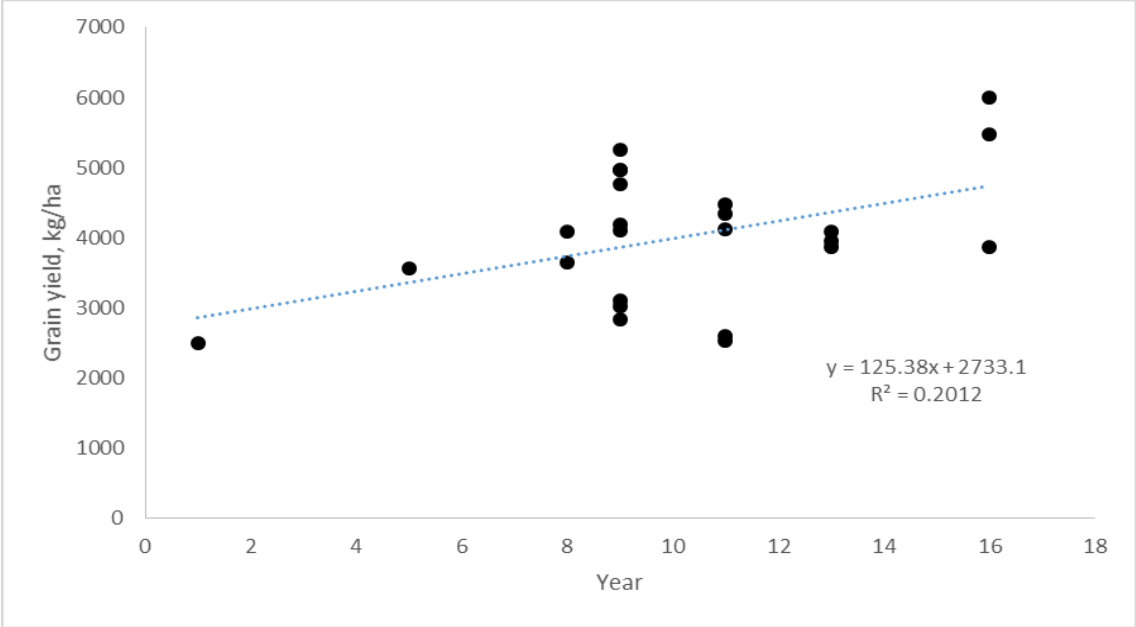


Fig. 1: Regression analysis of grain yield of released maize varieties over years of release

Table 5: Relative genetic gain of grain yield and other agronomic traits of released maize cultivars evaluated at Zaria in 2015 and 2016

Trait	Relative genetic gain (% per year)	R^2	a	b
Grain yield, kg/ha	4.59	0.201	2733.1	125.38
Days to anthesis	0.29	0.047	55.0	0.159
Days to mid-silk	0.28	0.058	57.1	0.158
ASI	-0.65	0.006	2.4	-0.015
Plant height, cm	0.43	0.030	140.6	0.607
Ear height, cm	0.99	0.036	57.9	0.571
Husk cover	-1.04	0.047	2.9	-0.031
Plant aspect	-0.18	0.005	2.8	-0.005
Ear aspect	-1.34	0.122	3.8	-0.051
EPP	-0.72	0.012	1.1	-0.008

ASI, anthesis-silking interval
EPP, number of ears per plant

Analysis of Variance and mean performance of maize cultivars

The combined analysis of variance for grain yield and other agronomic traits of the 24 maize cultivars reveled that cultivar mean squares were highly significant ($P < 0.01$) for all measured traits except plant height and husk cover at $P < 0.05$ and ASI $P > 0.05$ (Table 2). The presence of significant difference among the cultivars for all measured traits, except ASI indicated that adequate genetic variability existed among the cultivars released during the two decades of maize breeding. The existence of variability among the cultivars should allow significant progress from selection for improvements in most of the measured traits and identification of source of genetic materials for development of inbred lines and populations. Inbred lines could be extracted from the superior maize cultivars identified in the present study through pedigree breeding or the use of double haploid technique. Year and cultivar x year interaction mean squares were significant for all measured traits except ASI and husk cover. The presence of significant difference between the years for most of the measured traits indicated that each year was unique and that there is the need to extensively test cultivars in multiple years for identifying high-yielding cultivars. Similarly, the significant interaction between cultivars and years for grain yield and other measured traits indicated that the expression of these traits would not be consistent in varying years. This result suggests the need to evaluate cultivars in multiple years to identify genotypes with consistent performance in contrasting test years. This result in agreement with the finding of earlier workers [3; 14; 15]. The mean grain yield of the cultivars ranged from 2488 kg ha^{-1} for SAMMAZ 13 to 6007 kg ha^{-1} for SAMMAZ 48 with an average of 4065 kg ha^{-1}. The highest yielding cultivar, SAMMAZ 48 out-yielded the check 2 by 36%.

Correlation between grain yield and other agronomic traits

Results of this study revealed significant positive correlations between grain yield and plant height (r = 0.6**) and ear height (r = 0.5**) (Table 4). In contrast, grain yield had negative and significant correlations with husk cover (r = -0.3**), plant aspect (r = -0.8**), and ear aspect (r = -0.8**), indicating that increased grain yield was associated with improvement in husk cover, and plant and ear aspects. These results are in agreement with the findings of Badu-Apraku et al. [15; 16]. Plant height had significant and positive correlations with ear height (r = 0.8**). Days to anthesis had positive and significant correlations with days to silking (r = 0.9**), but negative and significant correlation with ASI (r = -0.4**) (Table 4). Plant aspect had significant positive correlations with husk cover, and ear aspect but negative correlation with plant and ear heights. These results are in agreement with the findings of Badu-Apraku et al. [3]. The authors reported that plant aspect had significant positive correlations with number of days to anthesis and silking, husk cover, and ear aspect but negative correlation with plant height and EPP under both drought and optimum growing environments. The lack of significant correlations between grain yield and days to silking and anthesis is in disagreement with the findings of Badu-Apraku et al. [3] who reported that grain yield had significant positive correlations with days to anthesis and silking

Genetic Gain in grain yield of released cultivars

Significant increase in the grain yield of released maize cultivars occurred during the breeding period (Table 5, Fig. 1). Mean grain yield of the released maize cultivars increased from 2488 kg ha^{-1} for SAMMAZ 13 for cultivar released in 2001 to 6007 kg ha^{-1} for SAMMAZ 48 (released in 2017) with an average of 4065 kg ha^{-1}. The R^2 values were low, ranging from 0.005 for plant aspect to 0.2 for grain yield, indicating low association between year of release and all measured traits except grain yield. The average rate of increase in grain yield was 125.4 kg $ha^{-1}yr^{-1}$ corresponding to a genetic gain of 4.6% yr^{-1} (Table 5). The genetic gain in grain yield under optimum growing conditions was associated with improved plant and ear aspects, short ASI, plant and ear heights, and improved husk cover. Plant aspect had a genetic gain of -0.2% yr^{-1}, while the values for ear aspect, husk cover, ASI, plant height and ear height were -1.3% yr^{-1}, 1.0 % yr^{-1}, -0.7% yr^{-1}, 0.4% yr^{-1}, 1.0% yr^{-1}, respectively. However, the lack of improvement in EPP of released cultivars in the present study was in disagreement with the finding of Badu-Apraku et al. [3; 17] who reported EPP to be a major component of increased grain yield in recurrent selection programs. The genetic gain of 2.7% yr^{-1} observed in grain yield under optimum growing condition in the present study is substantially greater than those reported by Badu-Apraku et al. [3;

12] for early maturing maize varieties. In a study conducted by Badu-Apraku et al. [3], the authors reported genetic gain of 1.3% yr^{-1} which was associated with improved plant and ear aspects, increased EPP, plant and ear heights, and improved husk cover. The differences in the observed genetic gain reported in the present study and those reported by Badu-Apraku et al. [3; 12] could be due to the environments under which the cultivars were evaluated, the type of material evaluated, methods of development of the cultivars, breeding periods, and number of cultivars involved in the evaluations. The high genetic gain in grain yield in the present study is not surprising because the cultivars used in the study are improved cultivars released over a breeding period of 16 years. The results of the present studies suggested that the breeding strategies including recurrent selection, backcrossing, hybridization and selection indices utilized in developing improved maize cultivars are effective. The results clearly indicated that new cultivars possess favourable genes that make them better performing than the old cultivars.

CONCLUSIONS

Substantial progress was made in breeding cultivars during the last two decades in Nigeria. The average rate of increase in grain yield was 125.4 kg $ha^{-1}yr^{-1}$ corresponding to annual genetic gain of 4.6% under optimum growing conditions. SAMMAZ 48 was the highest yielding and should serve as source of genetic materials for development of inbred lines, synthetic varieties, and populations. Grain yield had significant positive correlations with plant height and ear height but negative correlations with husk cover, plant aspect, and ear aspect. The substantial genetic gain in grain yield was associated with improvement in agronomic traits and this demonstrated progress made in breeding over the last 16 years.

REFERENCES

1. A.B. Olaniyan, Maize: Panacea for hunger in Nigeria. Afri. J. Plant Sci. 9 (2015) 55-174.

W.A. Russell, Agronomic performance of maize cultivars representing different eras of breeding. Maydica 29 (1984) 375–390.

2. FAOSTAT, Food and Agriculture Organization of the United Nations, Food Security Statistics, 2014. 2016.
3. B. Badu-Apraku, M. Oyekunle, A. Menkir, K. Obeng-Antwi, C.G. Yallou, I.S. Usman, H. Alidu, Comparative performance of early maturing maize cultivars developed in three eras under drought stress and well-watered environments in West Africa. Crop Sci. 53 (2013) 1298–1311.
4. D. N. Duvick, Genetic progress in yield of United States maize (*Zea mays* L.). Maydica 50 (2005) 193–202.
5. H. Campos, M. Cooper, G.O. Edmeades, C. Löffler, J.R. Schussler, M. Ibañez, Changes in drought tolerance in maize associated with fifty years of breeding for yield in the U.S. Corn Belt. Maydica 51 (2006) 369–381.
6. T. Y. Wang, X. L. Ma, Y. Li, D.P. Bai, C. Liu, Z.Z. Liu, X.J. Tan, Y.S. Shi, Y.C. Song, M. Carlone, D. Bubeck, H. Bhardwaj, E. Jones, K. Wright, S. Smith Changes in yield and yield components of single-cross maize hybrids released in China between 1964 and 2001. Crop Sci. 51 (2011) 512–525.
7. M. Tollenaar, Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1988. Crop Sci. 29 (1989) 1365–1371.
8. H. Tefera, A.Y. Kamara, B. Asafo-Adjei, K.E. Dashiell Improvements in grain and fodder yields of early maturing promiscuous soybean cultivars in the Guinea savannas of Nigeria. Crop Sci. 49 (2009) 2037–2042.
9. M.S. Lopes, M.P. Reynolds, Y. Manes, R.P. Singh, J. Crossa, H.J. Braun, Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a "Historic" set representing 30 years of breeding. Crop Sci. 52 (2012) 1123–1133.
10. Y.G. Xiao, Z.G. Qian, K. Wu, J.J. Liu, X.C. Xia, W.Q. Ji, Z.H. He, Genetic gains in grain yield and physiological traits of winter wheat in Shandong province, China, from 1969 to 2006. Crop Sci. 52 (2012) 44–56.
11. A.Y. Kamara, A. Menkir, M.A.B. Fakorede, S.O. Ajala, B. Badu-Apraku, I. Kureh, Agronomic performance of maize cultivars representing three decades of breeding in the Guinea savannas of West and Central Africa. J. Agric. Sci. 142 (2004) 567–575.
12. B. Badu-Apraku, M.A.B. Fakorede, M. Oyekunle, G.C. Yallou, K. Obeng-Antwi A. Haruna, I.S. Usman, R.O. Akinwale, Gains in grain yield of early maize cultivars developed during three breeding eras under multiple environments. Crop Sci. 55 (2015) 527-539.
13. SAS Institute, SAS user's guide. version 9.0. Cary, NC: SAS Institute Inc. (2002).
14. M.J. Moghaddam, S.S. Pourdad, Comparison of parametric and non-parametric methods for analyzing genotype x environment interactions in safflower (*Carthamus tinctorius* L.). The J. Agric. Sci. 147 (2009) 601-612.
15. B. Badu-Apraku, R. O. Akinware, S. O. Ajala, A. Menkir, M.A.B. Fakorede, M. Oyekunle,

Relationships among traits of Tropical Early Maize Cultivars in contrasting environments. Agron. J. 103 (2011) 717-729.

16. B. Badu-Apraku, R.O. Akinwale, J. Franco, M. Oyekunle, Assessment of reliability of secondary traits in selecting for improved grain yield in drought and low-nitrogen environments. Crop Sci. 52 (2012) 2050-2062.
17. B. Badu-Apraku, M.A.B. Fakorede, A.F. Lum, R.O. Akinwale, Improvement of yield and other traits of extra-early maize under stress and nonstress environments. Agron. J. 101 (2009) 381–389.

EVALUATION OF NINETEEN GENOTYPES OF COWPEA, *Vigna unguiculata* (L.) WALP FOR YIELD AND YIELD RELATED TRAITS

Showemimo, F. A., Eniseyin, O. O. and Porbeni, J. O.
Department of Plant Breeding and Seed Technology, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta. Nigeria.
Corresponding Author E-mail: showfa2002@yahoo.com

Abstract: The study was conducted to determine the variation among wild and domesticated genotypes of cowpea based on seed yield and yield related traits. The experiment was laid out in a randomized complete block design replicated 3 times at the Research Farm of the Federal University of Agriculture Abeokuta, Ogun state, Nigeria during the 2015/2016 and 2016/2017 cropping seasons. Data were taken on number of pods per plant, pod length, pod width, 100 seed weight, dried pod weight, shaft weight and seed yield (t/ha). There was analysis of variance, mean separation, computation of performance index (PI) and classification analysis. Genotypic differences were significant for all the traits evaluated. The variability among the genotypes in this study confirmed the inherent variability and the possibility of their exploitation for crop improvement. The superior wild cowpea genotypes for yield were NGB1158 (3.27t/ha), NGB001089 (2.43t/ha) and NGB1075 (2.40t/ha), and the best 3 genotypes among the improved cowpea varieties were IT07K-292-10 (3.53t/ha), TVNU1250 (3.33t/ha) and SOKOTO (3.27t/ha). Genotype seed yield/ha was plotted against their Performance Index Rank (PI) to classify or group the wild cowpea genotypes, cultivated cowpea and combined genotypes. The ideal cowpea genotypes that can be deployed and also for possible genetic manipulations were 3 wild and 5 cultivated cowpea genotypes viz NGB1158, NGB001089, NGB1075, SOKOTO, IT07K-292-10, TVNU1250, IFE BROWN and IT07K-318-33 having seed yield from 2.4t/ha to 3.5t/ha with good PI.

Key words: Performance, Wild, Domesticated, Yield, Cowpea Genotypes, Classification

INTRODUCTION

Cowpea is an important pulse crop in the savannah regions of West and Central Africa, where it is also an important vegetable and a valuable source of fodder. The cowpea (*Vigna unguiculata* [L.] Walp) is an important legume widely cultivated in tropics and subtropics for forage, green pods and grains [1]. Cowpea seed is reported to contain 24% crude protein, 53% carbohydrates, and 2% fat [2].

Cowpea is widely cultivated and consumed extensively in Asia and tropical Africa [3]. On the African continent, West Africa represents the largest production zone [4]. Cowpeas are mainly grown in the warm climates since they require warm soil temperatures for good establishment [5,6]. They are adapted to a wide variety of soils from heavy to light textured and from the humid tropics to the semi-arid tropics. It has the useful ability to fix the atmospheric nitrogen through its root nodules and it also grows well in poor soil conditions [4,7].

Several researchers have studied cowpea production and productivity in the temperate and tropical regions, including their value addition and adaptations [2,5,8,9]. However, genetic improvement of *V. unguiculata* is continuous, involving wild and cultivated genotypes. Germplasm evaluation is therefore necessary to provide baseline information for possible hybridization and/or selection. The objective of this study was the evaluation of cowpea gene pool of nineteen genotypes for genetic variability, performance analysis and classification.

MATERIALS AND METHOD

Nineteen cowpea genotypes (8 domesticated and 11 wild types) collected from the Germplasm Unit of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, were used for the study (Table 1). The experiment was conducted at the R*e*search Farm of the Federal University of Agriculture Abeokuta, in South-Western part of Nigeria with wet Savannah/Forest transition zone for two cropping seasons of 2015 and 2016. The annual

rainfall of 900 – 1650mm was recorded with a wet season of 130 – 250 rainy days, the soil type was sandy loam, relative humidity ranged between 70% and 85%, minimum and maximum temperature was 22^0C and 34^0C respectively.

The field layout was randomised complete block design (RCBD) with three replicates. The plot size was 5m long with inter and intra-row spacing of 75cm x 25cm. The 19 genotype seeds were sown at a depth of 4 – 5cm, at 2 seeds per hole. At 2 weeks after planting the seedlings were thinned to single plant stand per hole to maintain a total of 20 plants per plot. Manual weeding was carried out at the third week after planting and subsequent weeding was was done as the need arose. Cropthrin 10EC, which contains 100g/L of cypermethrin as emulsion concentrate was used to control insect pests especially at the onset of flowering and pod initiation. Ripe pods were handpicked at maturity during harvesting. Pods were spread for further sun drying before individual storage to avoid mix up.

The following data were obtained for statistical analysis from 10 plants per replicate: number of pods per plant, pod length (cm), pod width (cm), seed yield per pod, 100 – seed weight (g), dried pod weight (g), shaft weight (g) and seed yield (t/ha). The data collected were subjected to Analysis of Variance (ANOVA). There was mean separation using the Duncan Multiple Range Test (DMRT). Performance Index (PI) Rank was calculated for positive seed yield related traits. Classificatory analysis was done graphically by plotting seed yield against PI as used by Singh et al [5] and Manggoel et al [10].

RESULTS AND DISCUSSION

There was significant variation among the nineteen genotypes for the eight characters evaluated (Table 2). This suggests that variability exists among the cowpea genotypes and such variability can be exploited for crop improvement. This is in agreement with variability reported in cowpea populations [9,10]. These traits are also good performance indices that reveal the appreciable amount of variation among cowpea genotypes as reported by Khanpara [11] and Nwofia [12]. The combined analysis was in agreement with the findings of Nwosu et al [13].

In Table 3, the following genotypes IFE BROWN, SOKOTO, SAMPEA 10, IT90K-292-2, IT07K-292-10, IT07K-318-33, TVNU1250 and NGB001089 had seed yield above 2.4t/ha, they also had higher number of pod, seeds per pod, dried pod weight, shaft weight, pod width and pod length. The test genotypes exhibited appreciable amount of genotypic performance inherent in the best performing genotypes. Premium can therefore be placed on the phenotypic breeding or selection value. Similar result was reported by Nwofia [11] and Nwosu *et al* [12]. Five wild cowpea genotypes; NGB001067, NGB001067, NGB00975, NGB001009, NGB00063 and one improved cowpea genotype (Ife BPC) performed poorly across the 2 years of evaluations, these genotypes may not be for immediate deployment but can be retained for further breeding purposes.

According to Timko and Singh [6], Davies [14] and Singh *et al* [15], classification analysis of cowpea helped in identifying genotypes in several groups. In this study, genotypes with high seed yield and low value PI are more desirable. All the eleven wild cowpea genotypes had seed yield above 3t/ha (genotypic average), however, five wild cowpea genotypes had low PI (Fig. 1). Five wild cowpea genotypes in the first quadrant (Fig. 1) had excellent performance as in their high seed yield and low PI (lower than average) and they are excellent genotypes that can be selected for cowpea improvement programme.

The cultivated cowpea genotypes were grouped in to 3 quadrants (Fig. 2). The 4 cultivated cowpea genotypes had high seed yield with good PI. Khanpara *et al* [11] and, Sarath and Reshma [16] also emphasized the use of these traits as genotypic performance index in the identification, grouping and selection of superior genotypes of cowpea.

The combined classificatory analysis of all the genotypes is presented in Fig. 3. Nine genotypes performed poorly, having seed yield of less than the genotypic mean of 2.4t/ha combined with poor PI as grouped in A. Two genotypes (SAMPEA 10 and IT90K-212-2) were classified in group B (high seed yield but poor PI), group C has one genotype

(NGB001175) having less seed yield than the genotypic mean but good PI. Group D has the ideal genotypes showcasing 8 (3 wild and 5 cultivated cowpea) genotypes; NGB1158, NGB001089, NGB1075, SOKOTO, IT07K-292-10, TVNU1250, IFE BROWN and IT07K-318-33, having seed yield from 2.5t/ha to 3.5t/ha with good PI. These genotypes can be selected for crop improvement programme. Similarly, Ayanlere *et al* [9], Nwosu *et al* [13] and Gupta [17] also reported that plotting yield against computed performance index or rank is an easy and rapid method of selecting desirable genotypes.

Table 1: Description and source of 19 cowpea genotypes used for the study

S/N	Genotype	Plant type	Source
Wild/Local/Unimproved Cowpea Genotype			
1	NGB1075	Wild, local, unimproved Cowpea	NACGRAB
2	NGB001066	Wild, local, unimproved Cowpea	NACGRAB
3	NGB001099	Wild, local, unimproved Cowpea	NACGRAB
4	NGB1158	Wild, local, unimproved Cowpea	NACGRAB
5	NGB001075	Wild, local, unimproved Cowpea	NACGRAB
6	NGB001068	Wild, local, unimproved Cowpea	NACGRAB
7	NGB001067	Wild, local, unimproved Cowpea	NACGRAB
8	NGB00975	Wild, local, unimproved Cowpea	NACGRAB
9	NGB001163	Wild, local, unimproved Cowpea	NACGRAB
10	NGB001089	Wild, local, unimproved Cowpea	NACGRAB
11	TVNU 1250	Wild, local, unimproved Cowpea	NACGRAB
Domesticated/Cultivated/Improved Cowpea Genotype			
12	SAMPEA 6	Cultivated, improved, Cowpea	NACGRAB
13	SMAPEA 10	Cultivated, improved, Cowpea	NACGRAB
14	IT-90K-292-2	Cultivated, improved, Cowpea	IITA
15	IT 07K-318-33	Cultivated, improved, Cowpea	IITA
16	IT 07K-292-10	Cultivated, improved, Cowpea	IITA
17	IFE BPC	Cultivated, improved, Cowpea	NACGRAB
18	IFE BROWN	Cultivated, improved, Cowpea	NACGRAB
19	SOKOTO	Cultivated, improved, Cowpea	NACGRAB

NACGRAB – National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria.
IITA – International Institute of Tropical Agriculture, Ibadan, Nigeria.

Table 2: Mean Squares from combined analysis of variance of seed yield and yield related traits of cowpea grown in Abeokuta, Nigeria for 2 years

Source of variation	Df.	No. of Pods	Pod Length (cm)	Pod Width (cm)	Seed Per Pod	100 seed Weight (g)	Seed Yield (t/ha)	Dried Pod Weight(g)	Shaft Weight (g)
Rep.	2	119.42*	3.23	0.001	7.5	0.11*	3.27**	1.94	0.21
Genotypes (G)	18	123.16**	22.18**	0.17**	11.76*	0.5**	1.48**	2.12**	0.34*
Year (Y)	1	116.87	19.09	0.06	10.13	0.07	1.52	2.01	0.30
G x Y	18	103.15	20.04	0.11	10.86	0.34	1.27	1.99	0.29
Pooled Error	74	27.05	2.056	0.001	5.84	0.03	0.43	0.602	0.18

*, ** significance at 5% and 1% level of probability

Table 3: *Mean performance (across years) of seed yield and yield related traits in 19 cowpea genotypes grown in Abeokuta

Genotypes	No. of pods	Pod length (cm)	Pod width(cm)	Seed per pod	100 Seed wt(g)	Seed yield (t/ha)	Dried pod wt (g)	Shaft weight (g)
Wild/Local/Unimproved Cowpea Genotype								
NGB1075	24.33bc	8.83e-h	0.50de	8.00c-e	0.83c-e	2.40a-d	2.90b-g	0.50b
NGB001066	20.67b-d	8.00f-j	0.40fg	8.67a-e	0.90de	1.87c-e	2.30dg	0.43b
NGB001099	15.33cd	7.67f-j	0.33g	11.00a-e	0.77e	0.93e	1.40g	0.47b
NGB1158	30.33b	7.00h-j	0.40fg	9.67a-e	0.77e	3.27ab	3.97a-c	0.60b
NGB001075	21.67b-d	10d-g	0.43ef	13.00ab	0.70ab	2.30a-d	2.60b-g	0.30b
NGB001068	17.67cd	10.2d-f	0.47def	10.00a-e	0.70e	2.13b-e	2.50c-g	0.37b
NGB001067	14.67cd	8.67e-i	0.53d	13.33a	1.17cd	1.63c-e	2.03e-g	0.40b
NGB00975	19.67cd	9.00e-h	0.40fg	11.33a-e	0.77e	1.87c-e	2.37dg	0.50b
NGB001163	16.67cd	6.33h-j	0.50de	11.67a-e	0.80e	1.57de	1.93fg	0.37b
NGB001089	23.00b-d	8.00f-j	0.40fg	12.33a-d	0.87de	2.43a-d	2.87bg	0.43b
TVNU 1250	39.33a	5.33j	0.40fg	7.67de	0.67e	3.33ab	3.80a-d	0.47b
Domesticated/Cultivated/Improved Cowpea Genotype								
SAMPEA 6	14.33cd	13.00a-c	0.97a	8.33b-e	1.96de	2.30a-d	2.87b-g	0.57b
SMAPEA 10	16.67cd	11.67a-d	0.8b	10.00a-e	1.70ab	2.50a-d	2.93b-f	0.43b
IT-90K-292-2	14.00cd	7.33g-j	0.97a	7.00e	0.90de	2.90a-c	3.33a-f	0.43b
IT 07K-318-33	20.33cd	12.17a-d	0.83b	9.00a-e	1.348b	2.87a-c	4.77a	1.90a
IT 07K-292-10	16.00cd	14.67a	1.00a	9.00a-e	1.53b	3.53a	4.07ab	0.53b
IFE BPC	13.33d	6.00ij	0.70c	7.33e	1.50b	1.93c-e	2.50c-g	0.57b
IFE BROWN	24.33bc	13.93ab	0.80b	12.67a-c	1.43bc	2.90a-c	3.47a-e	0.57b
SOKOTO	21.67b-d	11.00cd	0.93a	10.33a-e	1.93a	3.27ab	3.90a-e	0.63b

*: Means followed by similar alphabets were not significantly different from one another according to Duncan's multiple range tests at 5% probability level.

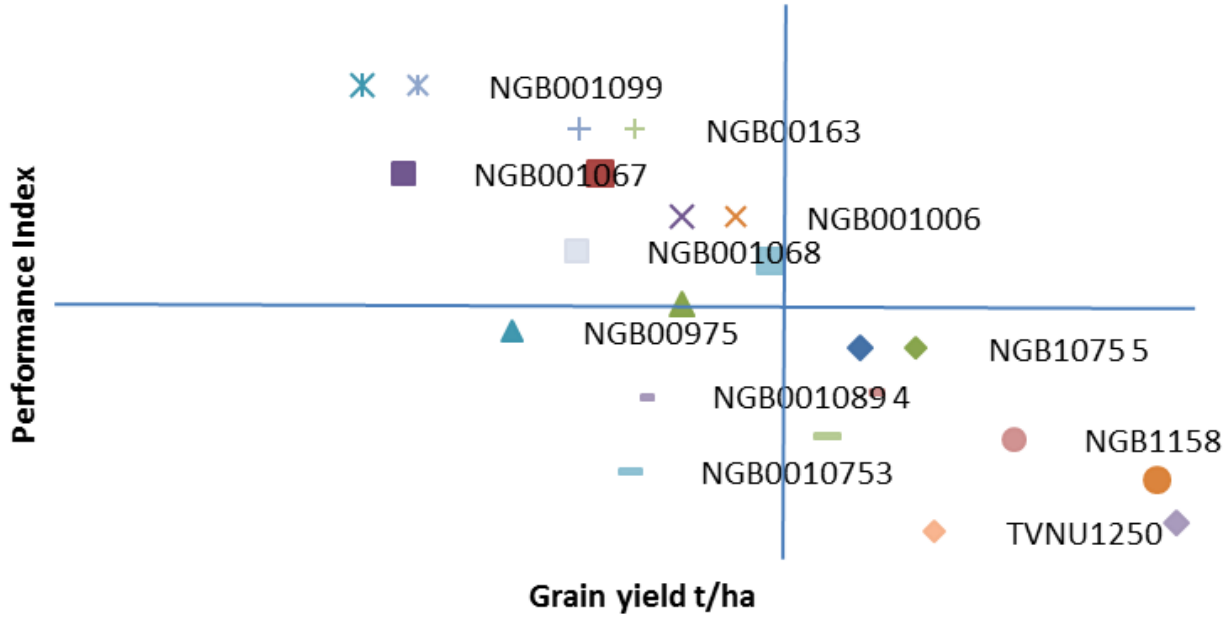


Fig. 1: Classification of Wild Cowpea Genotypes

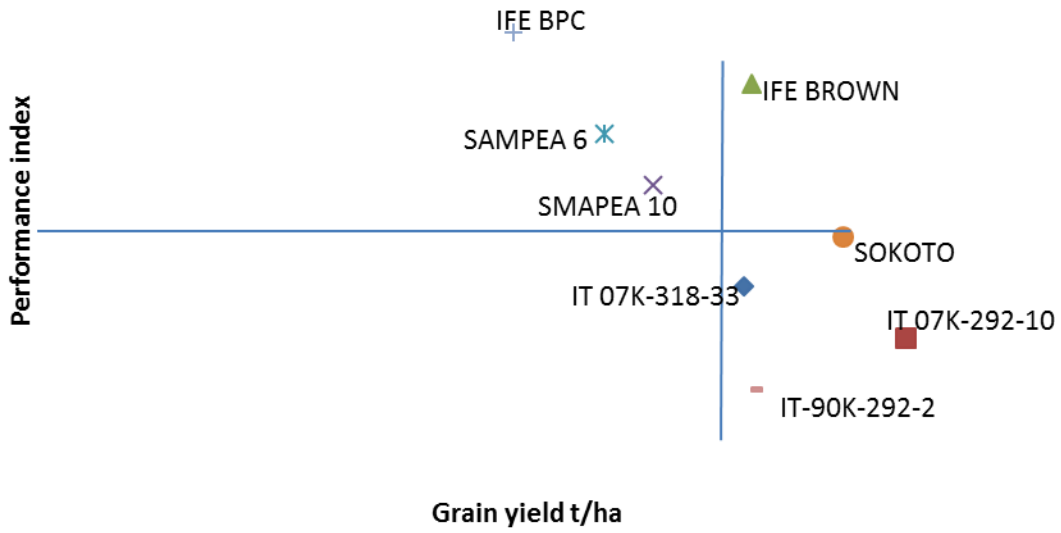


Fig.2. Classification of Cultivated Cowpea Genotypes

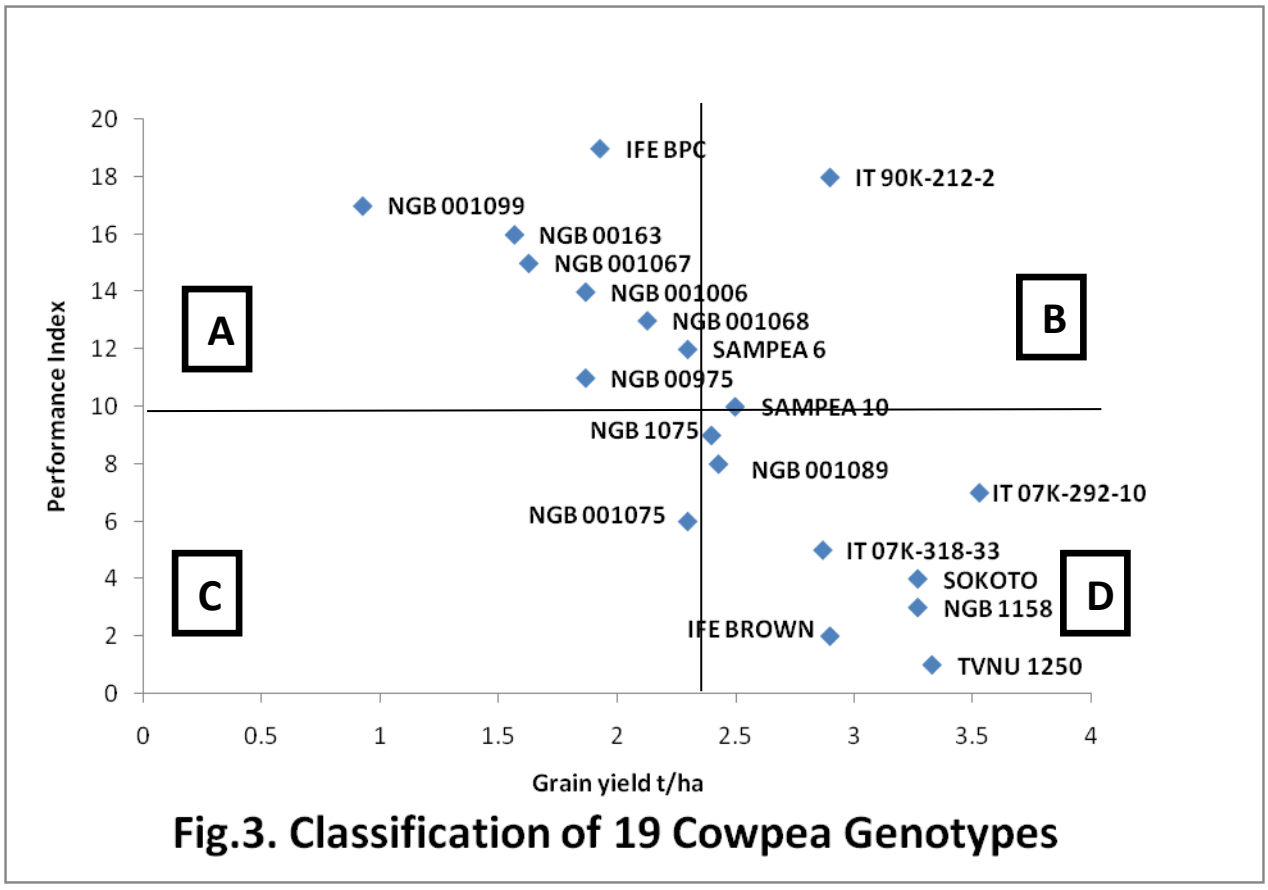


Fig.3. Classification of 19 Cowpea Genotypes

CONCLUSION

The study revealed that there was appreciable variation among the genotypes thus, good basis for crop improvement. The use of performance index along with seed yield classified genotypes into those with good seed yield combined with good performance index, good seed yield and poor performance index and out rightly poor performing genotypes. Eight genotypes (3 wild and 5 cultivated cowpea) viz:- NGB1158, NGB001089, NGB1075, SOKOTO, IT07K-292-10, TVNU1250, IFE BROWN and IT07K-318-33 having seed yield from 2.5t/ha to 3.5t/ha with good PI were identified as suitable genotypes for immediate deployment and can also be included in further crop improvement.

REFERENCES

[1] Ali, Y., Aslam, Z., Hussain, F., Shakur, A. (2004) Genotype and environmental interaction in cowpea (*Vigna unguiculata* L.) for yield and disease resistance. Inter. J. Environ. Sci. Technol., 1 (2): 119-123.

[2] Food and Agriculture Organization (FAO) (2012) Grassland species index. *Vigna unguiculata* fact sheet for 2011/2012 Production and Productivity data on www.fao.org

[3] Diouf, D. (2011) Recent advances in cowpea [*Vigna unguiculata* (L.) Walp.] research for genetic improvement, *African Journal of Biotechnology*, 10(15): 2803-2810.

[4] Pottorff, M., Ehlers, J. D., Fatokun, C., Philip, A., Close, R. T. J. (2012) Leaf morphology in cowpea [*Vigna unguiculata* (L.) Walp]: QTL analysis, physical mapping and identifying a candidate gene using synteny with model legume species BMC. Genomics, 13:234-236.

[5] Singh, B., Ajeigbe, H. A., Tarawali, S. A., Fernandez-Rivera, S., Abubakar, M. (2003) Improving the production and utilization of cowpea as food and fodder. Field Crops Research, 64: 145 – 150.

[6] Timko, M. P., Singh, R. (2008) Cowpea, a multifunctional legume. In: Moore, P. H., Ming, R. (Eds.), Genomics of tropical crop plants. Springer, New York, pp. 227-257.

[7] Tsegaye, E., Dechasa, N., Devakara, E. V. (2007) Genetic variability for yield and other agronomic traits in sweet potato. J. Agron., 6(1): 94-99.

[8] Kebe, K., Sembene, M. (2011) Cowpea (*Vigna unguiculata* (L.) Walp) field infestation by the bruchids (*Coleoptera: Bruchidae*) in the northern Senegal: preliminary biological and ecological data. J. Appl. Biosci., 41: 2788-2796.

[9] Ayanlere, A. E., Mohammed, A. B., Dutse, F., Abdullahi, M., Mohammed-Lawal, A. (2012). An assessment of the economics of maize-cowpea cropping system in Ogun area of Kwara State, Nigeria Biological and Environmental Sciences Journal for the Tropics, 9 (1): 39-43.

[10] Manggoel, W., Uguru, M. I., Ndam, O. N., Dasbak, M. A. (2012) Genetic variability, correlation and path coefficient analysis of some yield components of ten cowpea [*Vigna unguiculata* (L.) Walp] accessions. J. of Plant Breeding and Crop Science, **4**(5): 80-86.13.

[11] Khanpara, S. V., Jivani, L. L., Vachhani, J. H., Kachhadia, V. H. (2016): Genetic variability, heritability and genetic advance studies in vegetable cowpea [*Vigna unguiculata* (L.) Walp.] 7(2): 408 – 411.

[12] Nwofia, G.E. (2012) Yield and yield components in vegetable cowpea on an ultisol. *African Journal of Agricultural Research, 7(28):4097-4103.*

[13] Nwosu, D. J., Olatunbosun, B. D., Adetiloye, I. S. (2013) Genetic variability, heritability and genetic advance in cowpea [*Vigna unguiculata* (L.) Walp] genotypes in two agro-ecological environments. Greener J. Bio. Sci., **3**(5): 202-207.

[14] Davis, D. W., Oelke, E. A., Oplinger, E. S., Doll, J. D., Hanson, C.V., Putnam, D. (2003): Field crops manure, University of Minesota, St Paul, MN5510. University of Wisconsin-Madison, W1 53706.

[15] Singh, B. B., Ajeibe, H. A., Tarawali, B. A. (2009) Improving the production and utilization of cowpea as a food and fodder. Field crops Res., 84: 169-177.

[16] Sarath, P.S., Reshma. T. (2017) Genetic variability studies in cowpea (*Vigna unguiculata* L. Walp). *International Journal of Agricultural Science and Research,* 7(3): 129-132.

[17] Guptha, R. (2010) Characterisation of seed storage protein in high protein genotypes of cowpea (*V. unguiculata*). *Physiol. Mol. Biol., 16: 53-58.*

VARIABILITY IN YIELD TRAITS AND THEIR ASSOCIATION WITH GRAIN YIELD AMONG SELECTED RICE (*Oryza sativa* L.) GENOTYPES IN NIGERIAN AGRO-ECOLOGY

Osekita Oluwatoyin Sunday
Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Nigeria.
E-mail address: oluwatoyin.osekita@aaua.edu.ng

Abstract: The determination of yield traits that contribute significantly to final yield could be used effectively in initiating a rice breeding programme. Sixteen genotypes of lowland rice were evaluated under rain-fed conditions at three different locations for three planting seasons. The experiment was laid out in a randomized complete block design with three replications across the locations. Data were collected on morphological and yield component traits. There was a highly significant ($p < 0.001$) variation among the sixteen genotypes in the morphological and yield component traits. Highly positive genotypic and phenotypic correlations were observed between one thousand grain weight and grain yield in Environment 1 (0.69, 0.61), 2 (0.90, 0.89), 3 (0.61, 0.60) and 4 (0.63, 0.61), respectively. Mean performance of traits such as one thousand grain weight (26.1g), number of effective tillers (17.6), panicle length (24.9cm), panicle weight (4.6g) and number of grains per panicle (148.8) are the most varied traits in the test locations. Therefore, selection based on one thousand grain weight, number of effective tillers, panicle length, panicle weight and number of grains per panicle could be used for improving grain yield in rice.

Keywords: Yield Traits, Genotypes, Selections, Locations, Agro-Ecology

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop in the world and a model cereal species (Collard *et al.* 2008). It is a staple food in many parts of the world, including many developing countries in Asia, Africa and Latin America. In Nigeria, rice is the sixth most cultivated food crop after millet, sorghum, cowpea, maize and yam. Nigeria has between 4.6-4.9 million hectares of land suitable for rice production out of which only 1.7 million hectares is being cultivated. One of the perennial problems confronting rice production is low yield. One approach to combat the problem of poor yield is to breed for higher yielding genotypes through traits modification (Osekita, 2018). Yield is a complex trait that is controlled by many genes (Akinyele and Osekita, 2006), and the impact of the environment and genetic variations (Oladosu et al., 2018). Selection should not be based on yield alone because increased grain yield are combinations of interaction between yield and yield component traits. Knowledge of correlation that exists among important characters may facilitate the interpretation of results and provide a basis for planning more efficient breeding programme. An understanding of the association of yield with contributing characters is essential and their heritable variation has to be understood for efficient selection of a superior genotype through breeding. This can be achieved through the technique of "path coefficient analysis" which is a powerful multivariate statistical tool and which enables a researcher to understand the "path" through which causal factor (yield component traits) influence the yield (Therthappa, 2005). A path coefficient is a standardized partial regression coefficient that measures the direct influence of one variable upon another (Dewey and Lu, 1959). Path analysis allows the researcher to test theoretical propositions about cause and effect without manipulating variables. Variables may be assumed to be causally related and propositions about them tested (Acquaah, 2007). The establishment of a positive and negative relationship does not lead to a direct cause-and-effect interpretation, but a path coefficient analysis measures the direct influence of one variable upon another and permits the separation of the correlation into components of direct and indirect effects (Akinyele and Osekita, 2006). This study was conducted to select traits that would enhance higher yield in rice.

MATERIALS AND METHODS

Sixteen genotypes, which include six exotic and ten elite varieties were used for this study. The exotic varieties (MRQ 76, MR 269, MRQ 74, MR 220, MR

263 and MR 253) were developed by the Malaysian Agricultural Research and Development Institute (MARDI) Seberang Perai, Pinang Malaysia and the elite varieties from Africa Rice – International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The exotic genotypes were first raised and acclimatized to Nigerian agro-ecology and enough seeds were obtained prior the commencement the field evaluation in 2014. The field trials were conducted in five environments designated as Akungba 1, Akungba 2, Akure 1, Akure 2 and Okitipupa. These locations represented three different agro-ecologies with rainfall as the major source of water. The experiment was laid out in a randomized complete block design with three replications. Plot size was 440 m^2, with subplot size of 6 m^2 unit for each genotype in each replication. The seedlings were raised in the nursery for three weeks before transplanting in each location. Cultural practices such as weeding, fertilizer application in splits of three; 15, 55 and 75 days after transplanting, respectively were carried out.

Data collection: Five randomly selected hills for each genotype were used to record observations for plant height, number of tillers per hill, effective tillers per hill, flag leaf length, panicle length, panicle weight, number of grains per panicle, number of spikelet per panicle, one thousand grain weight, grain length, grain width , number of days to flowering, number of days to maturity and grain yield per hill. Analysis of covariance was carried out on pairs of variables which exhibited significance ($P \leq 0.05$). The generated components of the covariance were used to estimate the genotypic and phenotypic correlation coefficients as suggested by Singh and Chaudhary (1985).

Genotypic Correlation Coefficient (r_g) = $\frac{COV_{g(X1.X2)}}{\sqrt{\sigma_g^2 x_1 . \sigma_g^2 x_2}}$

Phenotypic Correlation coefficient (r_p) = $\frac{COV_{p(X1.X2)}}{\sqrt{\sigma_p^2 x_1 . \sigma_p^2 x_2}}$

Where; σ^2_g = genotypic variance, σ^2_p = phenotypic variance

$COV\ (x_1.x_2)$ = Covariance of characters x and y

Test of significance of correlation was done by comparing the computed values against table 'r' values given by Fisher and Yates (1963).

RESULTS AND DISCUSSION

The combined mean squares and coefficient of variation (CV) for fourteen agronomic and yield component traits of sixteen lowland rice genotypes evaluated in five locations were presented in Table 1. The analysis of variance for combined locations, the effect of replication, genotypes main effect and genotype by environment interaction were all highly significant ($P < 0.01$) for the fourteen traits evaluated. The grand mean values for plant height was (120.6cm), number of tillers (17.6), effective tillers (15.7), flag leaf length (27.1cm), panicle length (24.9cm), panicle weight (4.6g), number of grains per panicle (148.8), number of spikelet (11.5), one thousand grain weight (26.1g), grain length (0.9), grain width (0.3), number of days to flowering (88.0), number of days to maturity (118.0) and grain yield per hill (55.5g) suggesting that variation exist in yield traits among the genotypes with respect to locations. The coefficient of variation for the traits were generally low, it ranged from 0.0% in grain width to 17.4% in number of tillers/hill. The contributions of genotype x environment interaction (GEI) percentage proportion to total variation on quantitative traits measured indicated that plant height had (3.10%), while the contributions from number of tillers per hill, effective tillers per hill, flag leaf length, panicle length, panicle weight, number of grains per panicle, number of spikelets per panicle , one thousand grains weight, grain length, grain width, number of days to flowering, number of days to maturity, and grain yield per hill were 6.35%, 5.35%, 7.33%, 4.48%, 5.33%, 8.18%, 4.60%, 4.13%, 23.39%, 58.82%, 2.21% , 2.19%, and 4.89%, respectively. Mean performance of sixteen lowland rice genotypes in respect of fourteen agronomic and yield traits across locations were presented in Table 2. All the genotypes varied significantly ($p<0.05$) in all the traits. The mean panicle weight varied from 2.4g to 6.3g. BW 348-1 had the highest panicle weight of 6.3g and grain yield per hill of 85.8g, while WITA 4 with the lowest mean yield of 30.6g had a panicle weight of 4.3g which is affected by poor effective tiller number. Generally the genotypes were high yielding. This is in line with Akinwale *et al*. (2011) who reported significant variations among the rice genotypes for all the traits studied. Similar results

were reported on field evaluation of lowland rice genotypes by Osekita *et al*. (2015). The significant differences among the genotypes for all the traits investigated suggested that selection for these traits for further improvement is possible. This agrees with the findings of Jamal *et al*. (2009), Tahir *et al*. (2002), and Yadav (2000).

Genotypic and Phenotypic correlation coeffficients

The genotypic and phenotypic correlation coefficients among different traits in five environments are presented in Tables 3a and b respectively. In most cases, genotypic correlation coefficients is higher than phenotypic correlation coefficients in both magnitude and direction, similar results of correlation coefficients were observed in different environments where this research was conducted. Significant ($p<0.01$) positive genotypic and phenotypic correlations were observed between grain yield per hill and plant height in E1 and E3, panicle length in E5, panicle weight in E1, E2 and E3, number of grains per panicle in E5, number of spikelets per panicle in E5, one thousand grain weight in E1, E2 and E3.

Number of days to flowering and days to maturity exhibited positive significant genotypic and phenotypic correlations with plant height in the five locations. Also, number of grains per panicle and number of spikelets had positive significant ($p<0.01$) genotypic and phenotypic correlations with panicle length. It was observed that number of tillers per hill had perfect negative genotypic correlation with all the traits measured in location 2 only. Number of grains per panicle showed highly Significant ($p<0.01$) genotypic and phenotypic correlation with number of spikelets in all the environments. Positive significant ($p<0.01$) genotypic and phenotypic correlations of one thousand grain yield with grain yield per hill was obtained in locations 1, 2 and 3..

Number of days to flowering showed perfect positive genotypic and phenotypic correlation with days to maturity in all environments. Number of tillers per hill showed highly significant ($p<0.01$) positive phenotypic correlation with effective tillers in all the environments only. However, it exhibited negative significant genotypic correlation with effective tillers in environment 2. Flag leaf length showed highly significant genotypic and phenotypic correlations with panicle length in all the five environments. The positive and significant correlations among such characters as yield, plant height, number of tillers, panicle length and weight, number of spikelet/panicle, number of grains/panicle and one thousand grain weight of different genotypes across different locations are useful in designing an effective selection and breeding programme for rice.

Table 1a: Combined mean squares for agronomic traits of sixteen lowland rice genotypes evaluated in five environments

Source of variation	Degree of freedom	Plant height(cm)	Number of tillers	Effective tillers/ panicle	Flag leaf length (cm)	Panicle Length (cm)	Panicle Weight (g)	No of grains / panicle
Replication	2	487.0**	31.4**	38.1**	2.4**	1.0**	0.09	231.5^{ns}
Environment	4	4553.0**	88.1**	28.7**	17.8**	279.8**	6.0**	10461.8**
Genotypes	15	6646.4**	109.2**	65.0**	235.5**	111.8**	24.8**	14345.5**
Env * Gen	60	383.6**	20.9**	10.8**	21.0**	18.7**	1.8**	2303.3**
Env * Rep	8	168.6**	60.8**	46.7**	2.1**	2.6**	0.2**	56.0
Gen * Rep	30	74.9**	9.5**	6.6**	4.7**	1.8**	0.6**	488.5**
Pooled error	120	45.9	9.4	5.9	3.0	1.4	0.3	276.2
CV (%)		5.6	17.4	15.5	6.4	4.8	11.9	11.2
G$\bar{x}$		120.6	17.6	15.7	27.1	24.9	4.6	148.8
GEI Proportion (%)		3.10	6.35	5.35	7.33	4.48	5.33	8.18

*, **, ns significant at P = 0.05, 0.01 and non significant respectively.

Table 1b: Combined mean squares for agronomic traits of sixteen lowland rice genotypes evaluated in five environments

Source of variation	Degree of freedom	Number of spikelet/panicle	Thousand grain weight (g)	Grain length (cm)	Grain width (cm)	Number of days to flowering	Number of days to maturity	Yield per hill (g)
Replication	2	0.8**	0.1	0.04**	0.001**	1.3**	1.4**	49.7**
Environments	4	36.3**	70.1**	0.05**	0.02**	11.3**	13.1**	7026.7**
Genotypes	15	57.7**	154.0**	0.02**	0.01**	1105.6**	1112.1**	2532.0**
Env * Gen	60	4.7**	9.9**	0.04**	0.002**	25.3**	25.3**	457.4**
Env * Rep	8	1.3**	2.0**	0.01**	0.001**	0.66**	0.37	31.3**
Gen * Rep	30	0.7**	2.4**	0.01**	0.000	0.83**	0.76**	58.6**
Pooled error	120	0.6	1.3	0.001	0.000	0.8	0.7	40.1
CV (%)		6.7	4.4	3.5	0.0	1.0	0.7	11.4
G$\bar{x}$		11.5	26.1	0.9	0.3	88.0	118.0	55.5
GEI Proportion (%)		4.60	4.13	23.39	58.82	2.21	2.19	4.89

*, **, ns significant at P = 0.05, 0.01 and non significant respectively.

Table 2: Means of fourteen agronomic and yield traits of sixteen lowland rice genotypes across locations

Genotypes	PLHT	NT	ETP	FLL	PANL	PAWT	NGP	NSPK	TGW	GL	GW	NDF	NDM	GYD
MRQ 76	94.5^{ab}	20.3^{g}	17.1^{de}	23.4^{ab}	22.2^{a}	2.4^{a}	115.7^{a}	10.5^{bc}	21.8^{bc}	0.91^{bcd}	0.29^{de}	66.4^{a}	96.1^{a}	42.7^{b}
MR 269	98.5^{b}	16.3^{abcde}	14.5^{abc}	24.4^{bc}	25.2^{c}	3.0^{b}	144.7^{cde}	10.1^{b}	23.9^{d}	0.90^{bc}	0.29^{de}	74.7^{b}	104.8^{b}	52.9^{de}
MRQ 74	91.5^{a}	25.0^{h}	21.3^{f}	22.6^{a}	22.3^{a}	2.9^{b}	127.8^{ab}	10.8^{cd}	20.9^{ab}	0.94^{ef}	0.31^{f}	81.1^{c}	111.1^{c}	46.3^{bc}
MR 220	91.6^{a}	19.3^{efg}	16.2^{cde}	25.1^{c}	25.2^{c}	4.6^{f}	151.3^{def}	11.8^{e}	23.4^{d}	0.92^{bcde}	0.28^{c}	83.8^{d}	113.9^{d}	44.5^{b}
MR 263	96.4^{ab}	17.9^{cdefg}	16.6^{cde}	25.1^{c}	25.7^{c}	3.9^{de}	157.0^{ef}	11.3^{de}	21.3^{abc}	0.90^{bc}	0.31^{f}	86.6^{f}	116.7^{f}	49.8^{cd}
MR 253	100.5^{b}	19.3^{efg}	15.7^{bcde}	23.9^{abc}	23.5^{b}	3.8^{cd}	141.2^{bcd}	10.2^{bc}	20.8^{a}	0.94^{ef}	0.27^{b}	87.1^{f}	117.1^{f}	55.5^{ef}
Faro 57	125.9^{c}	14.8^{ab}	13.1^{a}	30.7^{ef}	27.2^{d}	6.1^{h}	178.9^{g}	12.9^{f}	25.9^{e}	0.94^{ef}	0.30^{def}	89.5^{g}	119.4^{g}	51.6^{de}
Faro 44	127.3^{c}	15.0^{abc}	13.6^{ab}	32.8^{g}	29.6^{e}	4.1^{de}	160.3^{f}	13.0^{f}	27.4^{g}	0.95^{f}	0.30^{def}	84.9^{e}	114.9^{e}	51.3^{de}
NL 19	149.7^{d}	14.1^{a}	12.7^{a}	31.4^{f}	30.1^{e}	6.2^{h}	216.2^{h}	14.7^{gh}	28.7^{h}	0.92^{bcde}	0.29^{cd}	94.4^{h}	124.4^{h}	59.8^{fg}
WITA 4	125.2^{c}	16.8^{a-f}	14.8^{abcd}	36.8^{h}	27.3^{d}	4.3^{ef}	177.8^{g}	14.9^{e}	21.9^{c}	0.89^{bc}	0.30^{def}	102.7^{j}	132.7^{j}	30.6^{a}
BW 348-1	151.0^{d}	15.2^{abc}	14.5^{abc}	29.8^{e}	29.9^{e}	6.3^{h}	211.1^{h}	14.3^{g}	29.5^{hi}	0.95^{f}	0.30^{def}	95.1^{i}	125.1^{i}	85.8^{i}
NL 30	129.5^{c}	19.4^{fg}	17.7^{e}	27.7^{d}	25.7^{c}	5.6^{g}	132.9^{bc}	9.9^{b}	26.6^{efg}	0.94^{ef}	0.30^{def}	95.5^{i}	125.5^{i}	56.4^{ef}
NL 34	127.4^{c}	17.4^{b-g}	15.7^{bcde}	26.6^{d}	22.6^{ab}	6.2^{h}	129.5^{ab}	10.0^{b}	27.3^{fg}	0.91^{cdef}	0.30^{def}	90.0^{g}	120.0^{g}	64.1^{g}
IRRI 154	126.6^{c}	16.1^{abcd}	14.6^{abc}	26.7^{d}	23.3^{b}	4.6^{f}	130.1^{b}	11.1^{d}	30.2^{i}	0.89^{bc}	0.29^{cd}	84.0^{d}	114.0^{d}	75.0^{h}
NL 11	124.7^{c}	17.8^{cdefg}	16.4^{cde}	24.5^{bc}	23.3^{b}	3.7^{cd}	115.6^{a}	8.1^{a}	26.3^{ef}	0.80^{a}	0.20^{a}	90.0^{g}	120.0^{g}	52.8^{de}
NL 25	148.6^{d}	18.7^{defg}	17.1^{de}	25.2^{c}	23.5^{b}	3.5^{c}	129.3^{ab}	10.1^{b}	27.5^{g}	0.91^{cdef}	0.30^{def}	89.8^{g}	119.8^{g}	63.9^{g}

Note: Means with the same alphabet in the same column are not significantly different by DMRT (P=0.05).

Table 3a: Genotypic correlation coefficients among fourteen traits of rice in five environments

TRAITS	ENV	NT	ETP	FLL	PANL	PANWT	NGP	NSPKP	TGW	GL	GWDT	NDF	NDTM	YDH
PLTHT	**1**	-0.85**	-0.63**	0.68**	0.88**	0.88**	0.67**	0.50*	0.92**	0.78**	0.54*	0.57*	0.56*	0.80**
	2	-1.00**	-0.85**	0.52**	0.63**	0.37	0.54*	0.44	0.74**	-0.15	0.18	0.53*	0.53*	0.25
	3	-0.41	-0.24	0.50*	0.58*	0.68**	0.19	0.03	0.84**	0.06	-0.75**	0.67**	0.67**	0.53*
	4	0.01	0.11	0.41	0.36	0.29	0.57*	0.39	0.73**	-0.21	0.01	0.58*	0.58*	0.39
	5	-0.64**	-0.62**	0.45	0.25	0.32	0.33	0.17	0.35	0.04	-0.05	0.61**	0.62**	0.45
NT	**1**		0.89**	-0.91**	-0.90**	-0.86**	-0.77**	-0.69**	-0.80**	-0.59*	-0.35	-0.25	-0.25	-0.54*
	2		-1.00**	-1.00**	-1.00**	-1.00**	-1.00**	-1.00**	-1.00**	-1.00**	-1.00**	-1.00**	-1.00**	-1.00**
	3		0.97**	-0.36	-0.38	-0.49	-0.46	-0.29	-0.49	-0.11	0.48	-0.37	-0.37	-0.09
	4		0.96**	-0.48	-0.52*	-0.70**	-0.52*	-0.67**	-0.3	-0.31	-0.32	-0.36	-0.36	0.18
	5		1.00**	-0.51*	-0.59*	-0.57*	-0.58*	-0.28	-0.41	0.28	0.24	-0.44	-0.44	-0.54*
ETP	**1**			-0.88**	-0.80**	-0.68**	-0.73**	-0.73**	-0.58*	-0.45	-0.07	-0.12	-0.12	-0.24
	2			-0.98**	-1.00**	-1.00**	-1.00**	-1.00**	-0.88**	-1.00**	-1.00**	-0.55*	-0.56*	-0.32
	3			-0.44	-0.36	-0.39	-0.47	-0.41	-0.4	-0.23	0.31	-0.15	-0.15	0.14
	4			-0.52*	-0.43	-0.82**	-0.49	-0.73**	-0.19	-0.43	-0.39	-0.3	-0.3	0.27
	5			-0.52*	-0.66**	-0.53*	-0.62**	-0.34	-0.41	0.34	0.3	-0.4	-0.4	-0.54*
FLL	**1**				0.88**	0.70**	0.82**	0.89**	0.49	0.80**	0.49	0.39	0.38	0.15
	2				0.80**	0.42	0.79**	0.87**	0.12	-0.14	0.28	0.84**	0.84**	-0.38
	3				0.67**	0.39	0.49	0.60**	0.24	0.45	-0.34	0.31	0.31	-0.32
	4				0.68**	0.52*	0.77**	0.85**	0.23	0.12	0.25	0.75**	0.75**	-0.28
	5				0.58*	0.22	0.36	0.33	0.08	-0.08	0.16	0.68**	0.68**	0.41
PANL	**1**					0.87**	0.87**	0.80**	0.73**	0.78**	0.46	0.62**	0.61**	0.45
	2					0.55*	0.82**	0.77**	0.31	0.21	0.45	0.64**	0.64**	0.03
	3					0.38	0.69**	0.51*	0.16	0.62	0.18	0.28	0.28	0.24
	4					0.38	0.69**	0.51*	0.16	0.62	0.18	0.28	0.28	0.24
	5					0.13	0.86**	0.76**	-0.07	-0.15	0.09	0.19	0.19	0.58*
PANWT	**1**						0.81**	0.64**	0.87**	0.82**	0.53*	0.59	0.58*	0.69**
	2						0.38	0.39	0.48	0.18	0.22	0.57*	0.57*	0.07
	3						0.66**	0.42	0.62**	0.25	-0.37	0.68**	0.68**	0.54*
	4						0.28	0.45	0.4	0.21	0.26	0.65**	0.65	-0.06
	5						0.33	0.08	0.11	0.36	0.23	0.3	0.3	0.38
NGP	**1**							0.94**	0.54*	0.74**	0.50*	0.66	0.65**	0.28
	2							0.93**	0.26	0.2	0.43	0.53*	0.53*	-0.03
	3							0.89**	-0.02	0.26	0.07	0.45	0.45	0.01
	4							0.86**	0.36	0.16	0.24	0.57*	0.57*	0.04
	5							0.84**	-0.24	0.09	0.13	0.16	0.16	0.78**
NSPKP	**1**								0.34	0.77**	0.49	0.48	0.47	0.03
	2								0.14	0.08	0.53*	0.56*	0.56*	-0.26
	3								-0.11	0.55*	0.33	0.15	0.15	-0.18
	4								0.34	0.16	0.49	0.63	0.63**	-0.28
	5								-0.13	-0.01	0.37	0.06	0.06	0.69**
TGW	**1**									0.71**	0.41	0.36	0.35	0.90**
	2									-0.16	0.17	0.21	0.21	0.61**
	3									0.15	-0.66**	0.44	0.44	0.63**
	4									-0.31	0.1	0.36	0.36	0.44
	5									-0.51*	-0.08	0.02	0.02	0.00
GL	**1**										0.90**	0.50*	0.48	0.48
	2										0.51*	-0.31	-0.31	0.27
	3										0.45	-0.12	-0.12	-0.18
	4										0.56*	-0.18	-0.18	0.2
	5										0.63**	-0.12	-0.12	0.33
GWDT	**1**											0.56*	0.56*	0.41
	2											-0.05	-0.05	0.04
	3											-0.51*	-0.51*	-0.3
	4											-0.07	-0.07	-0.03
	5											-0.08	-0.08	0.41
NDF	**1**												1.00**	0.36
	2												1.00**	-0.39
	3												1.00**	0.44
	4												1.00**	-0.27
	5												1.00**	0.07
NDTM	**1**													0.34
	2													-0.39
	3													0.44
	4													-0.27
	5													0.08

Table 3b: Phenotypic correlation coefficients among fourteen traits in five environments

Traits	Env	NT	ETP	FLL	PANL	PANWT	NGP	NSPKP	TGW	GL	GWDT	NDF	NDTM	YDH
PLTHT	**1**	-0.77**	-0.55*	0.68**	0.87**	0.86**	0.64**	0.52*	0.90**	0.65**	0.53*	0.56*	0.55*	0.79**
	2	-0.31	-0.35	0.47	0.56*	0.37	0.51*	0.42	0.72**	-0.16	0.19	0.52*	0.52*	0.26
	3	-0.34	-0.19	0.48	0.57*	0.66**	0.18	0.03	0.79**	0.05	-0.69*	0.66**	0.66**	0.52*
	4	0.03	0.1	0.39	0.35	0.28	0.56*	0.4	0.71**	-0.19	0.03	0.58*	0.58*	0.38
	5	-0.61**	-0.58**	0.45	0.25	0.32	0.33	0.16	0.35	0.04	-0.05	0.61**	0.61**	0.43
NT	**1**		0.89**	-0.80**	-0.79**	-0.76**	-0.64**	-0.61**	-0.74**	-0.47	-0.33	-0.24	-0.24	-0.47
	2		0.82**	-0.38	-0.50*	-0.36	-0.61**	-0.49	-0.36	-0.07	-0.04	-0.15	-0.15	-0.21
	3		0.95**	-0.29	-0.29	-0.39	-0.34	-0.23	-0.4	-0.04	0.29	-0.32	-0.32	0.02
	4		0.95**	-0.36	-0.34	-0.49	-0.36	-0.46	-0.2	-0.18	-0.16	-0.25	-0.25	0.12
	5		0.99**	-0.48	-0.56*	-0.52*	-0.56*	-0.27	-0.38	0.2	0.23	-0.42	-0.42	-0.46
ETP	**1**			-0.75**	-0.68**	-0.59*	-0.59*	-0.63**	-0.54*	-0.35	-0.06	-0.12	-0.11	-0.19
	2			-0.45	-0.56*	-0.49	-0.53*	-0.58*	-0.39	-1.00**	-1.00**	-0.27	-0.27	-0.11
	3			-0.35	-0.27	-0.29	-0.34	-0.32	-0.34	-0.15	0.15	-0.13	-0.13	0.19
	4			-0.35	-0.28	-0.51*	-0.3	-0.47	-0.11	-0.23	-0.19	-0.18	-0.18	0.18
	5			-0.47	-0.61**	-0.46	-0.59**	-0.31	-0.37	0.24	0.27	-0.37	-0.37	-0.43
FLL	**1**				0.86**	0.68**	0.77**	0.87**	0.48	0.66**	0.47	0.37	0.36	0.17
	2				0.78**	0.4	0.77**	0.83**	0.1	-0.09	0.26	0.81**	0.81**	-0.38
	3				0.66**	0.38	0.49	0.59*	0.23	0.41	-0.33	0.3	0.3	-0.29
	4				0.61**	0.50*	0.76**	0.83**	0.21	0.13	0.2	0.73**	0.73**	-0.27
	5				0.57*	0.22	0.35	0.32	0.08	-0.07	0.15	0.68**	0.68**	0.4
PANL	**1**					0.86**	0.85**	0.80**	0.72**	0.64**	0.44	0.60**	0.59*	0.47
	2					0.51*	0.78**	0.73**	0.28	0.23	0.4	0.59*	0.59*	0.02
	3					0.61**	0.58*	0.61**	0.38	0.36	-0.22	0.59*	0.59*	0.02
	4					0.35	0.66**	0.48	0.14	0.54*	0.19	0.27	0.27	0.23
	5					0.13	0.85**	0.75**	-0.07	-0.15	0.08	0.19	0.19	0.54*
PANWT	**1**						0.81**	0.66**	0.85**	0.66**	0.50*	0.56*	0.55*	0.69**
	2						0.37	0.38	0.47	0.17	0.21	0.57*	0.57*	0.67**
	3						0.66**	0.41	0.58*	0.21	-0.34	0.66**	0.66**	0.52*
	4						0.28	0.44	0.38	0.21	0.25	0.64**	0.64**	-0.05
	5						0.32	0.08	0.11	0.32	0.22	0.29	0.3	0.35
NGP	**1**							0.91**	0.50*	0.55*	0.45	0.59*	0.58*	0.31
	2							0.92**	0.24	0.22	0.41	0.52*	0.51*	-0.03
	3							0.85**	0	0.23	0.06	0.43	0.43	0.03
	4							0.85**	0.33	0.15	0.24	0.56*	0.56*	0.04
	5							0.82**	-0.24	0.09	0.12	0.16	0.16	0.73**
NSPKP	**1**								0.35	0.64**	0.47	0.46	0.45	0.07
	2								0.14	0.09	0.51*	0.55*	0.55*	-0.25
	3								-0.11	0.50*	0.29	0.15	0.15	-0.17
	4								0.33	0.15	0.46	0.62	0.62	-0.26
	5								-0.12	-0.02	0.36	0.05	0.05	0.61**
TGW	**1**									0.57*	0.4	0.36	0.35	0.89**
	2									-0.16	0.16	0.2	0.21	0.60**
	3									0.11	-0.58*	0.41	0.41	0.61**
	4									-0.29	0.1	0.35	0.35	0.43
	5									-0.47	-0.08	0.02	0.02	-0.01
GL	**1**										0.74**	0.42	0.4	0.41
	2										0.48	-0.3	-0.3	0.25
	3										0.34	-0.11	-0.11	-0.15
	4										0.52*	-0.18	-0.18	0.19
	5										0.57*	-0.1	-0.1	0.26
GWDT	**1**											0.56*	0.55*	0.4
	2											-0.04	-0.05	0.04
	3											-0.47	-0.47	-0.29
	4											-0.06	-0.06	-0.02
	5											-0.08	-0.08	0.39
NDF	**1**												1.00**	0.35
	2												1.00**	-0.39
	3												1.00**	0.43
	4												1.00**	-0.26
	5												1.00**	0.07
NDTM	**1**													0.33
	2													-0.38
	3													0.43
	4													-0.26
	5													0.07

Note: *, ** significant at 5% and 1% levels respectively.

PLTHT=plant height(cm), NT= No of tillers per hill, ETP= Effective tillers per hill, FLL(cm)= Flag leaf length, PANL (cm)= Panicle length, PANWT (g) = Panicle weight, NGP = No of grains per panicle, NSPKP = No of spikelet per panicle, TGW (g) = Thousand grain weight, GL(cm) = Grain length, GWD = Grain width (cm), NDF = No of days to flowering, NDTM = No of days to maturity, YHD(g) = Yield per hill.

REFERENCES

Acquaah, G. 2007. Principles of plant genetics and breeding. Blackwell publishing LTD. USA

Akinwale, M. G., Gregory, G., Nwilene, F., Akinyele, B. O., Ogunbayo, S. A. and Odiyi, A. C. 2011. Heritability and correlation analysis for yield and its components in rice (Oryza sativa L.). African Journal of Plant Science. 5(3): 207-212.

Akinyele, B. O and Osekita, O. S. 2006 Correlation and path coefficient analyses of seed yield attributes in okra (Abelmoschus esculentus (L.) Moench). African Journal of Biotechnology. 5 (14): 1330-1336.

Collard, B. C. Y., Cruz. C. M. V., McNally, K. L., Virk, P. S. and Mackill, D. J. 2008. Rice molecular breeding laboratories in the genomics era: Current status and future considerations. International Journal of Plant Genomics.doi:10.1155/2008/524847 pp.25

Dewey, D. R. and Lu, K. H. 1959. A correlation and path-coefficient analysis of components of crested wheat grass seed production. Agronomy Journal. 51: 515-518.

Fisher, G. and Yates, M. 1963. Statistical tables for biological, agricultural and medical research.Vol.6, pp: 62-63.

Jamal, Ifftikhar H. Khalil, Abdul Bari, Sajid Khan and Islam Zada 2009. Genetic variation for yield and yield components in rice. ARPN Journal of Agricultural and Biological Science. 4(6): 60-64.

Yusuff Oladosu, M. Y. Raffi, Usman Magaji, Norhani Abdullah, Gous Miah, Samuel C. Chukwu, Ghazali Hussin, Asfaliza Ramli and Isiaka Kareem 2018. Genotypic and phenotypic relationship among yield components in rice under tropical conditions. Hindawi BioMed Research International Vol. 2018: 1-10.

Osekita, O. S., Akinyele, B.O. and Odiyi, A. C. 2015. Evaluation of exotic rice varieties for genetic parameters in a Nigerian agro-ecology. International Journal of Plant and Soil Science. 5(6): 350-358.

Osekita, O. S. 2018. Genotype x environment interaction and molecular diversity studies of selected rice (Oryza sativa L.) across three Nigerian agro-ecological zones. Unpublished PhD Thesis submitted to The Federal University of Technology Akure 2018 Pp 194.

Singh, R. K. and Chaudhary, B. D. 1985. Biometrical methods in quantitative analysis. Kaljuni Publishers. New Delhi. 318pp.

Tahir M., Wadan D. and Zada A. 2002. Genetic variability of different yield characters in rice. Sarhad Journal of Agriculture. 18(2).

Therthappa, B. S. 2005. Application of Multivariate Techniques in Evaluation of Sunflower Genotypes (Helianthus annus L.). M.Sc. Thesis Submitted to the University of Agricultural Sciences. Dharwad.

Yadav R. K. 2000. Studies on genetic variability for some quantitative characters in rice (Oryza sativa L.) Advances in Agricultural Research in India. 13: 205 – 207.

VARIABILITY ASSESSMENT FOR SOME AGRO-MORPHOLOGICAL TRAITS AND OIL CONTENT IN Jatropha curcas L. GENOTYPES FROM NORTH WESTERN NIGERIA

Usman M., Usman A., Echekwu C. A., Katung, M. A. and Abdulmalik, R. O.
Department of Plant Science, Faculty of Agriculture, Institute for Agricultural Research, Ahmadu Bello University, Zaria, P.M.B. 1044, Kaduna State.

Abstract: *Jatropha curcas* L. is a shrub that is most suitable for production of biodiesel and can be grown as a quick yielding plant even in problem soils and adverse climatic conditions. In spite of the wide spread interest in planting Jatropha, information on the assessments of genetic variability necessary for the improvement of the crop in Nigeria is scarce. This study was carried out to assess the genetic variability of 57 genotypes of *Jatropha curcas* L. from the Northwestern Nigeria based on agro-morphological traits and oil content. The mean values for each trait from two years data were analysed. Genotypic coefficient of variation (*GCV*), phenotypic coefficient of variation (*PCV*), heritability in broad sense (H_b), genetic advance (*GA*) and genetic advance as percentage of mean (*%GA*) were estimated. The mean performance of the 57 genotypes across the two years for the 11 traits studied provides a clear indication of the superiority of some of the genotypes over others. All the 57 genotypes were high in oil content ranging from 32.6% to 42.5%. High heritability values coupled with high genetic advance were recorded for number of male flowers per inflorescence (MPI) 0.67, 51.10, number of inflorescence (NI) 087, 89.53 and number of branches (NB) 0.85, 87.96. The study indicated the presence of considerable variability in the 57 genotypes evaluated suggesting the possibility of improvement for the traits studied through appropriate breeding procedures such as biparental mating design.

Keywords: Biodiesel, Genetic Improvement, Genetic Variability

INTRODUCTION

Jatropha curcas L. is a plant that is widely distributed in almost all regions of the world (Fairless, 2007; Dias *et al*., 2012). It is a shrub that is most suitable for production of biodiesel as it can be grown as a quick yielding plant even in problem soils and adverse climatic conditions (Priyamvada *et al.,* 2010) and is thought to be native to Central America. The crop has drawn attention as a source of seed oil that can provide an economically viable substitute for motor fuel (Chen *et al*., 2006). Genetic improvement in Jatropha is needed to increase the seed yield, oil content, drought and pest resistance and to modify its oil composition so that it becomes a technically and economically preferred source for biodiesel production (Natarajan *et al.,* 2010). In spite of the wide spread interest in planting Jatropha, information of genetic variability necessary for the improvement of the crop in Nigeria is scarce. Plant breeders need to have a good knowledge about genetic variability, genotypic coefficient of variation (*GCV*), phenotypic coefficient of variation (*PCV*), heritability in broad sense (H_b), genetic advance (*GA*) and genetic advance as percentage of mean (*%GA*) and good understanding of correlation between the traits to be improved for the genetic material at hand. However, assessments of genetic variability of *J. curcas* in Nigeria are scarce. Moreover, breeding effort is still at an infancy state and in Nigeria currently there is no released variety of *Jatropha curcas* available. Thus, the objective of this study was to assess genetic variability of 57 genotypes of *Jatropha curcas* L. from the Northwestern Nigeria based on agro-morphological traits and oil content.

MATERIALS AND METHODS

Genetic Materials

The genetic materials for this research were 57 *Jatropha curcas* L. genotypes collected from North Western Nigeria and established in the Institute for Agricultural Research, Ahmadu Bello University, Zaria in 2009. The eco-geographical characteristics of the collection states which spanned across the Sahel, Sudan and Guinea Savannas are presented in Table 1.

Table 1: Eco-geographical characteristics of the areas where *Jatropha curcas* L. germplasm were collected

State	Latitude (N)	Longitude (E)	Zone	Soil type	Climate
Kaduna	9°11'-11°22'	8°21'-7°14'	NGS, SGS	Alfisols	Tropical wet and dry
Katsina	11°23'-13°07'	7°23'-7°47'	SSS	Entisols	Tropical wet and dry
Kano	11°06'-11o58'	9°-00'-9°02'	SS	Alfisols	Hot and semi-arid
Jigawa	11°00'-11o33'	9°30'-9°34'	SSS	Alfisols	Hot and semi-arid
Zamfara	11°56'-13007'	6°25'-6°43'	SSS	Entisols	Hot and semi-arid
Sokoto	12°03'-13o10'	5°46'-4°33'	SSS, NGS	Entisols	Hot and semi-arid
Kebbi	12°05'-12041'	4°07-4°26'	SSS	Entisols	Hot and semi-arid

SS: Sudan Savannah, NGS: Northern Guinea Savannah, SSS: Sudano-Sahelian Savannah

Table 2: List of Provenances, their collection states and accession numbers of *Jatropha caucus* used for the study

Accession Number	Collection State	Accession Number	Collection State	Accession Number	Collection State
IARJAT2009001	Kaduna	IARJAT2009020	Kano	IARJAT2009040	Kano
IARJAT2009002	Kaduna	IARJAT2009021	Zamfara	IARJAT2009041	Kebbi
IARJAT2009003	Jigawa	IARJAT2009022	Zamfara	IARJAT2009042	Kaduna
IARJAT2009004	Katsina	IARJAT2009023	Zamfara	IARJAT2009043	Kebbi
IARJAT2009005	Zamfara	IARJAT2009024	Katsina	IARJAT2009044	Kano
IARJAT2009006	Kebbi	IARJAT2009025	Kaduna	IARJAT2009045	Zamfara
IARJAT2009007	Katsina	IARJAT2009027	Kaduna	IARJAT2009046	Katsina
IARJAT2009008	Kebbi	IARJAT2009028	Jigawa	IARJAT2009047	Kano
IARJAT2009009	Zamfara	IARJAT2009029	Katsina	IARJAT2009048	Jigawa
IARJAT2009010	Kebbi	IARJAT2009030	Katsina	IARJAT2009049	Sokoto
IARJAT2009011	Katsina	IARJAT2009031	Kano	IARJAT2009050	Kaduna
IARJAT2009012	Kaduna	IARJAT2009032	Kaduna	IARJAT2009051	Kaduna
IARJAT2009013	Sokoto	IARJAT2009033	Kaduna	IARJAT2009052	Katsina
IARJAT2009014	Sokoto	IARJAT2009034	Katsina	IARJAT2009053	Zamfara
IARJAT2009015	Sokoto	IARJAT2009035	Kano	IARJAT2009054	Kano
IARJAT2009016	Kaduna	IARJAT2009036	Kano	IARJAT2009055	Sokoto
IARJAT2009017	Kebbi	IARJAT2009037	Kebbi	IARJAT2009056	Kaduna
IARJAT2009018	Katsina	IARJAT2009038	Kaduna	IARJAT2009057	----------
IARJAT2009019	Zamfara	IARJAT2009039	Kano		

Agronomic Practices

All the genotypes were laid in a Randomized Complete Design (RCBD) and maintained in the research field of the Institute for Agricultural Research, Ahmadu Bello University, Zaria. All agronomic practices were carried out including fertilizer application, weeding, spraying and adequate water application.

Data Collection

The agro-morphological data were taken from ten randomly selected plants from each of the 57 genotypes in 2015 and 2016 with each plant taken as a replication. Data were taken on plant height (PH), number of primary branches (NB), number of inflorescence (NI), collar height (CH), number of female flowers per inflorescence (FPI), number of branches on collar (NBC), number of male flowers per inflorescence (MPI), number of seeds per fruit (NSF), fresh seed weight and collar thickness (FSW) and oil content (OC).

Data Analyses

Combined analysis of variance for all the measured traits were performed using the fixed model of the PROC GLM in SAS version 9.3 (SAS Institute, 2004). The statistical model used for the ANOVA and expected mean square (EMS) was according to Snedecor and Cochran (1980) as follows: $Y_{ijk} = \mu + e_i + r/y_{j/i} + g_k + ge_{ki} + e_{ijk}$ Where: Y_{ijk} = The k^{th} observation on i^{th} entry in j^{th} replication, μ = The general mean, y_i = The effect of the i^{th} year; i = 1,2, $r/y_{j/i}$ = The effect of the j^{th} replication in the i^{th} year; j =

1,2,3…..10, g_k = The effect of the g^{th} genotype; k = 1,2,3…….57, ge_{ki} = The interaction effect between k^{th} genotype in the i^{th} year and e_{ijk} = The error associated with each observation. The form of combined analysis of variance for two years is presented in Table 3.

Table 3: Form of Combined Analysis of Variance with Expected Mean Square

Source of Variation	Df	MS	EMS
Year	(y-1)		
Replication (Year)	r(y-1)		
Genotypes	(g-1)	M_g	$\sigma_e^2 + r\sigma_{gy}^2 + ry\sigma_g^2$
Genotypes (Year)	(g-1)(y-1)	M_{gy}	$\sigma_e^2 + r\sigma_{gy}^2$
Pooled error	Y(g-1)(r-1)	M_e	σ_e^2

The component of variance and their standard errors were estimated from expected mean squares which is obtained by equating the observed mean squares with the expected mean squares as follows:

For individual year: $\sigma_e^2 = M_e with SE = \sqrt{\frac{2(M_e)^2}{df+2}}$, $\sigma_g^2 = \frac{M_g - M_e}{r} with$

$$S.E = \sqrt{\left(\frac{1}{2}\right)^2\left[\frac{2(M_{gy})^2}{df+2} + \frac{2(M_e)^2}{df+2}\right]}, \quad \sigma_g^2 = \frac{M_g - M_{gy}}{ry} with S.E = \sqrt{\left(\frac{1}{ry}\right)^2\left[\frac{2(M_{gy})^2}{df+2} + \frac{2(M_g)^2}{df+2}\right]}$$

Coefficient of Variability

Phenotypic (PCV) and genotypic (GCV) coefficient of variation were calculated as

PCV $= \frac{\sqrt{\sigma_p^2}}{\bar{x}}$ X 100 and GCV = $\frac{\sqrt{\sigma_g^2}}{\bar{x}}$ X 100, respectively.

Heritability estimates

Broad sense heritability was estimated for all traits as a ratio of genotypic variance (σ_g^2) to phenotypic variance (σ_p^2) (Singh *et al.,* 1993; Falconer and Mackay, 1996; and Holland *et al.,* 2003).

$H_b = \sigma_g^2 + \frac{\sigma_{gy}^2}{y} + \frac{\sigma_e^2}{ry}$ Where: H_b =broad sense heritability, σ_g^2 =genotypic variance, σ_e^2 = environmental variance

Estimation of expected genetic advance (GA)

The genetic advance i.e. expected genetic gain from selection of 5% superior individuals was estimated using the formula:GA = $\sigma_g^2 / \sigma_p^2 . K\sqrt{\sigma_p^2}$ Where: K is the selection differential at 5% selection intensity and K = 2.06 and $\sigma_g^2 / \sigma_p^2 = H_b$. Therefore, $GA = H_b . K\sqrt{\sigma_p^2}$ (Thakur, 2014).

Estimation of expected genetic advance as a percentage of mean

This was computed according Kaul and Bhan (1974) as; $(\Delta G / \bar{X})X100$ where ΔG = Expected genetic advance and $\bar{X}$ = Entry mean

RESULTS

Table 4: Combined Analysis of Variance for 10 Agronomic Traits Oil Content in 57 *Jatropha curcas* L. Lines in 2015 and 2016

Source	DF	PH	FPI	MPI	NB	NI	CH	CT	NBC	NSF	FSW	OC
Year	1	47094.2	0.01	0.2	50.4	8.7	0.9	0.8	0.01	0.02	0.02	
Rep(Yr)	18	241.2	0.9	61.8	437.4	393.5	11.2	172.4	1.8	0.01	0.02	
Prv	56	23496.7**	167.6**	70227.3**	74551.3**	57932.4**	757.2**	1632.9**	1.1**	1.0**	0.4**	
Prv*Yr	56	830.65	0.04	1.26	66.93	6.30	0.10	0.97	0.02	0.01	0.02	
Error	1008	243.78	0.838	47.43	260.48	134.54	3.72	87.22	0.63	0.01	0.02	
Year	1	-	-	-	-	-	-	-	-	-	-	0.14
Rep(Yr)	4	-	-	-	-	-	-	-	-	-	-	0.20
Prv	56	-	-	-	-	-	-	-	-	-	-	41.32**
Prv*Yr	56	-	-	-	-	-	-	-	-	-	-	0.04
Error	224	-	-	-	-	-	-	-	-	-	-	2.27
Total	341											0.14

* = Significant at 0.05 level of probability, ** = Significant at 0.01 level of probability, PH=Plant height (cm),FPI= number of female flower per inflorescence, MPI = number of male flower per inflorescence, NB= number of branches, NI = number of inflorescence, CH = collar height (cm), CT = collar thickness (cm), NBC = number of branches on collar, NSF = number of seeds per fruit, FSW= fresh seed weight and OC = oil content

Table 5: Mean performance for 10 Agronomic Traits and Oil Content for 57 Provenances of *Jatropha curcas* L. (2015 and 2016 Combined)

Provenance	PH	FPI	MPI	NB	NI	CH	CT	NBC	NSF	FSW	OC
IARJAT2009001	231.2	16.4	313.6	409.2	328.8	21.8	86.9	3.6	2.8	1.2	36.00
IARJAT2009002	229.3	11.7	206.6	445.3	339.5	12.4	82.0	3.4	2.8	1.2	37.00
IARJAT2009003	289.9	15.9	436.4	458.3	441.1	21.4	85.0	3.4	2.5	1.2	34.00
IARJAT2009004	292.8	14.8	218.0	435.5	362.1	15.9	85.0	4.1	2.3	1.7	34.00
IARJAT2009005	300.6	14.5	266.7	423.4	338.6	27.2	85.8	3.3	3.0	1.7	41.00
IARJAT2009006	295.3	11.1	179.0	363.1	304.2	35.1	90.3	3.7	2.4	1.2	37.00
IARJAT2009007	304.1	12.8	264.1	358.3	301.8	15.6	86.3	3.0	2.5	1.2	36.00
IARJAT2009008	321.8	13.3	239.0	410.5	314.7	21.4	75.9	3.4	2.3	1.7	34.00
IARJAT2009009	233.2	13.2	237.2	514.0	407.1	16.0	57.3	3.0	2.5	1.2	39.00
IARJAT2009010	231.1	13.0	239.2	521.9	426.0	21.3	76.6	3.4	2.3	1.4	36.00
IARJAT2009011	224.7	12.9	237.1	423.2	324.2	28.5	86.0	3.8	2.4	1.3	36.00
IARJAT2009012	225.1	10.5	202.6	456.1	364.6	33.3	93.9	3.2	3.1	1.2	42.00
IARJAT2009013	224.7	14.3	265.0	418.9	326.0	25.1	85.7	3.6	2.7	1.2	33.00
IARJAT2009014	230.0	11.8	222.9	416.4	416.7	19.7	74.3	3.6	2.3	1.4	39.00
IARJAT2009015	231.7	9.5	166.1	482.1	370.5	17.7	82.2	3.9	2.4	1.4	40.00
IARJAT2009016	215.1	10.6	202.6	506.8	419.4	21.0	92.6	3.3	2.5	1.3	39.00
IARJAT2009017	246.4	7.5	154.1	401.4	319.8	22.0	105.4	3.4	2.4	1.4	37.00
IARJAT2009018	226.7	12.6	233.2	414.6	320.6	29.0	88.3	3.4	2.7	1.2	33.00
IARJAT2009019	218.5	12.1	203.1	509.9	404.7	21.5	90.2	3.6	2.6	1.3	37.00
IARJAT2009020	243.4	12.6	223.7	549.5	436.8	12.7	75.5	3.5	2.4	1.3	40.00
IARJAT2009021	241.2	11.6	204.0	425.3	322.6	13.8	85.5	3.5	2.3	1.4	37.00
IARJAT2009022	211.2	13.3	251.0	422.4	337.9	20.0	81.9	3.4	2.4	1.3	38.00
IARJAT2009023	240.4	10.9	188.0	320.3	255.6	14.8	87.4	3.5	2.7	1.5	41.00
IARJAT2009024	223.2	16.4	315.5	419.9	328.4	12.0	81.3	3.8	2.3	1.6	41.00
IARJAT2009025	204.6	15.0	274.7	419.9	321.0	14.9	82.7	3.4	2.3	1.4	42.00
IARJAT2009026	225.9	16.2	315.3	401.0	320.6	14.6	83.6	3.7	2.6	1.3	39.00
IARJAT2009027	213.2	7.8	150.2	407.9	320.5	21.9	79.9	3.4	2.4	1.4	35.00
IARJAT2009028	241.0	12.8	259.1	399.6	319.4	26.0	81.0	3.0	2.3	1.2	36.00
IARJAT2009029	244.3	13.1	247.4	502.0	404.1	30.3	83.2	3.3	2.4	1.3	40.00
IARJAT2009030	242.8	13.7	243.3	510.1	417.0	30.2	80.3	3.5	3.1	1.2	34.00
IARJAT2009031	250.5	12.3	221.0	421.2	326.6	24.5	88.8	3.4	2.4	1.3	40.00
IARJAT2009032	215.2	8.3	167.6	423.6	339.4	31.3	90.6	3.2	2.3	1.7	39.00
IARJAT2009033	168.9	12.6	202.6	502.8	400.8	30.2	72.8	3.1	2.5	1.2	40.00
IARJAT2009034	173.7	13.5	259.0	397.2	319.0	30.7	70.6	3.5	2.3	1.7	40.00
IARJAT2009035	250.8	13.1	238.5	514.0	413.5	25.2	96.0	3.4	2.4	1.3	40.00
IARJAT2009036	202.6	16.6	313.2	340.5	271.1	15.8	71.2	3.1	2.7	1.3	39.00
IARJAT2009037	190.5	8.9	164.4	423.7	329.2	23.9	73.4	3.8	2.4	1.3	35.00
IARJAT2009038	172.8	9.2	165.8	512.2	403.9	17.4	85.8	3.5	2.5	1.2	39.00
IARJAT2009039	166.6	17.5	327.9	427.2	435.7	21.6	76.9	3.8	2.7	1.5	33.00
IARJAT2009040	169.9	13.6	233.4	447.8	366.1	22.5	85.3	3.5	2.5	1.2	35.00
IARJAT2009041	238.3	14.6	257.8	428.2	340.0	17.5	83.9	3.8	2.5	1.2	38.00
IARJAT2009042	205.5	8.3	147.4	529.5	327.5	17.7	85.1	3.4	2.3	1.5	39.00
IARJAT2009043	228.6	15.5	273.6	447.3	360.8	22.1	91.4	3.5	2.3	1.2	40.00
IARJAT2009044	171.2	8.3	166.0	340.3	255.5	31.0	86.4	3.5	2.3	1.3	41.00
IARJAT2009045	241.0	12.6	226.9	301.5	235.2	17.4	76.8	4.0	2.4	1.3	39.00
IARJAT2009046	245.0	18.2	330.1	399.7	320.3	17.9	56.2	3.4	2.4	1.2	39.00
IARJAT2009047	207.4	17.6	321.9	303.1	240.5	20.3	79.4	3.9	2.7	1.2	41.00
IARJAT2009048	248.7	17.6	319.2	320.6	250.3	29.8	88.2	3.4	2.7	1.3	41.00
IARJAT2009049	260.5	17.4	324.9	319.8	250.2	17.8	95.1	3.7	2.8	1.3	40.00
IARJAT2009050	239.7	13.2	223.8	320.4	249.2	21.6	94.6	3.6	2.4	1.3	40.00
IARJAT2009051	229.9	11.6	204.7	408.1	319.0	17.3	75.0	3.3	2.7	1.2	35.00
IARJAT2009052	241.9	9.0	166.3	409.5	328.1	17.4	82.2	3.4	2.4	1.3	36.00
IARJAT2009053	230.1	12.1	222.5	419.7	331.5	25.6	92.6	3.4	2.4	1.2	33.00
IARJAT2009054	271.2	12.8	238.4	351.5	283.1	23.5	105.4	3.5	2.8	1.2	34.00
IARJAT2009055	194.8	18.2	331.6	359.0	280.2	24.9	88.3	3.6	2.9	1.4	35.00
IARJAT2009056	263.1	17.5	314.4	401.3	320.3	34.5	90.2	3.6	2.3	1.4	36.00
IARJAT2009057	256.8	18.1	318.9	427.1	339.9	33.6	75.1	3.5	3.0	1.2	36.00
Mean	232.2	13.2	242.8	421.6	337.6	22.3	83.7	3.5	2.5	1.3	37.7
CV	6.7	7.0	2.8	3.8	3.4	8.7	11.2	22.8	3.3	9.8	2.7

Table 6: Mean, estimates of variance components, GCV, PCV, broad sense heritability, genetic advance and genetic advance as %mean in 10 agronomic traits in 57 *J. curcas* L. genotypes

Traits	Variance Components					*GCV*	*PCV*	H_b	*GA*	%*GA*
	σ^2_{prov}	σ^2_y	σ^2_{pxy}	σ^2_e	σ^2_{ph}					
PH	125.90	85.85	63.70	282.12	171.86	4.83	5.65	0.73	17.05	7.34
FPI	8.02	0.02	-0.08	0.84	12.33	21.54	26.70	0.65	4.05	30.83
MPI	1234.78	-11.33	-2099.40	33089.60	1839.56	14.47	17.66	0.67	51.10	21.04
NB	2903.20	0.20	-19.31	260.01	3432.01	12.78	13.90	0.85	87.96	20.86
NI	2913.70	0.20	-12.80	134.28	3337.11	15.99	17.11	0.87	89.53	26.52
CH	37.86	0.01	-0.36	3.72	40.01	27.60	28.38	0.95	10.62	47.66
CT	81.84	0.13	-8.61	87.07	97.07	10.81	11.77	0.84	14.74	17.62
NBC	0.06	0.03	-0.06	0.63	0.06	6.78	6.83	0.99	0.42	11.94
NSF	0.05	0.07	0.03	0.01	0.09	8.91	11.86	0.56	0.30	11.89
FSW	0.02	0.06	0.03	0.02	0.04	10.96	15.04	0.53	0.19	14.18
OC	7.00	0.00	-0.74	2.27	7.72	7.04	7.39	0.91	5.19	13.80

PH=Plant height (cm), FPI= number of female flower per inflorescence, MPI = number of male flower per inflorescence, NB= number of branches, NI = number of inflorescence, CH = collar height (cm), CT = collar thickness (cm), NBC = number of branches on collar, NSF = number of seeds per fruit, FSW= fresh seed weight and OC = oil content.

RESULTS (Contd.)

Mean squares

Mean squares from the analysis of variance for all the morphological traits and oil content studied were presented in Tables 4 and 5 respectively. There was highly significant variation ($P \leq 0.01$) among the genotypes for all the morphological traits studied. No significant variation was observed due to year and genotype by year interaction for all the traits.

Mean performance

The means for all the agronomic traits and oil content is presented in Table 5. The mean for plant height (PH) was 232.22cm and ranged from 166.6cm for genotype IARJAT2009039 to 321.8 cm for genotype IARJAT20090008. The highest number of female flowers per inflorescence (FPI; 18.2) was recorded for genotype IARJAT2009055 while genotype IARJAT2009017 exhibited the least (7.5) FPI with an average of 13.2The mean for MPI was 242.8 and ranged from 147.4 for IARJAT2009042 to 436.4 for IARJAT2009003. Number of branches has a mean of 421.6 and ranged from the genotype with the lowest number of branches, IARJAT2009045 (301.5) to the genotype with the highest number of branches, IARJAT2009020 (549.5). The mean for NI was 337.6 and ranged from 235.2 for IARJAT2009045 to 441.1 for IARJAT2009003. Collar height has a mean of 22.3cm and ranged from 12.0cm for IARJAT2009024 to 35.1cm for IARJAT2009006.

On the other hand, collar thickness has a mean of 83.7cm and ranged from 56.2cm for IARJAT2009046 to 105.4cm for IARJAT2009017 and IARJAT2009054. Number of branches on collar has a mean of 3.5 and ranged from the genotype with the lowest number of branches on collar IARJAT2009007, IARJAT2009009 and IARJAT2009028 (3) to the genotype with the highest number of branches on collar IARJAT2009004 (4.1). NSF has a mean value of 2.5. Two genotypes, IARJAT2009012 and IARJAT2009030 have a value of 24% above the mean. The mean for FSW is 1.3. Five genotypes, IARJAT2009004, IARJAT2009005, IARJAT2009008, IARJAT2009032 and IARJAT2009034 have fresh seed weight of 31% each above the mean. Oil content has a mean of 37.7%. IARJAT2009013 has the lowest oil content (33%) while two genotypes IARJAT2009012 and IARJAT2009025 have oil content of 42% each.

Estimates of variance components

The genotypic, σ^2_{pro} and phenotypic σ^2_{pr} variances as well as genotypic and phenotypic coefficients of variation (GCV and PCV) for all the traits studied are presented in Table 6. Genotypic variances depicted a wide range of values from 0.02 (FSW) to 2913.70 (NI). Similar observation was recorded for phenotypic variances. The minimum value was recorded for fresh seed weight (0.04) and the maximum for number of branches (3432.01). Traits related to seed yield (number of seeds per fruit and fresh seed weight) presented low genotypic (0.05, 0.02) and phenotypic (0.09, 0.04) variances respectively.

All the genotype by year interactions were negative for all traits except for plant height, number of seeds per fruit and fresh seed weight (Table ..). The highest genotype by year interaction estimate was obtained in plant height while the lowest genotype by year interaction was obtained in NSF and FSW.

The GCV varied from 4.83 (PH) to 27.60 (CH) and PCV varied from 5.63 (PH) to 28.38 (CH). High GCV and PCV (>20%) were observed only in FPI and CH. Moderate estimates of PCV and GCV were observed for MPI, NI and NB. PH, NBC and NSF recorded low value (<10%) of GCV and PCV although NSF showed moderate PCV value. The PCV estimates were higher than GCV estimates for all the traits. However, the magnitude of difference was low for all the traits.

Heritability and Genetic advance

Broad sense heritability (H_b), genetic advance estimates (GA) as well as genetic advance as percentage of mean ($\%GA$) is presented in Table 6. Broad sense heritability estimates were generally very high (>60%) for all traits except NSF and FSW which showed moderate heritability (50%-60%). The highest heritability estimates were observed for number of branches on collar and collar height (99% and 95% respectively). The lowest heritability estimate (53%) was however observed for FSW.

Genetic advance refers to the expected gain from selection. The genetic advance estimates were very

high for number of branches and number of inflorescence (87.96 and 89.53 respectively). Very low genetic advance estimates (less than unity) were observed for number of branches on collar, NSF and FSW (0.42, 0.30 and 0.19 respectively). When expressed as a percentage of mean (%GA), the highest estimates were observed for collar height and FPI (47.66% and 30.83% respectively). NBC, NSF and FSB have low genetic advance as a percentage of mean (11.94%, 11.89 and 14.18 respectively). Plant height however has the lowest *%GA* (0. 73) estimate.

DISCUSSION

The highly significant variability in the results obtained in the present study suggests the existence of differences in the Jatropha genotypes for all the agro-morphological and oil content traits included in this study. This indicates the possibility of improvement for these traits through biparental mating design. Previous studies have also reported a high variability for morphological traits and seed oil content in *J. curcas* in Senegal (Diédhiou *et al.* (2007) and Ouattara *et al.* (2013). The non-significant mean square value obtained for the year for all traits may suggest that the conditions in the two years were similar for all the traits.This suggests why the genotypes performed in the same way in the two years for all the traits. The non-significant effects of genotype × year interaction mean squares that were observed for all traits also suggest the stability of the genotypes in the two years of study. The mean performance of the 57 genotypes across the two years for the 11 traits studied provides a clear indication of genetic differences among the genotypes. The result showed that all the genotypes are good sources of biodiesel. IARJAT2009012 and IARJAT2009025 demonstrated potentials for both oil content and yield components in this study.

Estimation of GCV and PCV suggested the presence of considerable variation among the genotypes and among the traits considered in this study. The high GCV and PCV for FPI and CH are indications of high variability in the two traits. The Moderate PCV and GCV for MPI, NI and NB indicate the presence of moderate levels of variability among genotypes for the traits. However, the low PCV and GCV obtained for PH, NBC and NSF indicate the presence of narrow genetic variability for these traits. The narrow difference between PCV and GCV suggests the low effect of environment on the expression of these traits.

Most of the traits studied were highly heritable as indicated by high broad sense heritability estimates. High heritability values coupled with high genetic advance recorded for MPI, NI and NB suggest a highly significant role of additive gene action for the inheritance of these traits. The moderate heritability values coupled with low genetic advance obtained in NSF and FSW indicate that additive and non-additive gene effects are both important in the expression of these traits.

CONCLUSION

This study indicated the presence of considerable variability in the 57 genotypes evaluated suggesting the possibility of improvement for the traits studied through appropriate breeding procedures like biparental mating design. The evidence of this variability is promising regarding the existence of genetic diversity among the *J. curcas* genotypes studied. These results are important for Jatropha improvement programs in Nigeria in order to obtain cultivars with high seed and oil yield.

REFERENCES

Adama Z., Abel T. N., Mahamadou S., Abdourasmane K. K., Philippe S., Bonny R. N. and Haile D. (2017). Variability and Correlations among Groundnut Populations for Early Leaf Spot, Pod Yield, and Agronomic Traits. *Agronomy* 2017, 7, 52; doi: 10.3390/agronomy7030052

Adebowale K.O., Adedire C.O. (2006). Chemical composition and insecticidal of underutilized *Jatropha curcas* seed oil. *Afr. J. Biotechnol*. 10:901-906.

Chen Y.X., Mao J.Q., Wu Z.B., Zhu H.P., Tang Z.Y. (2006). Comprehensive exploitation and utilization *Jatropha* oil plants. China Oils Fats 31:63-65.

Fairless D. (2007). *Biofuel*: the little shrub that could maybe. Nat. 449: 652-655.

Freitas R. G., R. F. Missio, F. S. Matos, M. D.V. Resende and L. A. S. Dias (2011). Genetic evaluation of *Jatropha curcas*: an important oilseed for biodiesel production. *Genetics and Molecular Research* 10 (3): 1490-1498 (2011).

Montes O. L. R., Andres F. T. S., Raymond E. E. J., Cesar A. A. P., Julio E. B. S., Luisa M. T., Richard G. F. V. and Eibertus N. L. (2014). High level of molecular and phenotypic biodiversity in *Jatropha curcas* from Central America compared to Africa, Asia and South America. BMC *Plant Biology* 2014, 14:77

Natarajan, P., Kanagasabapathy, D., Gunadayalan, G., Panchalingam, J., Shree, N., Sugantham, P. A., Singh, K. K. & Madasamy, P. (2010). Gene discovery from *Jatropha curcas* by sequencing of ESTs from normalized and full-length enriched cDNA library from developing seeds. *BMC Genomics,* 11**,** 606.

Openshaw K. (2000). A review of *Jatropha curcas*: An oil plant of unfulfilled promise. *Biomass Bioener*. 19:1-15.

Priyamvada S., Singh S., Mishra S., and Bhatia S. K. (2010). Genes, Genomes and Genomics. *Global Science Books.*

ASSESSMENT OF GENETIC DIVERSITY IN GROUNDNUT (*Arachis hypogaea* L.) GERMPLASM USING PRINCIPAL COMPONENT ANALYSIS AND CLUSTER SEGMENTATION

Celestine U. Aguoru[1], Olalekan J. Olasan[1], Lucky O. Omoigui[2], [3]Ebenezer J. Ekefan

[1]Department of Botany, University of Agriculture, Makurdi, Nigeria.

[2]Department of Plant Breeding and Seed Science, University of Agriculture, Makurdi, Nigeria.

[3]Department of Crop & Env. Protection, University of Agriculture, Makurdi, Nigeria.

Corresponding Author E-mail: celeaguoru@yahoo.com

Abstract: Genetic diversity of 118 groundnut germplasms were assessed in Nigeria using 35 agronomic and fruit traits. Planting materials were sourced from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Principal Component Analysis (PCA) and Cluster Segmentation were used to determine the pattern and level of genetic variability in the germplasms. The Thirteen principal components that were extracted explained 71.87% of the overall cumulative variance. The first three principal components contributed 15.3%, 7.287% and 6.6% respectively. Extraction cummunality values identified twenty characters as influential traits contributing to the recorded genetic diversity in the PCA. Most attributes are cultivar-dependent. This yielded 53 levels of genetically diverse cultivars (Level A to Level BB) belonging to six clusters and two sub clusters. Sixteen cultivars are highly divergent in the dendrogram. They include: ICGV-IS-13865, SAMNUT-24, ICGV-IS-141151, ICGX-IS-11003-F2-B1-B1, ICGV-99241, ICGV-IS-09992, ICGV-97182, ICGV-IS-141156, ICGV-IS-13867, ICGV-IS-09828 and HAUSA-KANO. Selected cultivars should be exploited to facilitate improvement program. Traits such as Leaf pubescence, Days to maturity, Seed weight, Days to 50% flowering, Number of seeds, Pod sizes, Plant height and Number of pod are valuable in groundnut Breeding and Taxonomy. The information provided in this report is useful in the efficient management and the global sustainable utilization of the crop genetic resources.

Key words: Crop improvement, Genetic diversity, Germplasm, Groundnut, Taxonomy

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is ranked thirteenth among the world food crops, fourth as the most important source of edible oil, and third as the most important source of vegetable protein (FAOSTAT, 2017). The seed is rich in all classes of phytonutrients of health benefits including essential vitamins and minerals (Asibuo *et al*., 2008; Atasie *et al*., 2009). Nigeria is the largest producer of groundnut in Africa. Many landraces and cultivars are localized in different parts of the country (Ladodo *et al*., 2015; Mshelmbula *et al*., 2017) where they are cultivated at both subsistence and commercial levels (ICRISAT, 2017).

The extent and distribution of genetic diversity in a plant species depend on its evolution and breeding system, ecological and geographical factors as well as anthropogenic influences (Aguoru *et al*., 2014, 2015c). Genetic variability in groundnut is low due to the origin of the crop through a single hybridization event between two diploid species followed by a chromosome doubling and crossing with wild diploid species (Janila *et al.,* 2013). The cultivated groundnut is an allotetraploid (Bertioli *et al*., 2011; Lavia *et al*., 2014; Wang *et al*., 2016). The low genetic diversity reported in the crop has been a setback in groundnut breeding (Janila *et al*., 2013). The cultivated accession of *Arachis hypogaea* in the gene banks and the advanced breeding lines are the most frequently used parents in hybridization. Intensification of breeding efforts to produce varieties that combine high yield attributes with other agronomic qualities has engineered huge collections that should be properly managed and evaluated (Janila *et al.,* 2013; Saghiri and Abdel-Salam, 2015; Wang *et al*., 2016).

Genetic diversity represents the heritable variation within and between population (Belamka *et al*., 2011; Aguoru et *al*., 2015b; Wang *et al*., 2016). This pool of genetic variation within an inter-mating population is the basis for selection as well as for crop improvement (Oboh et al., 2008; Aguoru et *al*., 2015b).

Establishment and maintenance of groundnut genetic resources is essential for sustainable development (Janila *et al*., 2013). It is important to understand the distribution and extent of genetic diversity in the crop available to humans so that they can be adequately managed to prevent genetic erosion (Aguoru et *al*., 2015c). It is known that plant genetic diversity changes in time and space (Janila *et al*., 2013). Diversity studies facilitate selection, promote crop improvement and necessitate systematic action (Aguoru et *al*., 2015a). Due to low level of genetic variability reported on the crop, assessment checks are important on groundnut collections across the globe. The present study aimed at assessing the pattern and level of genetic diversity in landraces and cultivars of groundnut germplasms in Nigeria. The outcome may accelerate groundnut breeding not only in Nigeria but also at the global scale. The information may also be applied in the systematic audit of crop and in the determination of phylogenetic relationship, classification and naming of taxa.

MATERIALS AND METHODS

Plant materials and Study design

Seeds of 200 cultivars of groundnut were sourced from the Nigeria out-station of the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), a global leader in groundnut breeding and research with its Headquarters in India. 118 cultivars were randomly selected from the 200 germplasms for genetic diversity studies and assigned accession number G1-G118. Planting of seeds was done on ridges at the University of Agriculture Makurdi Research Farm on a farm size of 891.75m^2. The planting of the seeds of each cultivar was replicated 5 times with a spacing of 1m.

Data collection and analysis

Phenotypic data was collected in the field and in the laboratory. Thirty-five characters were studied. Genetic diversity of the cultivars based on PCA was carried out on SAS software (V.9.4 versions). The accessions were considered in two batches to avoid the overlap of cultivars in the plotted prediction ellipses. The first batch consisted of 60 cultivars (G1-G60) while 58 cultivars were in the second batch (G61-118). The Eigen matrix and cummunality values were also obtained from the PCA. Dendrogram was constructed using the Average Linkage (Between Group) method of the SPSS software (20.0 versions).

RESULTS AND DISCUSSION

The Eigen matrix of the 13 principal components Principal Component Analysis (PCA) generated an Eigen matrix consisting of 13 components is presented in Table 1. The amount of variance explained by the thirteen components as computed from the matrix is presented in Table 2. The Thirteen principal components explained 71.87% of the overall cumulative variance in the groundnut germplasms. The first three components explained 15.29%, 7.287% and 6.62% of the overall variance respectively. Number of seed had Eigen values (0.843) and 0.218 in the first and second components respectively. Pedicel length was low (0.122) in the first component but 0.632 in the third component. Other traits with high variability in the first component are pod weight (0.804), number of pod (0.736) and plant biomass (0.647). Day to maturity had Eigen value of 0.398 in component 1.

The number of components contributing to the cumulative variance depicts a huge variation within the germplasms. This contradicts the earlier reports of low variability on groundnut (Malti, 2002; Janila *et al*., 2013). However, the present work agrees with other authors who found high diversity in groundnut germplasm (Balota *et al*., 2012; Albert, 2014; Aworinde *et al*., 2007; Saghiri and Abdel-Salam, 2015; Wang *et al*., 2015) and in other crops (Atangana, 2002; Oboh *et al*., 2008; Rao *et al*., 2011; Ogunniyan and Olukojo, 2015; Somta *et al*., 2016). The high level of genetic diversity observed in this study appears to have come from the interplay of the different many traits involved. This variability should be explored to maximize the full benefits the crop offers. Extraction cummunality from PCA picked twenty characters as highly influential traits on the observed genetic diversity. Pedicel length has the highest cummunality value of 0.958 followed by hairiness of mature and young leaflets with values of 0.936 and 0.924 respectively. Others ranged from 0.834 in Days to maturity to 0.701 in Days to first flowering.

Characters such as Leaf pubescence, Days to maturity, Seed weight, Days to 50% flowering, Number of seeds, Pod sizes, Plant height and Number of pod contributing

to high variation in the crop have opened windows of opportunity in many areas. Cultivars with early maturity time such as ICGV-IS-141091, ICGV-IS-141178, ICGV-IS-14898, ICGV-IS-13967, ICGV-IS-13887, ICGS-44, ICGV-02144, ICGV-87378, ICGV-02189 and ICGV-97182 should be selected for planting or hybridization. Many authors have emphasized on the need to explore varieties with quick maturity time to improve others (Janila *et al*., 2013; Mshelmbula *et al*., 2017). Timing is a crucial factor to achieve high production in groundnut especially when short raining season is predicted and in drought condition (Ceccarelli *et al*., 2007; Balota *et al*., 2012). Early flowering portends quicker pod production than other cultivars and this may correlate with early maturity time (Andargie *et al*., 2013). Higher agronomic values in traits like number of pods or larger pod and seed sizes promotes the market values of the cultivars for a good return (Atangana, 2002; Aworinde *et al*., 2007).

Therefore, cultivars with high pod yields such as SAMNUT 24, ICGV-IS-13867, ICGV-IS-09828, ICGV-IS-141071, ICGV-12991, SAMNUT 23, ICGV-IS-13897, ICGV-IS-14867, ICGV-02022, ICGV-IS-13865, ICGV-IS 13896, and ICGV-IS-13893 should be selected. Some of these cultivars have been reported by many authors as high yield breeds. Selection based on seed sizes should consider cultivars such as: ICGV-IS 09828, ICGV-99241, ICGV-02022, ICGV-12991, ICGV-IS-13863, ICGV-IS-13874 and SAMNUT 23. Cummunality values have pointed out some vegetative characters such as plant height, stem pigmentation, stem hairiness, leaf hairiness and leaf sizes that systematically valuable in the description, circumscription and classification of taxa (Krapovickas and Gregory, 2007). The present study supports earlier taxonomic reports on groundnut based only on the use of vegetative characters (Krapovickas and Gregory, 2007). Apart from breeding applications, most reproductive traits with high cummunality values appear to be an excellent source of taxonomic evidence.

Characters that contributed less to the variability explained in the germplasm based on the low cummunality values are contained in Table 4. They include days to emergence, seed colour, pod beak, leaf sizes and branching pattern. This implies that they have less application in the breeding and taxonomy of groundnut but some varieties were earlier described based on the basis of growth habit and branching pattern (Krapovickas and Gregory, 2007). Traits such as days to emergence, diseases, stem circumference and plant biomass could be influenced by the prevailing environmental conditions such as water and soil factors (Ceccarelli *et al*., 2007; Musa *et al*., 2010; Ladodo *et al*., 2015).

Figure 1 explains the diversity of first 60 cultivars (G1-G60). Based on 95% prediction ellipse of component 1 and 2, some cultivars are clearly separated as genetically diverse accessions. Very distant cultivars such as ICGV-IS-13943, ICG-4750, ICGV-02144 and ICGV-03179 (G28, G42, G52 and G55 respectively) are those that did not produce pods in field. G10 (ICGV-IS-13867) and G15 (ICGV-IS-13881) were fertile but very distant from other cultivars (not within the ellipse). Cultivars that are widely separated from others are: G41 (ICGV-IS-13887), G54 (ICGV-02189), G56 (ICGV-97182), G43 (ICG-4729), G47 (ICGS-11060), G32 (ICGV-IS-13955), G58 (ICGV-02022), G13 (ICGV-IS-13877), G8 (ICGV-IS-13862), G51 (ICGV-99241), G17 (ICGV-IS13892), G9 (ICGV-IS-13865) and G35 (ICGV-IS-13968). Others cultivars in the circle are clustered together. Figure 3 explains the genetic diversity of second set of 58 cultivars (G61-G118). Prediction ellipse of component 1 and 2 revealed cultivars that are either more clearly separated or more clustered than those in Figure 2. Some accessions such as G-2-52, ICGV-SM-07441, ICGV-IS 07965, ICGV-IS-13811, ICGV-IS-07831, and ICGV-IS-09926 showed more diversity to an extent than the highly clustered cultivar.

Table 1: Eigen Matrix of 13 Principal Components Extracted from the agronomic and fruit characteristics of the 118 cultivars of groundnut

	Character												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
DTE	-.123	-.143	-.024	-.123	.680	-.046	.164	-.272	-.055	-.102	-.147	-.161	-.102
DFF	.323	.411	-.214	.128	-.387	.107	.238	.148	-.063	-.313	.020	-.074	.067
D50%F	-.147	.117	-.115	-.049	.578	-.027	.153	-.134	-.007	.391	.226	.270	.275
Survival (%)	-.097	.312	.377	-.150	-.053	-.047	.077	.097	-.289	.095	-.369	-.019	-.333
Days to maturity	.398	-.022	-.484	.367	-.216	.311	.037	.296	.050	.080	-.005	-.021	.258
Growth habit	.212	-.110	-.032	-.165	-.055	.519	-.272	-.148	.115	-.216	.303	-.111	-.105
Branching pattern	.289	-.008	.187	.046	-.116	.349	-.106	-.012	.110	.293	-.472	-.072	.029
Stem pigm.	.027	.182	.061	.021	.361	.306	-.020	.278	-.274	-.299	-.018	.358	-.040
Stem hairines	.109	-.193	-.026	.116	.100	-.508	-.023	.139	.177	.000	.458	-.135	-.176
Leaf colour	-.003	.113	-.140	.104	-.230	.052	.435	-.475	.355	.149	-.070	.282	-.025
HaIriness YL	-.154	.452	.329	.601	.222	.171	-.263	-.023	.185	-.040	.155	.056	-.088
Hairiness ML	-.086	.494	.394	.560	.180	.146	-.264	-.062	.257	-.057	.098	.009	-.066
No of flowers	.454	.149	-.006	-.028	.347	-.264	-.139	-.213	.006	-.164	-.165	-.477	.098
Pedicel Length	.122	-.456	.632	.108	.022	.266	.401	.142	.027	-.032	.121	-.108	.021
Flower Length	.186	-.495	.556	.106	.078	.332	.329	.148	-.002	.094	.090	-.176	-.032
Leaflet texture	-.011	.061	-.027	.333	.022	-.334	.541	.137	.260	-.081	.029	-.084	.202
Traces of disease	.210	.204	-.148	-.151	.129	.411	.226	.154	-.121	.273	.076	.011	-.104
No of branch	.204	-.096	-.300	-.085	.268	.123	-.253	.314	.390	.333	-.014	.079	-.237
Plant height	-.020	.101	.477	-.118	-.053	-.370	-.056	.267	.287	-.156	-.312	.264	.128
Leaflet length	-.147	.128	.401	-.495	-.015	-.016	-.129	.252	.087	-.147	.185	.105	.439
Leaflet width	-.153	.155	.370	-.406	-.265	-.141	-.066	-.060	.195	.441	.134	.018	-.002
Stem circum	.006	-.261	.028	.295	.119	-.322	.112	.240	-.358	.117	.093	.232	-.304
Petiole length	.169	-.241	.195	.153	-.465	-.203	-.296	-.047	-.186	.070	.161	.010	-.157
Plant biomass	.647	.119	.143	-.092	.098	.082	.018	.149	-.159	.114	.077	-.181	.191
No of pod	.736	.149	.150	-.102	.256	-.085	.016	-.172	.046	.040	-.005	-.160	-.011
Pod length	.740	.072	.047	-.047	.154	-.080	.107	-.053	-.038	-.218	-.044	.332	-.040
Pod width(cm)	.670	-.027	-.112	.056	-.120	-.161	-.118	.016	-.255	.072	.059	.055	.114
Pod weight	.804	.183	.081	-.122	.012	-.010	.090	-.148	.018	-.074	.066	.232	-.130
Pod beak	.071	-.168	.124	.539	-.128	-.043	.058	-.274	-.074	.061	-.130	.041	.068
Pod constriction	.037	-.080	.216	.255	.041	-.016	-.251	-.309	-.454	.232	.042	.117	.391
No of seeds	.843	.218	.020	-.029	-.061	-.117	.003	.025	.023	.133	.038	-.057	-.032
Seed weight	.778	.326	.082	.045	-.073	-.162	.055	.127	.037	.165	.033	-.027	-.074
Seed length	.556	-.519	-.057	-.123	.117	.032	-.110	-.122	.144	-.165	-.021	.235	.000
Seed width	.462	-.583	.093	.019	-.086	.058	-.182	-.190	.239	-.117	-.099	.159	.018
Seed colour	.083	-.354	-.171	.296	.254	-.150	-.233	.407	.118	.117	-.296	-.038	.155

Extraction Method: Principal Component Analysis. a. 13 components extracted.

Legend: DTE = day to emergence; DFF = day to first flowerin; Stem pigm. = stem pigmentation; Hairiness YL = hairiness of young leaflet; Hairiness ML = hairiness of mature leaflet; Stem circum = stem circumference

Table 2: Total variance explained using Eigen values of Principal Component Analysis

Component	Initial Eigenvalues		
	Total	**% of Variance**	**Cumulative %**
1	5.351	15.288	15.288
2	2.550	7.287	22.575
3	2.317	6.620	29.196
4	2.119	6.055	35.251
5	2.032	5.807	41.057
6	1.874	5.353	46.410
7	1.552	4.434	50.844
8	1.466	4.189	55.033
9	1.376	3.931	58.964
10	1.243	3.553	62.516
11	1.148	3.280	65.796
12	1.097	3.136	68.932
13	1.027	2.934	71.865
14	.989	2.825	74.690
15	.943	2.694	77.384
16	.882	2.519	79.903
17	.832	2.377	82.279
18	.753	2.151	84.430
19	.685	1.958	86.388
20	.622	1.776	88.164

Table 3: First twenty traits that contribute to variability among the 118 cultivars of groundnut

Characters	**Extraction Cummunality value**	**Variability Rating**
Pedicel length	.958	1st
Hairiness of mature leaflets	.936	2nd
Hairiness of young leaflets	.924	3rd
Days to maturity	.834	4th
Flower length	.826	5th
Seed weight	.819	6th
Days to 50% flowering	.808	7th
Number of seeds/plant	.800	8th
Seed width	.799	9th
Pod length	.786	10th
Pod weight	.782	11th
No of flowers	.772	12th
Plant heigth@60ds	.756	13th
Seed length	.747	14th
Number of pod/plant	.734	15th
Stem pigmentation	.733	16th
Pod constriction	.733	17th
Stem hairiness	.725	18th
Leaflet length	.719	19th
Days to first flowering	.701	20th

Table 4: Traits of low contribution to variability among the 118 cultivars of groundnut

Characters	Extraction Communality value
Days to emergence	.697
Seed colour	.690
Length of floral whorl	.687
Leaflet width	.679
Number of branches @ 60days	.669
Growth habit	.666
Percent survival	.659
Stem circumference	.658
Leaf petiole length	.629
Pod width	.606
Stem branching pattern	.604
Leaflet texture	.602
Plant biomass	.571
Traces of diseases	.503
Pod beak	.501

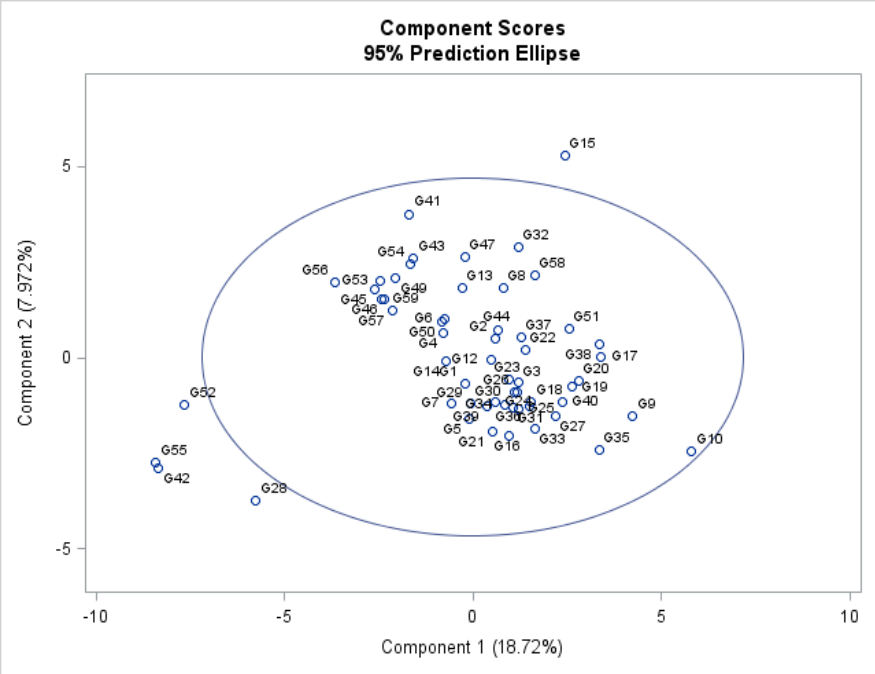


Figure 1: Prediction Ellipse of Component 1 and 2 (First 60 cultivars, G1-G60)

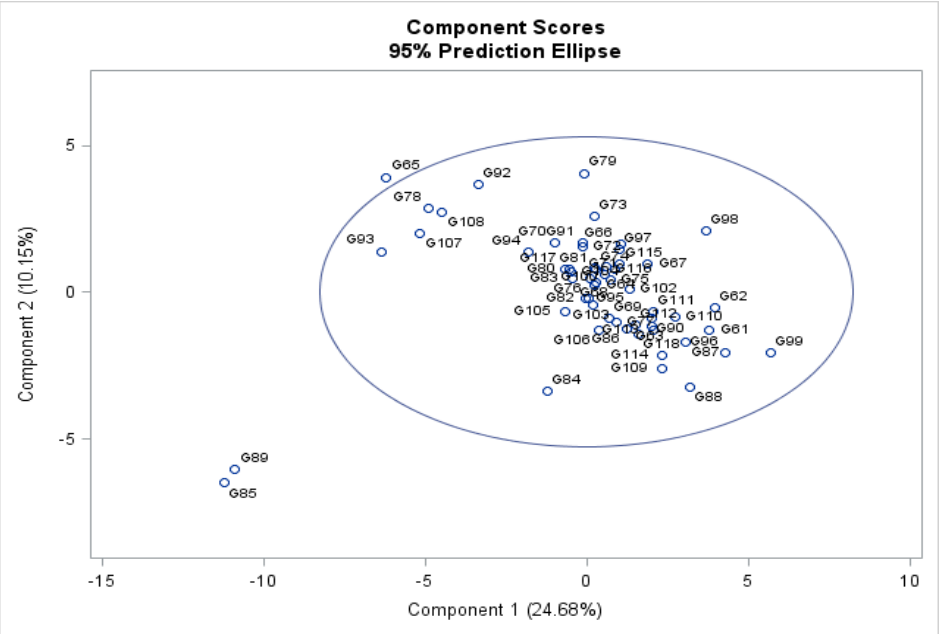


Figure 2: Prediction Ellipse of Component 1 and 2 (Second 58 cultivars, G61-G118)

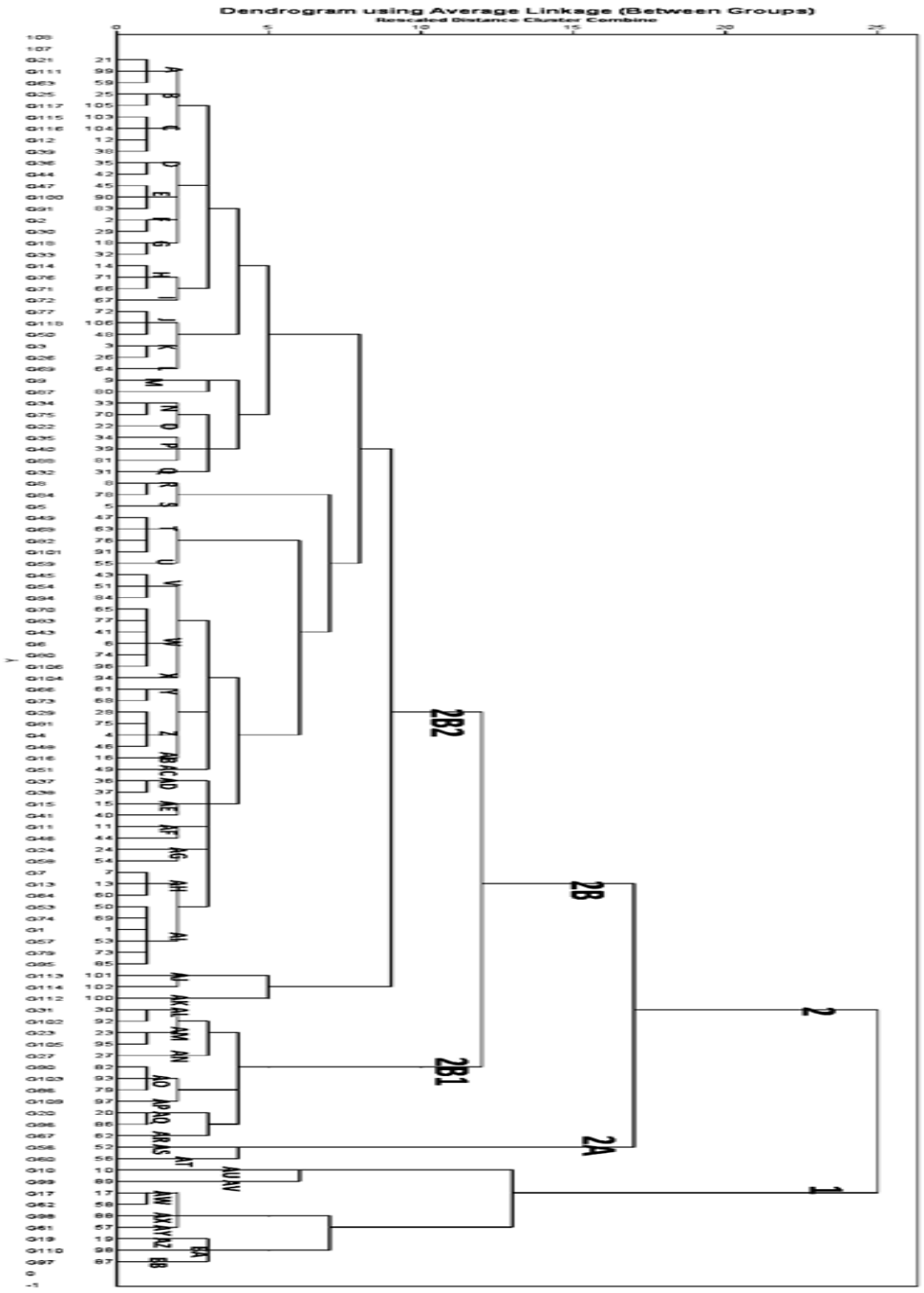


Figure 3: Dendrogram explaining the pattern of genetic diversity using Average Linkage method

Cultivars such as ICGV-IS-13967, ICGV-IS-141144, HAUSA KANO, ICGV-IS-09828, ICGV-12991, ICGV-IS-89767, ICGV-94379 and ICGV-93305 are genetically diverse cultivars. They are represented by accession codes G79, G73, G98, G99, G88, G84 and G81 respectively. Cultivars such as ICGV-IS-141213, J-11, ICGV-IS-96909, ICGV-SM-01711, TG-39, Dh-86, EX-DAKAR and ICGV-IS-13988 did not produce pods, therefore far distant from the rest, although ICGV-IS-96909 and ICGV-SM-01711 are too far distant from this group. Some of these cultivars such Dh-86 and EX-DAKAR have been reported as high yielding varieties (ICRISAT, 2017), but this contradicts the present findings. It

thus appears that these cultivars are not sterile but they may be vulnerable to some disease conditions affecting pod formation in the field (Musa *et al*., 2010).

Cluster analysis also explains the nature and level of genetic diversity among the cultivars (Figure 3). The level of genetic diversity was high based on the pattern of convergence and divergence of accessions. Most attributes could be described as cultivar-dependent attributes. Divergence could be attributed to cultivar differences in those characters with high cummunality values in the PCA. They have yielded 53 levels of genetically diverse cultivars (Level A to Level BB) belonging to six groups and two sub groups. Some levels consist of cultivars that shared many attributes in common (very closely related). They include cultivars that are convergent in Levels A, B, C, D, E, F, G, H, J, K, R, T, V, W, Z, AD, AE, AF, AG, AH, AI, AJ, AL, AM, AO, AQ and AW. Divergent cultivars shared limited number of characters with other members of the same or different groups. They include cultivars in levels: I (G72), L (G69), O (G22), P (G35, G40, and G88), S (G5), U (G59), X (G104) and AB (G16). 16 cultivars were highly divergent in the dendrogram. They are: G9, G87, G59, G104, G51, G112, G56, G60, G10, G99, G98, G61, G19, G110, G97 and G17. They represent cultivars: ICGV-IS-13865, SAMNUT-24, ICGV-IS-141151, ICGX-IS-11003-F2-B1-B1, ICGV-99241, ICGV-IS-09992, ICGV-97182, ICGV-IS-141156, ICGV-IS-13867, ICGV-IS-09828, HAUSA-KANO, ICGV-IS-14867, ICGV-IS-13896, ICGV-IS-09926, SAMNUT-25 and ICGV-IS-13892 respectively. They are parts of the widely separated cultivars in PCA prediction ellipse.

CONCLUSION

The present study observed wide genetic diversity in the groundnut germplasm assessed using two technical approaches. The recommended cultivars (ICGV-IS-13865, SAMNUT-24, ICGV-IS-141151, ICGX-IS-11003-F2-B1-B1, ICGV-99241, ICGV-IS-09992, ICGV-97182, ICGV-IS-141156, ICGV-IS-13867, ICGV-IS-09828 and HAUSA-KANO) and the influential traits (Leaf pubescence, Days to maturity, Seed weight, Days to 50% flowering, Number of seeds, Pod sizes, Plant height and Number of pod) may be utilized to facilitate improvement program and systematic studies in groundnut. The information provided in this report is relevant in the efficient management and the global sustainable utilization of groundnut genetic resources.

REFERENCES

Aguoru, C.U., Uduaga, B.E and Olasan, J.O. (2014). Ecological pattern and distribution of economic trees in the Federal University of Agriculture, Makurdi in the North Central Guinea Savannah, Nigeria. *International Journal of Tropical Agriculture and Food Syst*ems, 8(1): 10-11.

Aguoru, C.U., Omoigui, L.O and Olasan, J.O. (2015a). Molecular Characterization of *Solanum* Species (*Solanum aethiopicum* Complex; *Solanum macrocarpon* and *Solanum anguivi*) using Multiplex RAPD Primers. *Journal of Plant Studies*, 4: 1-8.

Aguoru, C.U.,Omoigui, L.O and Olasan, J.O. (2015b). Population Genetic Study of Eggplants (*Solanum*) Species in Nigeria, Tropical West Africa, Using Molecular Markers. *International Journal of Plant Research*, 5(1): 7-12.

Aguoru, C U., Azua E. T and Olasan J. O. (2015c) Approaches to mininmizing and overcoming current biodiversity loss. *British Journal of Environmental Sciences*, 3(3): 12-26.

Albert, A. (2014). Analysis of genetic diversity of some groundnut (*Arachis hypogaea* L.) cultivars using Principal Component Analysis. MSc thesis. Ahmadu Bello University, Zaria, Nigeria. 76pp.

Andargie, M., Rem, S. P., Geoffrey, M.M., Michael, P.T. (2013). Quantitative Trait Loci of flowering time related traits identified in recombinant inbred lines of cowpea (*Vigna unguiculata*). *Genome*, 56: 289-294.

Asibuo, J. Y., Akromah, R., Safo-Kantanka, O., Adu-Dapaah, H.K., Ohemeng-Dapaah, S. Agyeman, A. (2008). Chemical composition of groundnut, *Arachis hypogaea* (L) landrace. *African Journal of Biotechnology*, 7: 2203-2208.

Atangana, A. R. (2002). Domestication of *Irvingia gabonensis*: phenotypic variation in fruit and kernels in two populations from Cameroon. *Agroforest Systems*, 53:55-64.

Atasie, V. N., Akinhanmi, T. F. and Ojiodu, C. C. (2009). Proximate analysis and physico chemical properties of groundnut (*A.hypogaea*). *Pakistan Journal of Nutrition*, 8: 194-197.

Aworinde, D. O., Jayeola, A. A. and Ayodele, M.S. (2007) Fruit morphological characterization among some varieties of *A. hypogaea* L. in Nigeria. *Advances in Science and Technology*, 1(2): 155-160.

Balota M. T., Isleib G., Tallury, S. (2012). Variability for drought related traits of Virginia-type peanut cultivars and advanced breeding lines. *Crop Science,* 52: 2702–2713.

Belamka, V., Selvaraj, M. G., Ayers, J. L., Payton, P. R., Puppala, N. and Burow, M. D., (2011). A First Insight into Population Structure and Linkage Disequilibrium in the US Peanut Minicore Collection.

Bertioli D. J., Seijo G., Freitas F. O. (2011). An overview of peanut and its wild relatives. *Plant Genetic Resources,* 9: 134–149.

Ceccarelli S., Grando S., Baum M. (2007). Participatory plant breeding in water-limited environments. *Experimental Agriculture,* 43: 411–435.

Food and Agricultural Organization (FAO) (2017). Available at: http://faostat.org/peanut. Retrieved on 12/04/2017, 15:10 GMT.

ICRISAT (2017). International Crops Research Institute for the Semi-Arid Tropics Annual Report of 2016. Retrieved from: www.icrisat.org. On 21/02/2017, 08:50 GMT.

Janila, P., Nigam, S.N., Manish, K.P, Pandey, Nagesh, P. and Ranjeev, K.V. (2013). Groundnut improvement: use of genetic and genomic tools. *Frontier in Plant Science*, 4 (23): 1-33.

Krapovickas, A. And Gregory, W.C. (2007). Taxonomy of the genus Arachis (Leguminosae). *Bonplandia*, 16:1-205.

Ladodo, A., Husaaini, M.A.and Muhammed, S.G. (2015). Evaluation of tillage practices on weed control and yield of groundnut (*A.hypogaea* L.) in Sudan savanna Agroecology of Nigeria. *American Journal of Experimental Agriculture*, 6(6): 361-371.

Lavia, G. and Aveliano, F. (2014). Karyotypic studies in *Arachis hypogea* L. *International Journal of Cytology, Cytosystematics and Cytogenetics*, 57(4): 353-359.

Malti, R.K. (2002). Peanut crop (*Arachis hypogea*) crop. Science publishers inc, enfield. Pp1-11.

Mshelmbula, B.P., Jummai, B.F., Mallum, S.M. and Zacharia, R. (2017). Studies on four cultivars of groundnut grown in Mubi, Adamawa State Nigeria. Journal of Technological Research, 12(1)

Musa, A.K., Kalejaiye, D.M., Ismaila, L.E. and Oyerinde, A.A. (2010). Proximate composition of selected groundnut varieties and their susceptibility to *Trogoderma granarium* E. attack. *Journal of Stored Products and Postharvest Research*, 1(2): 13-17.

Oboh, B., Ogunkanmi, L. and Olasan, L. (2008). Phenotypic Diversity in *Terminalia Catappa*. *Pakistan Journal of Biological Sciences*. 11(1): 135-138.

Ogunniyan, D.L. and Olukojo, S.L. (2015). Genetic variation, heritability, genetic advance and agronomic character association of yellow elite inbred lines of maize (*Zea mays* L). *Nigerian Journal of Genetics*, 28(2): 24-28.

Rao, G.R., Arun, K.S., Korwar, G.R., Venkateswailu, B., Srinivas, I. (2011). Diversity and variability in seed characters and growth of *Pongamia pinnata* L. Pierre accessions. *Trees*, 25(4): 725-734.

Saghiri, M.G. and Abdel-Salam, A.G. (2015). Genetic Diversity of Peanut (*Arachis hypogea* L.) Cultivars as Revealed by RAPD Markers. *American Journal of Plant Sciences*, 6: 2303-2308.

Somta, P., Chank, S., Rungnoi, O., Srinive, P. (2016). Genetic diversity of the Bambara groundnut (*Vigna subterranea* (L.) Verdc.) as assessed by SSR markers. *Genome*, 54(11): 898-910.

Wang, H., Khera, P., Huang, B., Yuan, M., Katem, R., Zhuang, W., Harris-Schultz, K., Moore, K.M. (2015). Analysis of genetic diversity and population structure of peanut cultivars and breeding lines from China, India and the U.S. using SSR markers. *Journal of Integrative Plant Biology*, 58(5): 452-46.

ASSESSMENT OF GENETIC DIVERSITY AMONG SELECTED MAIZE LANDRACES AND HYBRIDS USING SSR MARKERS LINKED TO QTLS FOR DROUGHT AND NITROGEN TOLERANCE

Oyenike A. Adeyemo, Olatunde. A. Bhadmus, Olumide A. Adebesin, and Uzezi E. Okinedo

Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria

***Corresponding Author:** adeyemona@gmail.com; aoadeyemo@unilag.edu.ng; +2348055073665; +2349074619574

Abstract: In this study, seventeen quantitative trait loci (QTLs) linked simple sequence repeat (SSR) markers of maize were used to assess genetic diversity in selected 20 maize genotypes adapted to Southern Nigeria. The morpho-physiological traits considered include stay-green characteristics, days to anthesis, days to silking and anthesis-silking interval, leaf number and high nitrogen regime, ear setting and yield components, while the maize genotypes comprised of 9 landraces, 5 commercial hybrids grown by local farmers and 6 recently developed IITA hybrids. A total of 57 polymorphic alleles were amplified and the mean allele number was 3.35 and a range of 2 to 5 alleles per locus. The average polymorphic information content (PIC) value of 0.46, with a minimum and maximum of 0.16 and 0.72 were observed. The genetic similarity ranged from 0.45 to 0.91 with a mean of 0.58. The UPGMA dendrogram indicated that the 20 maize genotypes could be divided into two major groups, of which each had two subgroups. Principal component analysis also depicted diversity among the lines. Based on the grouping, lines from landraces were clustered together, while the commercial had close genetic relationship with IITA hybrids. Genetic diversity was moderately high among the landraces than in the hybrids. This study reveals the efficiency of trait-linked SSR markers to estimate the extent of the genetic variation of the maize varieties for drought/nitrogen regime adaptive traits and will be a contribution to further application of marker assisted selection in maize breeding.

Keywords: Hybrids, Genetic Diversity, Landraces, Maize, SSR linked to QTLs

INTRODUCTION

Maize is (*Zea mays* L.) is one of the most economically important tropical crops worldwide because it is a staple food for humans and animals (Reif *et al.,* 2005). Presently, maize is still largely grown by indigenous small-scale farmers in southern Nigeria using landraces that have wide adaptability to local growing environments and commercial hybrids or varieties developed from the breeding program of the International Institute of Tropical Agriculture. The high usage of commercial hybrids with enhanced yield and resistance to biotic and abiotic stresses have substantially decreased genetic diversity of landraces (Yong-Bi, 2015). The lack of protection of genetic resources of the original maize landrace varieties, is also a major contributing effect on the erosion of its genetic variability (Westengen *et al.,* 2012).

Knowledge of the genetic diversity among farmers' varieties and hybrid maize lines is useful for monitoring germplasm conservation, utilization of diverse genetic resources for cultivar improvement and protection (Smith *et al.,* 1997; Bernardo, 2002). Molecular markers provide means of measuring genetic relationships and variations among genotypes. DNA markers like microsatellites (simple sequence repeats, SSR) markers have been widely used to evaluate genetic diversity (Liu *et al.,* 2003; Menkir *et al.,* 2004; Warburton *et al.,* 2008). Furthermore, SSR markers have also been applied in the classical linkage/QTL mapping to study the genetic basis of important quantitative traits in many different population structures of various maize germplasm (Szalma *et al.,* 2007).

Several previous linkage mapping studies have identified quantitative trait loci (QTL) associated with some morphological and physiological traits in maize (Smith *et al.,* 1995; Veldboom and Lee, 1996; Li *et al.,* 2003; Zhang *et al.,* 2004; Ribaut *et al.,* 2007;

Wu *et al*., 2008). Wang *et al.* (2012) examined the QTLs for stay-green related traits such as green leaf area per plant at 30 d after flowering (GLA2), green leaf area per plant at the grain-ripening stage (GLA3), and left green leaf number per plant at the grain-ripening stage (LLN), green leaf area per plant in the whole growing period (GLA1) and total leaf number per plant in the whole growing period (TLN). Szalma *et al.* (2007) performed the QTL mapping for days to anthesis, days to silking, and anthesis-silk interval in a collection of maize inbreds and hybrids lines. A mapping study for 3 traits (plant height, ear height, and leaf number) associated with plant architecture under the high nitrogen regime and low nitrogen regime have been conducted on a group of maize genotypes (Liu *et al.,* 2003; Zheng and Liu, 2013).

To our knowledge, very little study is known on the relationship in maize landraces and hybrids using molecular characterization. The specific objectives of this study were: (a) to evaluate polymorphism of QTL linked SSR markers to assess the genetic diversity of the selected maize varieties and (b) to compare the diversity of the markers in 9 landraces with the diversity of 5 commercial hybrids and 6 hybrids from the IITA.

MATERIALS AND METHODS

Plant materials: A collection of twenty maize accessions was used for this study. A total of 5 commercial varieties/hybrids and 9 landraces adapted to the south-western part of Nigeria were collected from the local farmers from four States: Oyo, Ogun, Osun and Lagos and 6 newly developed hybrids, adapted to the south-western part of Nigeria were collected from IITA (Table1).

DNA extraction: Ten plants of each genotype were planted and at two weeks, the young leaf tissue of 8 randomly selected plants of each genotype were bulked into a single Eppendorf tube and approximately 1 g of leaf tissue from each bulk was used to isolate genomic DNA using a modified method of Dellaporta *et al.* (1993). The quality and quantity of the extracted DNA for each sample were determined using the Nano drop spectrophotometer.

SSR markers: Genetic diversity among the maize varieties was assessed using a total of 20 QTL linked SSR markers chosen from the previous linkage mapping studies of maize. These markers were selected based on tight linkage to the QTLs for the morpho-physiological traits (Table 2). The primer sequence was obtained from maize genome database (www.maizegdb.org) and synthesized (Inqaba Biotec, South Africa).

PCR Amplification of QTL linked SSRs: Each primer pair was optimized to know the accurate annealing temperature (55 to 65°C) for specific and good amplifications. PCR amplifications were performed in 25µL reaction volumes consisting of 13.85 µL sterile dd (H_20), 2.5µL 10X PCR buffer, 1.0 µL $MgCl_2$, 2.5 µL 100 ng template DNA, 0.10 1unit Taq DNA polymerase, 1.0 µL of forward primer (5pMol), 1.0 µL of reverse primer (5pMol), and 1.0 µL DMSO. Reactions were performed in an ABI 1000 thermocyler with the thermal cycling program consisted of initial denaturation at 94 °C for 2 min, 9 cycles at 94 °C for 15 s, 65 °C for 20 s, and 72 °C for 30 s, followed by 35 cycles at 94 °C for 15 s, 55°C for 20 s, 72 °C for 30 s, and a final extension at 72 °C for 7min. Samples were held at 10 °C until their removal from the thermocycler. Electrophoresis was carried out on 2% (wt/vol) SFR agarose gels. Scoring analyses were performed relative to a 50 base pairs DNA ladder from Thermo Scientific.

Data Analysis

Genetic diversity assessment

For each polymorphic SSR locus, number of allele per locus, Polymorphic Information Content (PIC), as well as total number of alleles were calculated using the formula of Smith *et al.* (1997). Variation in the number of heterozygosity within lines in the light of genetic backgrounds was examined. Genetic distances (GD) were estimated using the modified Rogers' method (Warburton *et al.,* 2008) and the genetic relationship among the genotypes was assessed using unweighted pair group method of arithmetic average (UPGMA) clustering algorithm and Principal Component Analysis (PCA) in the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) package version 2.2 (Rohlf, 1997).

RESULTS

Genotyping of maize varieties

17 out of the 20 SSR QTL linked markers produced clear and polymorphic PCR product (Figure 1) while the remaining ones were monomorphic SSRs (15 %). The 17 markers generated a total of 57 alleles among the genotypes (Table 1). Out of the 57 SSR alleles (average of 3.35 alleles per locus), 2 SSR markers produced the highest number of allele (5), while others ranged between 2-4 alleles. The polymorphic information content (PIC) of the observed alleles showed values from 0.16 to 0.72 with a mean of 0.46. Some of the SSR markers (47 %) had PIC higher than 0.5. Variation in the number of heterozygosity within lines in the light of genetic backgrounds is shown in figure 1. Observed heterozygosity within the maize landraces and recently developed IITA hybrids is higher even compared than the observed heterozygosity within the commercial maize lines.

Clustering of SSR Data

The UPGMA dendrogram (Figure 2) revealed the level of genetic relatedness among 5 commercial varieties/hybrids, 9 landraces and 6 newly developed hybrid lines. The 20 maize genotypes were clustered into 2 main groups. Group I consisted of a total of 9, with 3 and 1 landraces from Oyo and Lagos States, respectively; 2 and 1 commercial hybrids from Ogun and Lagos States, respectively and 2 IITA hybrids. Group II included 5 landraces of which 2 were from Oyo State, 2 from Ogun State, and 1 from Osun State. The remaining members of the group were 2 commercial hybrids from Ogun State and 4 IITA hybrids. The two groups were comparatively diverse since each consisted landrace, commercial hybrid and newly developed IITA hybrid.

The two IITA hybrids were identified to be similar and one landrace and one commercial hybrid collected from Lagos had very close relationship in Group I.

The generated Roger's genetic distance matrix was used to construct principal component analysis PCA) to decipher the genetic relationship among the genotypes as shown in figure 3. The PCA showed clear separation among the genotypes. The first two principal axis accounted for 12.87 % and 15.52 % variation, respectively. The PCA supports the results obtained from UPGMA cluster analysis. Genetic relationship among landraces tended to associate with commercial hybrids.

Table 1: Details of maize varieties used in this study

S/N	Maize varieties	States (town)/Institution	Kernel colour	Genetic background
1	MZY01	Oyo (Ogbomoso)	Yellow	Landrace
2	MZY02	Oyo (Ogbomoso)	White	Landrace
3	MZY03	Oyo (Lanlate)	White	Landrace
4	MZY04	Oyo (Lanlate)	White	Landrace
5	MZY05	Oyo (Iseyin)	Yellow	Landrace
6	MZS06	Osun (Ife)	Yellow	Landrace
7	MZG07	Ogun (Owode)	White	Commercial hybrid
8	MZG08	Ogun (Owode)	Yellow	Commercial hybrid
9	MZG09	Ogun (Owode)	Yellow	Landrace
10	MZG10	Ogun (Ifo)	White	Landrace
11	MZG11	Ogun (Abeokuta)	Yellow	Commercial hybrid (Oba super 6)
12	MZG12	Ogun (Abeokuta)	Yellow	Commercial hybrid (Oba super 2)
13	MZL13	Lagos (Badagry)	Yellow	Commercial hybrid (Oba super 1h)
14	MZL14	Lagos (Lagos)	White	Landrace
15	MZH15	IITA, Nigeria	White	(4)EXL05 X ADL36
16	MZH16	IITA, Nigeria	White	(1)ADL47X ADL41
17	MZH17	IITA, Nigeria	White	(3)EXL16 X EXL02
18	MZH18	IITA, Nigeria	White	(2)EXL15 X ADL35
19	MZH19	IITA, Nigeria	White	(2)EXL16 X ADL07
20	MZH20	IITA, Nigeria	White	(2)ADL32 X EXL06

Table 2: Characteristics of polymorphic SSR markers linked to QTL used in the study

SSR markers	Bin no	No of alleles	PIC	Traits reported to QTLs	Previous studies
bmc1208	5.04	3	0.54	DTA	(Szalma *et al.,* 2007)
umc1557	5.03	3	0.48	DTA and DTS	(Szalma *et al.,* 2007)
dupssr12	1.08	3	0.44	DTA and DTS	(Szalma *et al.,* 2007)
bmc1712	10.03	3	0.33	DTS, DTA and ASI	(Szalma *et al.,* 2007)
umc1221	5.04	2	0.36	DTA, DTS and ASI	(Szalma *et al.,* 2007)
bnlg197	3.06	4	0.6	DTA, DTS and ASI	(Szalma *et al.,* 2007)
umc1822	5.05	4	0.55	DTS, DTA and ASI	(Szalma *et al.,* 2007)
bnlg1520	2.09	3	0.33	DTS, DTA and ASI	(Szalma *et al.,* 2007)
umc2151	1.00	3	0.41	Stay green	(Wang *et al.*, 2012)
bmc 1429	1.00	5	0.72	Stay green	(Wang *et al.*, 2012)
bnlg 1755	4.00	3	0.53	Stay green	(Wang *et al.*, 2012)
bnlg 1337	4.00	3	0.52	Stay green	(Wang *et al.*, 2012)
umc 2281	4.00	4	0.56	Stay green	(Wang *et al.*, 2012)
bmc1375	9.07	5	0.56	Leaf number with Low nitrogen regime	(Zheng and Liu, 2013)
bmc1792	7.02	3	0.46	Leaf number and low nitrogen regime	(Zheng and Liu, 2013)
umc1295	7.04	2	0.16	Leaf number and high nitrogen regime	(Zheng and Liu, 2013)
umc 1415	8.03	4	0.37	Ear length	Veldboom and Lee, 1996
Mean		**3.35**	**0.46**		

PIC-polymorphic information content
DTA-days to anthesis
DTS-days to silking
ASI- anthesis-silking interval

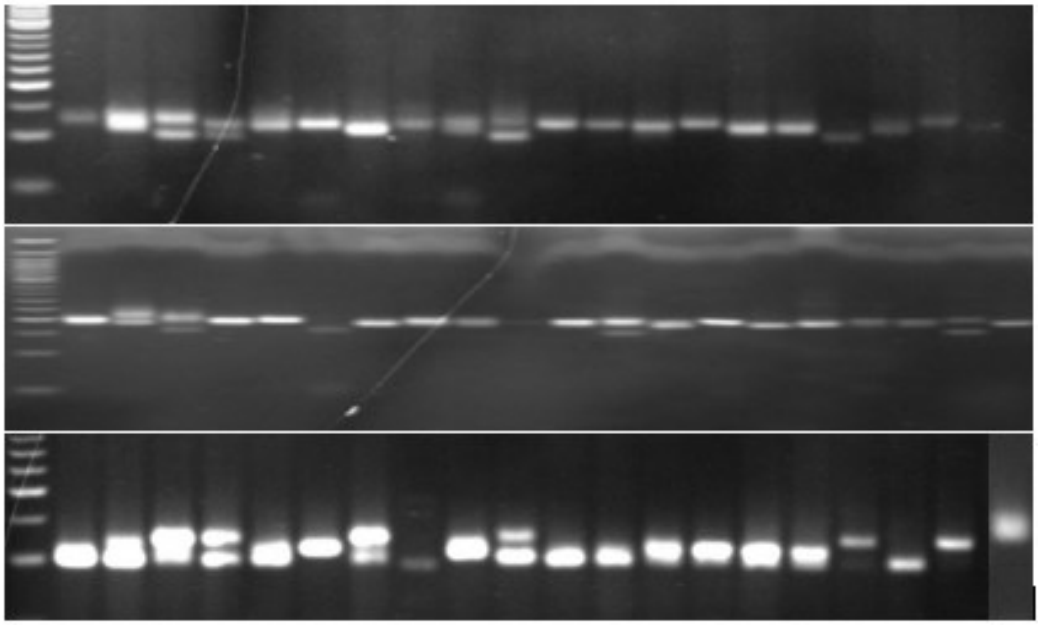


Figure 1: Gel photograph showing amplification of 3 QTL linked SSR markers (a. bmc1375 b. umc2881 and c. bmc1208) in 20 maize genotypes

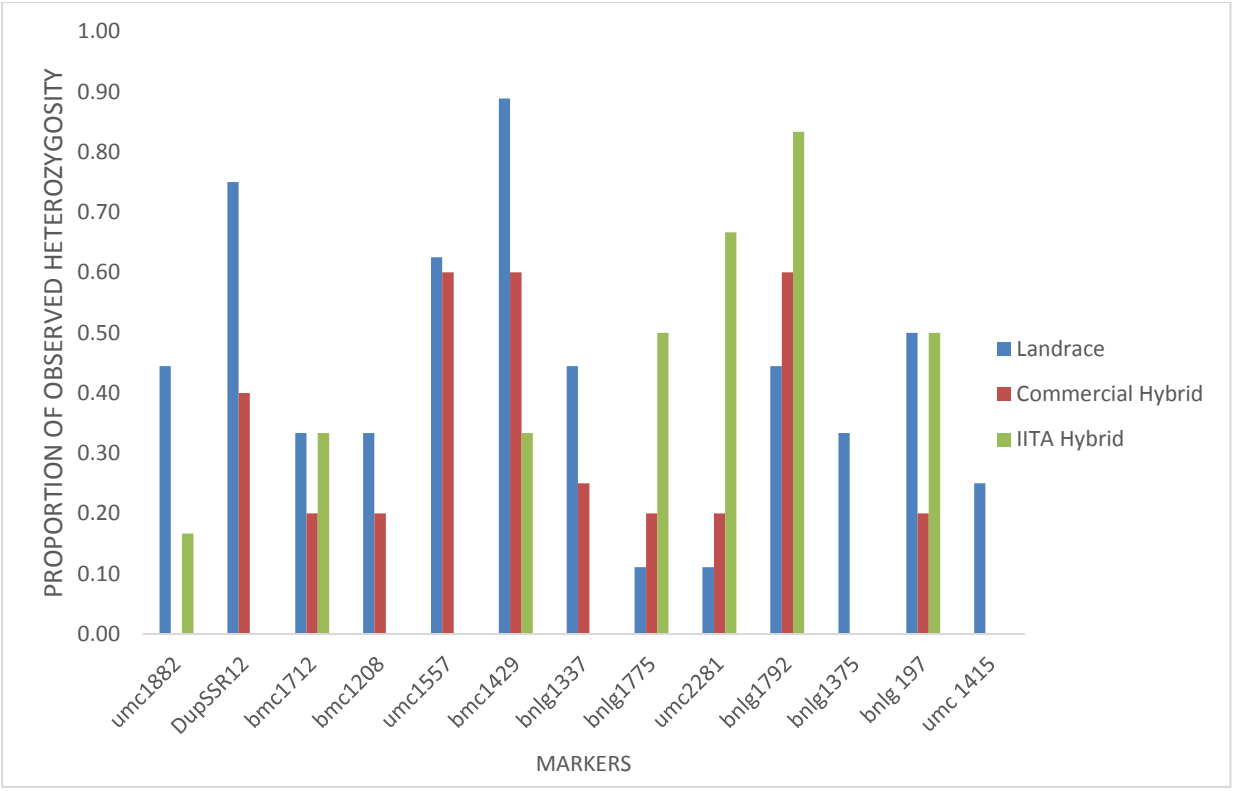


Figure 2: The proportion of observed heterozygosity of QTL linked SSR markers among landrace, commercial hybrids and IITA Hybrids

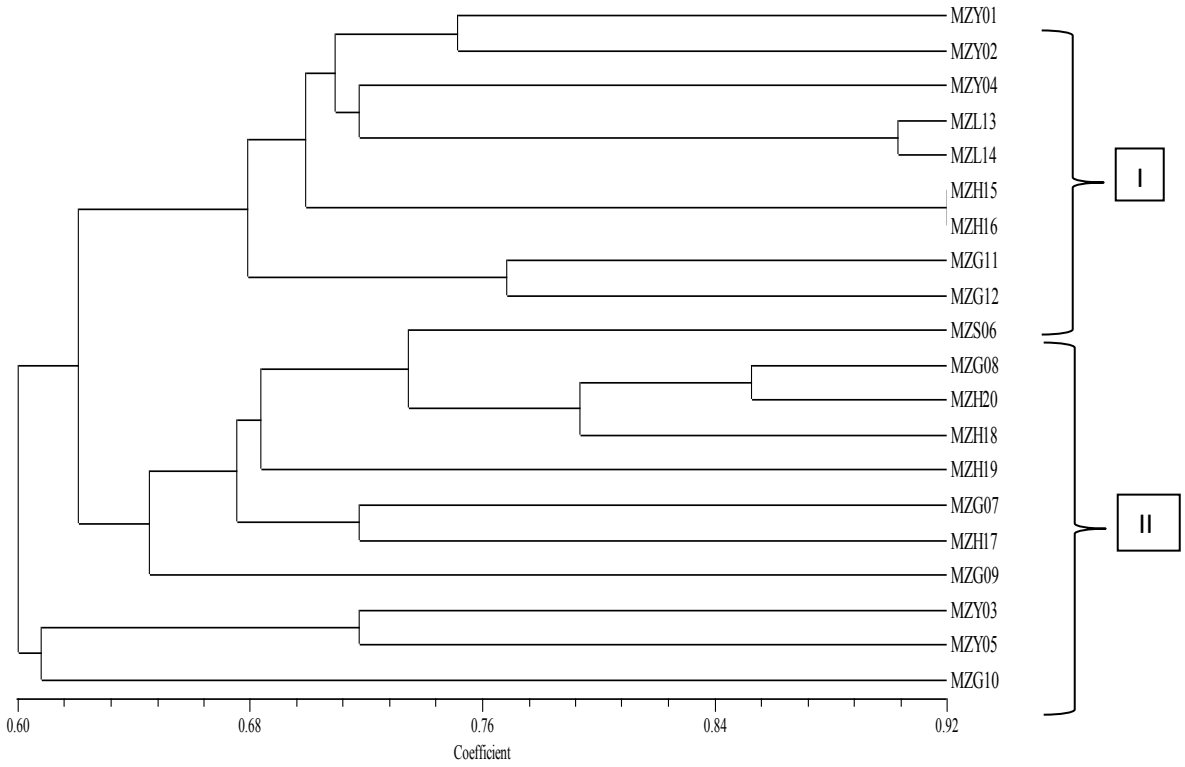


Figure 3: Dendogram of 20 maize genotypes constructed from UPGMA Cluster analysis based on QTL linked SSR markers

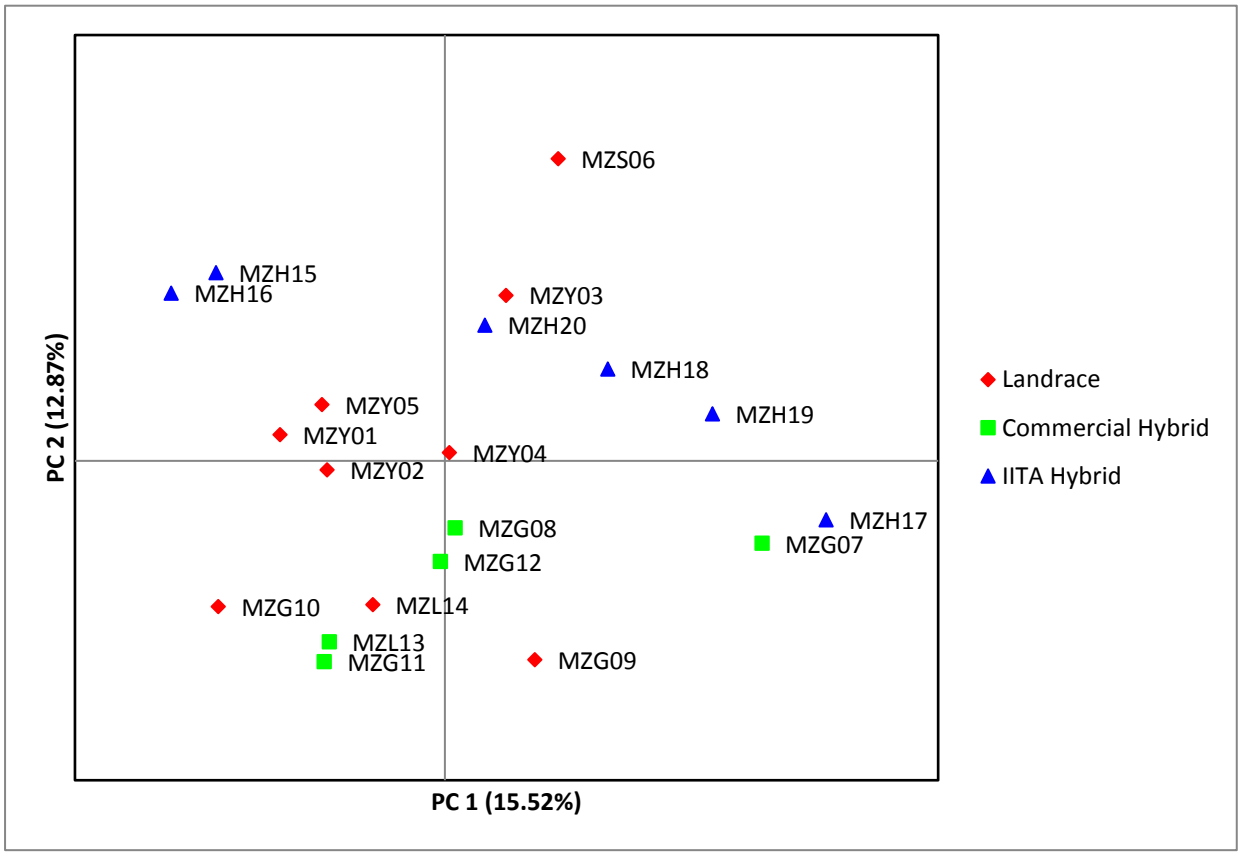


Figure 4: Principal Coordinate Analysis of 20 maize genotypes based on QTL linked SSR markers

DISCUSSION

The study of genetic diversity in specific regions of maize genome using molecular characterisation has importance for the improvement of the crop. This can facilitate the application of marker assisted selection (MAS) in maize breeding programs (Smith *et al.,* 1997; Prasanna *et al.,* 2010). QTL linked SSR markers have been used to evaluate diversity in few plant species (Ganie *et al.,* 2016). Also, quite number of genetic diversity and relationship analyses of various maize germplasm have been reported using genomic SSR analysis (Adeyemo *et al.,* 2011; Adeyemo and Omidiji, 2014). The present study represents the first report of genetic relationship in maize landraces and hybrids using QTL linked SSR loci for molecular characterization. Across the maize genotypes used in this study, we obtained a mean allele number 3.35 and a range of 2 to 5 alleles. This indicates moderate level of polymorphism of the markers among the genotypes. When compared to previous study having either genotypes, these values were close to mean number per locus generated by each marker varied from 2 to 4 with an average of 3.13 alleles per locus detected in the 13 rice varieties using the eight SSR markers tightly linked to the QTLs for aroma and cooked kernel elongation (Kioko *et al.,* 2015). However, other studies revealed lower average alleles per loci in maize inbred lines (Barbosa *et al.,* 2003; Garcia *et al.,* 2004) and in maize landraces (Molin *et al.,* 2013) using the genomic SSR markers. This implies that the genetic diversity in the set of lines studied is moderately large, and likely has been due to the landrace germplasm. SSR loci have good potential to discriminate between closely related maize genotypes and are polymorphic due to the multi-allelic variations (Veldboom and Lee, 1996; Inghelandt *et al.,* 2010; Sharma *et al.,* 2010; Yang *et al.,* 2011).

The PIC value of each locus usually demonstrates an estimate of the discriminatory power of the locus, considering the number of alleles and its relative frequencies (Smith *et al.,* 2000). In this study, the mean value of PIC of 0.46 also revealed considerable genetic variability among the 20 maize genotypes which is lower to that of 0.51 using 30 SSR markers for genotyping 79 elite maize inbred lines (Nyaligwa, 2015). The low level of PIC observed in the present study could be as a result of types of the SSR markers used and the genetic differences among the maize hybrids and landraces. In addition, some eight primer pairs (47 %) had PIC higher than 0.5, indicating their usefulness in the detection of polymorphism among the lines and also for marker-assisted selection program. Moreover, the four out of the five SSR markers linked to stay green trait QTLs (Wang *et al.,* 2012) analysed in this study showed moderate level polymorphisms among the genotypes, implying high genetic variation in the trait.

Heterozygosity provides an understanding of the information about the SSR loci and their potential to detect differences between lines based on their genetic diversity. In this study, seven out of thirteen markers that showed high allelic variants were found higher in landraces than in the hybrids. This result suggests a wide gene pool of heterozygous of the landraces which can be useful source of genes for novel alleles (McCouch *et al.,* 1997; Hossain *et al.,* 2012). The cluster analysis revealed two main groups within the maize genotypes, which was in close agreement with the results of PCA. There was no clear structure of grouping of the maize lines along genetic backgrounds (landrace, commercial and hybrids). The mixed or dispersed and close genetic relationship of the genotypes used in this study suggests that the continuous natural or human selection process of maize landraces has not brought any massive divergence from the hybrids. This may account for the large usage of hybrids by local farmers nowadays. The moderate genetic diversity further suggested that the lines used in this study may have originated from the same adapted environment.

In this study too, genotypes with lowest genetic similarity were identified MZL13 (commercial hybrid, yellow, Lagos) and MZL14 (landrace, white, Lagos). This shows similarity of commercial hybrid with landraces and the landraces may have been source population, from which inbred parents of the hybrids were extracted. The similarity between MZH15 and MZH16 at the loci assessed indicated that they had a common parent or ancestry. However, this is not unlikely due to the limited SSR markers used and narrow difference between the lines under study (Bhawna *et al.,* 2015).

CONCLUSION

The present study has demonstrated moderate amount of genetic variability among landraces, commercial hybrids and recently developed IITA hybrid lines based on a set of QTL linked SSR loci. This result indicated the intermixed grouping of the lines used. The information will be important for marker applications in maize breeding for traits such as drought and nitrogen tolerance. In addition, further analysis of molecular diversity within and among the landrace accessions adapted to the south-western Nigeria is recommended to facilitate proper classification and utilization.

REFERENCES

Adeyemo, O and Omidiji, O. (2014). SSR-based and carotenoid diversity assessment of tropical yellow endosperm maize inbred lines. *Plant Gen. Res.* **12(1)**: 67-73.

Adeyemo, O., Menkir A., Melaku, G. and Omidiji, O. (2011). Genetic diversity assessment and relationship among tropical yellow endosperm maize inbred lines using SSR markers. *Maydica* **56**: 1703-1709.

Bernardo, R. (2002). Breeding for quantitative traits in plants. Stemma Press, Woodbury, 400p.

Barbosa, A.M.M., Geraldi, I.O., Benchimol, L.L., Garcia, A.A.F, Souza Jr. C. L. and Souza, A. P. (2003). Relationship of intra- and interpopulation tropical maize single cross hybrid performance and genetic distances computed from AFLP and SSR markers. *Euphytica.* **130**: 87-99.

Bhawna, A.M.Z., Arya, L., Ram, C., Sureja, A.K., Verma, M. (2015). Development of novel gene-based microsatellite markers for robust genotyping purposes in *Lagenaria siceraria*. *Sci Hortic.* **191**:15-24.

Dellaporta, S. L., Wood, J. and Hicks, J. B. (1983). "A plant DNA mini-preparation: version II. *Plant Mol. Biol. Rep.* **1(4)**: 19-21.

Flint-Garcia, S.A., Thuillet, A.C., Yu, J. Pressoir, G., Romero, S. M., Mitchell, S. E., Doebley, J., Kresovich, S., Goodman, M.M. and Buckler, E. S. (2005). Maize association population: a high-resolution platform for quantitative trait locus dissection. *Plant J.* **44**:1054-106.

Ganie, S. A., Borgohain, M. J., Kritika, K., Talukdar, A., Pani, D.R., and Mondal,T.K. (2016). Assessment of genetic diversity of Saltol QTL among the rice (Oryza sativa L.) genotypes. *Physiol. Mol. Biol. Plants*. **22(1)**:107-114.

Garcia, A.A.F., Benchimol, L.L., Barbosa, A.M.M., Geraldi, I.O., Souza Jr. C. L and de Souza, A. P. (2004). Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines. *Genet. Mol. Biol*. **27**: 579-588.

Hamblin, M.T., Warburton, M.L. and Buckler, E.S. (2007). Empirical comparison of simple sequence repeats and single nucleotide polymorphisms in assessment of maize diversity and relatedness. *PLos ONE.* **2(12):** e1367.doi: 10.1371/journal.pone.0001367.

Hossain, M.M., Islam, M.M., Hosai, H., Ali, M.S., Teixeira da Silva, J.A., Komamine, A., and Prodhan, S.H. (2012). Genetic diversity analysis of aromatic landraces of rice (Oryza sativa L.) by microsatellite markers. *Genes Genom Genomic*. **6**:42-47.

Inghelandt, D.V., Melchinger, A.E., Lebreton, C and Stich, B. (2010). Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. *Theor. Appl. Genet.* **120(7)**: 1289-1299.

Kioko, Wambua., F., Musyoki, M. A., Piero, N. M., Muriira, K. G., Wavinya, N. D., Rose, L., Felix, M. and Ngithi, N. L. (2015). Genetic Diversity Studies on Selected Rice (Oryza sativa L) Populations Based on Aroma and Cooked Kernel Elongation. . J. *Phylogen. Evolution.* Biol. **3**: 158. doi:10.4172/2329-9002.1000158.

Li, X.H., Liu, X.D., Li, M.S. and Zhang, S.H. (2003). Identification of quantitative trait loci for anthesis-silking interval and yield components under drought stress in maize. *Acta Botanica Sinica,* **45**: 852-857.

Li, Y.C., Korol, A.B., Fahima, T., Beiles, A., Nevo, E. (2002). Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. *Mol Ecol.* **11 (12)**: 2453-2465.

Liu, K., Goodman, M.M., Muse, S., Smith, J.S., Buckler, E., Doebley, J. (2003) Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. *Genetics.* **165**:2117–2128.

Menkir, A., Melake-Berhan, A., The, C., Ingelbrecht I and Adepoju, A. (2004). Grouping of tropical mid-altitude maize inbred lines on the basis of yield data and molecular markers. *Theor. Appl. Genet.* **108**: 1582-1590.

McCouch, S.R., Chen, X. and Panaud O. (1997). Microsatellite mapping and applications of SSLP's in rice genetics and breeding. *Plant Mol Biol.* **35**:89–99.

Molin, D., Coelho, C.J. Máximo, D.S., Ferreira, F.S., Gardingo, J.R. and Matiello, R.R. (2013). Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. *Genet. Mol. Res.* **12 (1)**: 99-114.

Nyaligwa, L., Shimelis, H., Beyene, A. and Habteab, G. (2015). Genetic diversity analysis of elite maize inbred lines of diverse sources using SSR markers. *Maydica.* **60**:1-9.

Prasanna, B. M., Pixley, K., Warburton, M.L. and Xie, C-X (2010). Molecular marker-assisted breeding options for maize improvement in Asia. *Mol. Breeding.* 26:339–356.

Ranum, P., Peña-Rosas, J. P. and Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Ann. N.Y. Acad. Sci*. **1312**: 105–112. doi:10.1111/nyas.12396.

Reif, J.C., Hamrit, S. and Heckenberger, M., (2005). Trends in genetic diversity among European maize cultivars and their parental components during the past 50 years. *Theor. Appl. Genet.* **111**: 838–845.

Ribaut J.M., Fracheboud Y., Monneveux P., Banziger M., Vargas M. and Jiang C. (2007). Quantitative trait loci for yield and correlated traits under high and low soil nitrogen conditions in tropical maize. *Mol. Breed.* **20**: 15-29.

Rohlf, F.J. (1997). NTSYS-pc 2.1. Numerical Taxonomy and Multivariate Analysis System. Setauket, NY: Exeter Software

Sharma, L., Prasanna, B. M and Ramesh. B. M. (2010). Analysis of phenotypic and microsatellite- based diversity of maize landraces in India, especially from the North East Himalayan region. *Genetica.* doi:10.1007/s10709-010-9436-1.

Smith J.S.C., Chin E.C.L., Shu H., Smith O.S., Wall S.J., Senior M.L., Mitchell, S.E., Kresovich, S. and Ziegle, J. (1997). Evaluation of the utility of SSR loci as molecular markers in maize (Zea mays L.): comparisons with data from RFLPs and pedigree. *Theor Appl Genet.* **95**: 163-173.

Smith, J.S.C., Ertl, D.S. and Orman, B.A. (1995). Identification of maize varieties. In: Wrigley CW (ed) Identification of food grain varieties. *Am. Assoc. Cereal Chemists.* St. Paul, pp 253-264.

Szalma S.J., Hostert B.M., LeDeaux J.R., Stuber C.W and Holland J.B. (2007). QTL mapping with near-isogenic lines in maize. *Theor. Appl. Genet.* **114**:1211-1228.

Veldboom, L.R. and Lee, M. (1996). Genetic mapping of Quantitative Trait Loci in maize in stress and non-stress environments: I Grain yield and yield components. *Crop Sci.* **36**:1310–1319.

Veldboom, L.R., Lee, M., Woodman, W.L. (1994). Molecular marker facilitated studies in an elite maize population: I. Linkage analysis and determination of QTL for morphological traits. *Theor. Appl. Genet*. **88**:7-16.

Vignal, A., Milan, D., SanCristobal M and Eggen A (2002) A review on SNP and other types of molecular markers and their use in animal genetics. *Genet. Sel. Evol*. **34**:275-305.

Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S, Smith JS, Jaqueth J, Smith OS, Doebley J. (2005). An analysis of genetic diversity across the maize genome using microsatellites. *Genetics.* 2005:**169(3)**:1617-1630.

Wang, A., Li, Y, Zhang C. (2012). QTL mapping for stay-green in maize (*Zea mays*). *Can. J. Plant Sci.* **92**:249-256.

Warburton, M.L., Reif, J.C., Frisch, M., Bohn, M., Bedoya, C., Xia, X.C., Crossa, J., Franco, J., Hoisington, K. D., Pixley, S., Taba. and Melchinger A. E. (2008). Genetic diversity in CIMMYT nontemperate maize germplasm: Landraces, open pollinated varieties, and inbred lines. *Crop Sci.* **48(2)**: 617–624.

Westengen, O. T., Berg, P. R., Kent, M. P., and Brysting, A. K. (2012). Spatial structure and climatic adaptation in African maize revealed by surveying SNP diversity in relation to global breeding and landrace panels. PLoS ONE 7:e47832. doi: 10.1371/journal.pone.0047832.

Wright, S. (1978). Evolution and genetics of populations, vol IV. Chicago: The University of Chicago Press. p. 91.

Wu, J.W., Liu, C., Shi, Y.S., Song, Y.C., Chi, S.M., Ma, S.Y., Wang, T.Y. and Li, Y. (2008). QTL analysis of flowering related traits in maize under different water regimes. *J. Maize Sci.* **16(5)**: 61-65.

Yang, X., Xu, Y., Shah, T., Li, H., Han, Z., Li, J and Yan, J. (2011). Comparison of SSRs and SNPs in assessment of genetic relatedness in maize. *Genetica.* DOI 10.1007/s10709-011-9606-9.

Yong-Bi Fu. (2015). Understanding crop genetic diversity under modern plant breeding. *Theor. Appl. Genet.* **128(11)**: 2131-2142.

Zhang, J.M., Liu, C., Shi, Y.S., Song, Y.C., Bai, B.Z., Li Y. and Wang, T.Y. (2004). QTL analysis of parameters related to flowering in maize under drought stress and normal irrigation condition. *J. Plant Genet. Resour.* **5**:161-165.

Zheng, Z. P and Liu, X. H. (2013). Genetic analysis of agronomic traits associated with plant architecture by QTL mapping in maize. *Genet. Mol. Res.* **12(2)**: 1243-1253.

GENE COMPLEMENTATION VIA HETEROKARYON FORMATION IN *Aspergillus flavus*

Lugard Eboigbe
Plant Biology and Biotechnology Department, Faculty of Life Sciences,
University of Benin, PMB 1154, Benin City Edo State
Email: lugard.eboigbe@uniben.edu, lugar2004@yahoo.com

Abstract: Formation of heterokaryon in Ascomycetes fungus *Aspergillus flavus* is a very important step in the exchange of genetic information through parasexual cycle. In this research, heterokaryon formation has been used to showcase gene complementation in which detail genetic analysis is possible as in other sexual organism. Here, two color mutants (nit3 and nit4) were selected from five auxotrophic mutants as genetic markers which were derived as chlorate resistant sectors that were not able to utilize nitrate as sole nutrients. Attempting to fuse these mutants reveal an intriguing result; that the recombination of the mutants can show complementation and at the same time, in some cases, could also show no complementation. Analysis of the results indicated that one may be dealing with coupling-repulsion (cis-trans) positional effect. In which case, complementation of gene only occurred in the cis configuration thus suggesting that we are dealing with a locus with two alleles (functional alleles). Attempt also made to classified the mutants into vegetative compatibility groups revealed two compatible groups which left us still with the question of how many loci are involved in heterokaryosis of*A. flavus*

Keywords: Heterokaryon, Complementation, Auxotrophic Mutant, Genetic Marker

INTRODUCTION

Heterokaryon the first necessary step in the parasexual cycle is known to be a valuable tool in genetic analysis of certain imperfect fungi. Formation of heterokaryon is a means of genetic recombination in Ascomycetes fungus, *Aspergillus flavus.* Heterokaryosis is cells fusion that leads to cytoplasmic mixing and vegetative heterokaryon (i.e cells containing different nuclear types (Papa, 1973). Though, mating types under laboratory condition has been reported in *A. flavus* (Horn and Moore, 2009; Grubisha and Cotty, 2015), heterokaryon remains the means by which more than one nuclear can exist in a cell. According to Saupe, (2000) the viability of heterokaryons is genetically controlled by specific loci termed *het* loci (for heterokaryon incompatibility loci).

Heterokayon incompatibility leads to self-destruction mechanism commonly known as programme cell death (PCD), which is very common in *A. flavus* and other Ascomycetes fungi (Robson, 2006; Sharon *et al*. 2009). However, the formation of stable heterokaryon has valuable applications in pharmaceutical, agriculture and environmental remediation (Bushley and Strom 2016). This was the information that stimulated interest in this area of research. Different heterokaryon incompatibility loci have been identified in many Ascomycetes fungi including *A. niger, A. nidulans, A. flavus* etc. For *A niger* and *A. nidulans* the number of het loci has been identified (Dales, Moorhouse and Croft, 1993; Diepeningen *et al*. 2008). However, for *A. flavus* there is no clear statement on the number of het loci. Unfortunately, researchers are also silent about the genes responsible for stable heterokaryon. Therefore, the major focus in this work was to obtain a stable heterokaryon and to understand the genes involved. Thereafter, study the recombination and attempt to identify the genes responsible for stable heterokaryon formation. Though heterokaryon is rare in nature, it could be forced to occur in the laboratory (Smith, Gibbs and Milgroom 2006; Horn *et al.* 2016). However, the limitation is finding a compatible partner as a result of widespread incompatible haploid and the rigor of identifying diploids.

Viable heterokaryons have been produced using incompatible auxotrophic strains (Moorhouse, 1977) and techniques of protoplast fusion (Dale and Croft, 1977; Dale, 1978). If heterokaryon is stable, it means there are no differences in the alleles of the loci involved. On the other hand, if the resultant heterokaryon leads to degradation of mycelia strand, it invariably shows that there are differences in the alleles of the loci involved in the formation. This implies that,

two nuclei being able to fuse together is mediated by level of homogeneity of the het loci. This process may involve gene complementation as a result of allelic/non-allelic interactions. Strom and Bushley (2016) in their review stated that complementation tests are often used to visualize heterokaryon formation between two fungal isolates expressing different genetic or auxotrophic markers. In this work, we have used this concept to evaluate heterokaryon formation in *A. flavus*. Here we report on genetic complementation through heterokaryon formation in *A. flavus*. In this, we hope to join other researchers in search of how many het loci exist in *A. flavus.*

MATERIALS AND METHODS

Strains and growth conditions

Aspergillus flavus strains used in this work was isolated in University of Benin, Benin City, Edo State, Nigeria. Potato-dextrose agar (PDA Difco, Detroit, Michigan) was routinely used as a complex medium. The growth of cultures were maintained at ~25^{oC}. The minimal medium (MM) was prepared following the procedure according to Correll *et al*., (1987).

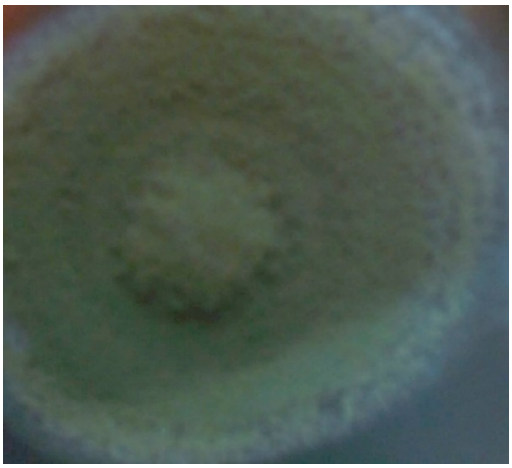


Plate 1: Pure culture of *Apergillus flavus.* This was isolated in the wild. Identification according to Carlson, 2012

Isolation of *nit* mutants

Chlorate resistant sectors were generated on PDA containing 10gl^{-1} potassium chlorate ($KClO_3$) as PDC and incubated at 25^{0}C for 14 to 21 days. The Chlorate resistance sectors thus formed were classified as nit mutants using Nitrate ($NaNO_3$), Nitrite ($NaNO_2$) and Ammonium tartrate ($(NH_4)2C_4H_4O_6$) (Eboigbe and Oijagbe 2017)

Nit mutants were isolated using the method of Bayman and Cotty (1991).The chlorate served as a toxic analogue of nitrite (Correll *et al.,* 1987). Sectors with sparse mycelium unable to utilize nitrate were considered as *nit* mutants and isolates that reverted to a dense aerial mycelium on MM were considered as chlorate-resistant. Nitrate-utilizing mutants were discarded. The *nit* mutants were grown on three phenotyping media (Correll *et al.,* 1987) which are sodium nitrate ($NaNO_3$) - 0.84gl^{-1}, sodium nitrite ($NaNO_2$) - 0.69gl^{-1},ammonium tartrate [$(NH_4)2C_4H_4O_6$] – 1.0gl^{-1}.

Plate 2: Show Chlorates resistant mutants that were nitrate-non-utilizing mutants. The growth of the mutants were restricted and sectorial (Eboigbe and Oijagbe, 2017).

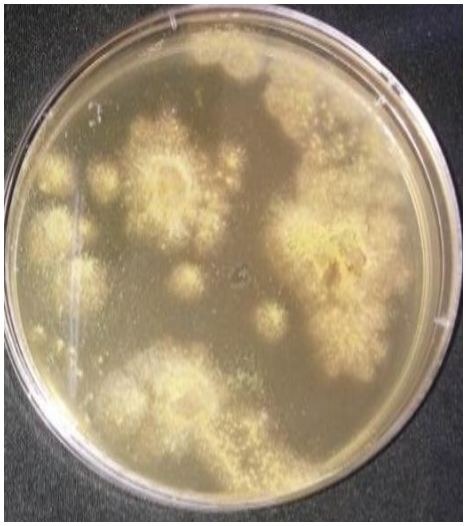


Table 1: The five auxotrophic mutants from *A. flavus* strain (Eboigbe and Oijagbe, 2017)

Medium	Class	Mutants
$NaNO_3$	1	*nit*1
$NaNO_2$	1	*nit*2
$NaNO_3$	2	*nit*3
$NaNO_2$	2	*nit*4
[$(NH_4)2C_4H_4O_6$]	3	*nit*5

Mutants Haploids Strains Constructed for Heterokaryons: From the auxotrophic mutants constructed (table1), *Nit*3 with white color (wh) and *Nit*4 with grey color (gy) were selected. Their distinct phenotypic status was confirmed after several generations of sub culturing.

Compatibility/Complementation test: *Nit* mutants recovered from fungal isolates were paired on minimal media (MM) containing $NaNO_3$, $NaNO_2$ and $[(NH_4)2C_4H_4\ O_6$ as a sole nitrogen sources. MM without the respective nitrogen sources were used as control. Each isolate was paired with two other isolates in different order of combinations (*nit*1× *nit*2 ×*nit*3, *nit*4×*nit*5×*nit*3 etc.) to verify the heterokaryon self- incompatibility isolate. Test mutants were placed approximately 1 cm apart. Mutant cultures were incubated in a dark condition at 25 ^{0}Ċ for 7-14 days. The pairs were also used to assign them into vegetative compactibility groups (VCGs). The isolates in the same VCG showed a dense mycelia growth at the line where colonies merged that is known for heterokaryon wild-type growth, often accompanied by microsclerotium formation. Isolates which complemented each other were assigned to the same VCG. All positive reactions were repeated for verification.

Standard method for the production of balanced heterokaryons: Heterokaryons are not stable in filamentous fungi such as *Aspergillus nidulans* unless they are maintained by selection pressure. To produce them, isolates bearing complementary auxotrophic mutations were used. Conidial colour mutants were used to visualize parental contributions to the heterokaryons. Spore suspensions for each of the heterokaryons or VCGs were grown in a liquid broth and kept in a shaker to allow for growth of the conidia. Turbid suspensions of conidia were obtained from heterokaryons growing on the liquid broth. Complete medium were seeded with 0.1ml of the original suspensions (Stromnaes and Garber, 1962).

RESULTS AND DISCUSSION

A flavus strain used in this work was isolated in pure culture with green coloration (Plate1) which was identified following the procedure according to Carlson, (2012). From this strain, five auxotrophic mutants were constructed (table1). The mutants were the chlorate resistant sectors which were unable to utilize nitrate as a sole source of Nitrogen, therefore the colonies were thin and transparent with no aerial mycelium on MM and were designated as *nit* mutants. *Nit* mutants emerged from restricted growth on chlorate media after 7–14 days, showing the normal sectoring as seen in chlorate resistant mutants (Plate2). However, the one that had wild-type colony morphology on MM and were considered as chlorate-resistant nitrate-utilizing mutants were discarded.

A mycelial fragment of the *nit* mutants was sub-cultured on each of the three-media containing different nitrogen sources: $NaNO_3$, $NaNO_2$ and $[(NH_4)2C_4H_4\ O_6]$. Mutants were transferred to each phenotyping medium and were incubated at room temperature for 7 days before colony morphology was scored. Colony with abundant aerial mycelium (fluffy) was considered as having a "+" growth while colony with sparse, transparent mycelium was considered as having a "–" growth. The *nit* mutants (*nit*1, *nit*2, *nit*3, *nit*4 and *nit*5) were classified into three phenotypic classes (table1) according to Correll *et al*. (1987).

In order to classify the above auxotrophic mutants into compatibility groups, incompatibility assays were performed by confronting strains (Table 1) in all pair-wise combinations on PDA using the color of mutants as indicator. A total number of 30 pairs showed compatibility at various levels while 32 pairs were incompatible strains. From the 30 compatible strains two vegetative compatibility groups (VCGs) emerged nit5*nit1, nit3*nit1/nit2*nit4, nit3*nit4 (table 2). No zone line was evident in

Table2: **Show results of the compatibility test. Two vegetative compatibility group emerged**

	***nit*1**	***nit*2**	***nit*3**	***nit*4**	***nit*5**
***nit*1**	+	-	-	-	+
***nit*2**		+	-	+	+
***nit*3**			+	+	-
***nit*4**				+	+
***nit*5**					+

Key: + represents point of compatibility
- represents point of no compatibility

pairing between strains with the same VC genotype. However dark lines (barrage formation) were associated with incompatible pairings which is presumably due to cell death and lysis after fusion-usually a sign of heterokayon incompatibility. Thus, mycelia incompatibility appears correlated with heterokaryon incompatibility function in this fungus.

Among the vegetative compatibility groups, a stable heterokaryon was formed in which the two mutants (wh/wh and gr/gr) involved gave rise to progeny expressing the wild phenotypes (plate3A). Further plating of this progeny resulted in segregation in which both mutants were recovered alongside with the wild progeny. This wild progeny was always found at the point where the two mutants meet or joined on culture plate (plate3A indicated by the arrow). Since the two mutants that gave rise to the wild progeny, they were considered as haploids.This resulting wild progeny was therefore considered as a diploid progeny. The two mutants is said to have genetically complemented one another thus restoring the wild type phenotypes. To prove this fact, the green diploid progeny was repeatedly subcultured. The result of the progeny showed segregation, in which the two color mutants were recovered (plate3B). However, there were cases in which the combination of the two mutants did not result in the wild progeny though there was evidence of compatibility (plate3)

Heterokaryon formation as a means of genetic interaction is rear in the natural realm in fungi. However this phenomenon is possible if there is hyphal fusion between compatible strains (Huber, 1996). In this study, color mutants as genetic markers was use to demonstrate heterokaryon formation (plate3A) which resulted from the ability of the color mutants to complement thus given rise to the wild strain. According to Strom and Bushley (2016), this phenomenon is known as genetic complementation.

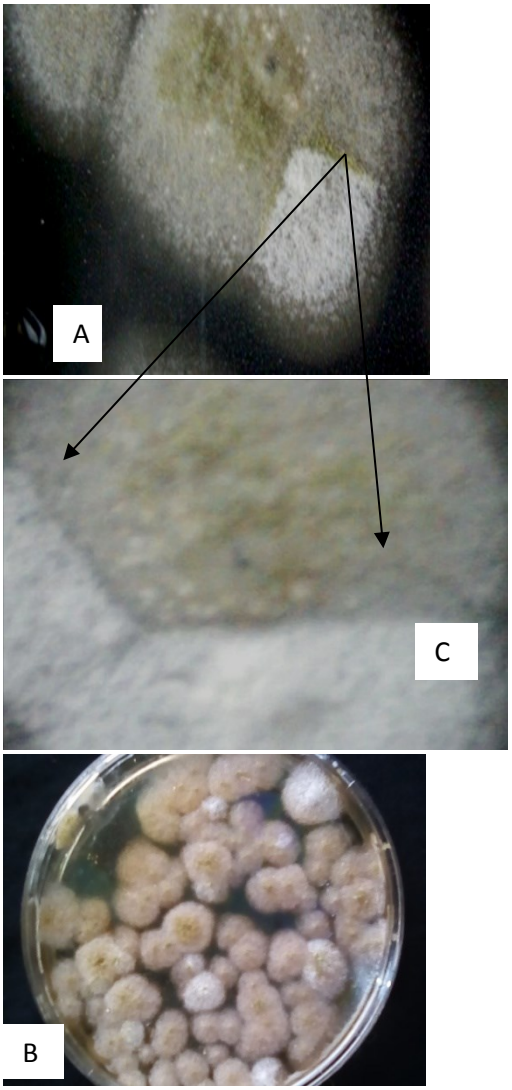


Plate 3:**(A) Heterokaryon** formation through parasexual crossing in *Aspergillus flavus*, this resulted in wild phenotype at the point in which the two mutant met as indicated by the arrow **(B)** highlighted the results of the segregation of the diploid subcultured from the point the arrow met; which was where the heterokaryon was formed . Plate B indicate the presence of nit3 white Mutant (**wh**), nit4 grey mutant (**gy**) as haploid, grey-grey mutants, white-green-grey as diploid heterozygous (indicating heterokaryon formed when the mutation is in the cis configuration), white-grey mutants (showed absence of heterokaryon formation probably due to mutation trans configuration **(C)** One of the pairing of the white and Grey mutants which showed compatibility without heterokaryon formation.

Gene complementation test has been used to define allelism. The interest in this work, was to know if the

two mutations (wh, gy) affected the same locus (allelic) or different loci (nonallelic). As result, the two mutants were repeatedly subculture in order to ensure that there were no segregation and hence were haploids nuclei prior to combination for hypha fusion of the two recessive mutants. The homozygous mutants were inoculated short distance from one another on the same plate so as to facilitate the fusion of their mycelia and thus recombination of their nuclei. Following the already well-established procedure for functional allele, we postulated that if mutations occurred in the same gene the resultant combination ought to give mutant phenotype only (fig1A) which was not the case.

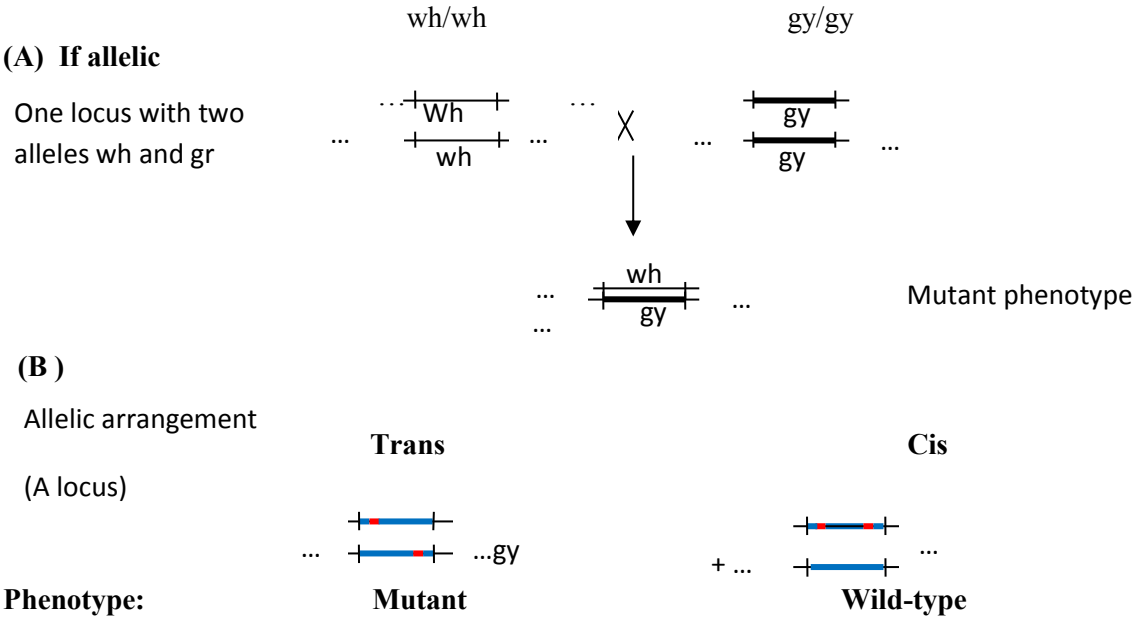


Fig 1 Figure 1: **(A)** If the mutation occurred in the same gene the combination of the two will result in mutants **(B)** if on the other hand there is coupling-repulsion (cis-trans) effect in the allelic arrangement mutants will result when the combination is the trans direction while the wild progeny will result when the combination is in the cis direction. This will happen irrespective of where the chromosome is found whether in the same or different conidia

On the other hand, if mutations occurred in different genes as in the case of nonallele, it is expected that there will be complementation in which only the phenotype of the wild (green color) will be produced as the diploid progeny. Interestingly, heterozygote diploids were obtained, that in some cases were complemented while in others they were not (plate3). However, complementation of genes is an indication of nonallelic trait. In an attempt to clearly underpin the allelic status, segregation of the heterokaryon by subculturing (selfing) repeatedly, were carried out.. As stated in the result above, both mutants were recovered including the complemented and uncomplemented diploids. The simple explanation to this occurrence might be in the cis-trans positional effect in which case, in the trans position the phenotypes remain as mutant, while in cis configuration it results in the wild (fig1B).The subject of complementation is not new, however, this is the first time a coupling-repulsion (cis-trans) model is clearly demonstrated in *A. flavus.* Since the mutation fail to complement while in the trans configuration, the alleles are termed functional alleles. Each of these color mutants was also classified as auxotrophic markers since their recombination resulted in stable heterokaryon formation.

The vegetative compatibility test used in this work were absence of barrage between strains as experienced when the same mutant is paired. The most obvious evidence for diploidy as stated in the result above, is in the tendency of the green-spored colonies to produce sectors of growth with either grey or white conidia. According to (Berg and Garber, 1962) such sectors were not in abundant, but one or two were present on most plate culture. However, from our

segregation of color marker analysis, this statement showed some reservations. It is the white colonies that gave such results which were always fewer (in most cases three) than the grey colonies. From the onset of this work, we had intended to check the number of loci involved in stable heterokaryon formation in *A. flavus*. However, It is obvious that the functional gene complementation show that we are dealing with a locus with two alleles (allelic system). The argument here, is purely on the bases that the color mutants markers exhibited complementation in cis confuguration.

In conclusion, this study reveals that gene complementation can be carried out via heterokaryon formation and that detail genetic analysis is possible as in sexual reproduction. At least, a locus with two alleles has been putatively identified from the *A. flavus* mutant strains constructed. This will be cloned and sequenced in the near future. Here we conclude that, a lot of information still remains untapped in many asexual system of which it is only through heterokaryon formation and thus parasexual cycle in combination with the right molecular approach that these can be unraveled.

REFERENCES

Bayman, P. and Cotty P.J. (1991). Vegetative compatibility and Genetic variation in the *Aspergillus flavus* population of a single field, Can. J. Bot. **69**: 1707- 1711

CarlsonN.(2012)SustainableMycology:sustainablemycology.blogspot.com/2012/12aspergillus-flavus.html

Correll *J.C.,* Klittich R. J.C., Leslie J.F.(1987) Nitrate nonutilizing mutants of Fusarium oxsporum and their use in vegetative compatibility test., Phytopathology **77**: 1640-1646

Dale R. B. G. (1978). Heterokaryon incompatibility in *Aspergillus nidulans*. Ph.D thesis, Birmingham University, UK.

Dale R.B. G. and Croft J.H. (1977) Protoplast fusion and the isolation of heterokaryons and diploids from vegetatively incompatible strains of *Aspergillus nidulans,* FEMS Microbiology Letters **1**:201-204

Dales R.B. G, Moorhouse J. and Croft J.H. (1993) Evidence for a multi-allelic heterokaryon incompatibility (het) locus detected by hybridization among three heterokaryon-compatibility (h-c) groups of *Aspergillus nidulans* Heredity **70 :** 537-543

Diepeningen A.Van, Pal K, Van der Lee J.A.T., Hoekstra F.R and Debets A.J.M.(2008) The het-c heterokaryon incompatibility gene in *Aspergillus niger*. Mycologia **113**:222-229

Eboigbe L. and Oijagbe R. E.(2017) Construction of nitrate non-utilizing mutants in *Aspergillus flavus* and its application in vegetative compatibility groups, Nigeria Journal of Life Sciences **7**: 84-93

Grubisha L.C. and Cotty P.J. (2015). Genetic Analysis of the *Aspergillus flavus* Vegetative Compatibility Group to which a Biological Control Agent that Limits Aflatoxin Contamination in U.S Crops Belongs, Applied and Environmental Microbiology **81**: 5889-5899

Horn B.W., Gell R.M., Singh R., Sorensen R.B. and Carbone I.(2016) Sexual reproduction in *Aspergillus flavus* sclerotia: Acquisition of novel alleles from soil population and uniparental mitochondrial inheritance PloS One **11**(1): e0146169

Horn B.W., Moore G.G. and Carbone I. (2009). Sexual reproduction in *Aspergillus flavus* Mycologia **101**:423-429

Huber D.H. (1996) Genetic analysis of vegetative incompatibility polymorphisms and horizontal transmission in the chestnut blight fungus *Cryphonectria parasitica*. Ph.D Dissertation, Michigan State University, East Lansing, MI

Moorhouse J. (1977) The genetical basis of heterokaryon incompatibility in *Aspergillus nidulans.* Ph.D thesis, Birmingham University, UK.

Papa, K. E. (1973) The parasexual cycle in *Aspergillus flavus* . Mycologia **65**:1201-1205

Robson D.G. (2006) Programmed cell death in the aspergilla and other filamentous fungi, Medical Mycology **44 :** S109-S114

Stromnaes O. and Garber E.D. (1962) Heterokaryosis and the parasexual cycle in *Aspergillus fumigates,* Genetics **48**: 653-660

Saupe S.J. (2000) Molecular genetics of heterokaryon incompatibility if filamentous ascomycetes. Microbiol Mol Biol Rev **64**: 489-502

Strom N. B. and Bushley K. E. (2016) Two genomes are better than one: history, genetics and biotechnological applications of fungal heterokaryons, Fungal Biol Biotechnol 3:4 DOI 10.1186/s40694-016-0022-x

Sharon A., Finkelstein A., Shlezinger I. H. (2009) Fungal apoptosis: Function, genes and gene function FEMS Microbiology Reviews 33: (doi.org/10.1111/j.1574-6976.2009.00180.x)

Smith M.L., Gibbs C.C. and Milgroom G. M. (2006) Hetrokaryon incompatibility function of barrage-associated vegetative incompatibility genes(vic) in *Cryphonectria parasitica* Mycologia **98**: 43-50.

SERUM PROTEIN ANALYSIS OF LOCAL AND EXOTIC CHICKEN USING SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

W. A. Olaniyi♠[1], O. Bamidele[2], B.H. Ajao[2], G.S. Arowojolu[1], A. Babatunde[2], M.A. Salau[3], and O. G. Omitogun[2]

[1]Department of Environmental Biology and Fisheries,
Adekunle Ajasin University, PMB 001, Akungba-Akoko, 342111, Ondo State, Nigeria;
[2]Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, 220005, Osun State, Nigeria.
Department of Agricultural Technology, The Polytechnic, Ibadan, Saki Campus, Oyo State, Nigeria.
♠Corresponding Author: wasiu.olaniyi@gmail.com; waolaniyi@gmail.com; +2348033799789

Abstract: The recent global loss of animal biodiversity has raised a serious concern on the unknown risk status of African chicken. In this study, blood sera from local and Harco breeds of exotic chicken were analyzed using 10% Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) for genetic similarity using the relative mobility of the protein bands. After the electrophoretic separation, the gel was stained, de-stained, scored and analyzed using PAleontological STatistics (PAST) software for similarity association between the samples. Results obtained from the pooled data showed 51% genetic similarity, which suggests differences within and/or between exotic and local chickens. Few individuals had 100% similarities irrespective of sexes and chicken types. These serve as indicators that could be implication for successful crossbreeding program between the two breeds.

Keywords: African Chicken, Biodiversity, Crossbreeding, Electrophoresis, Phylogeny

INTRODUCTION

Chickens are the most popular poultry species primarily used as source of meat and egg by man (Ashraf *et al.,* 2003). The local chicken constitutes a larger percentage (70-80%) of the total chicken population in the developing countries (Adebambo *et al.,* 2015). They serve as cheap source of animal protein and provide income to households (Lambio *et al.,* 1998; Lingaya *et al.,* 2007; Carbales *et al.,* 2012). The local chicken, also referred to as village, indigenous or traditional chicken are usually raised by local farmers and plays importance in the economy, religious and socio-cultural lives of people in the developing countries (Tadelle *et al.,* 2000; Nosrollah, 2008; Besbes, 2009; Carbales *et al.,* 2012). The local chickens are tolerant to diseases, parasite and harsh weather conditions (Besbes, 2009; Ambaye *et al.,* 2012), and also survive on little or no inputs and can adjust to changes in food availability (Adebambo *et al.,* 2015). The exotic breeds and improved hybrids are of high economic value, but they are low in supply in these local environments due to the effect of harsh environmental factors (Ambaye *et al.,* 2012); However, cross non-targeted, unplanned breeding activities exist between the local and exotic species in these local areas. In view of this, proper characterization is necessary on available chicken collections for conservation of biodiversity and improved breeding programmes. Recently the global loss of animal biodiversity has been of a serious concern (Banerjee, 2012). The unknown risk status of African chicken were reported to be 60% of the avian breeds, while that of Latin America and the Caribbean were estimated to be 81% (FAO, 2007). Genetic/molecular characterization of local chicken is necessary to protect biodiversity, and planning an effective conservation programme.

Electrophoresis is a technique used in the separation of charged particles such as ions, molecules and macromolecules which may be nucleic acids or protein molecules in a liquid medium using an electric field applied to a gel matrix. The electromotive force is used to move the molecules through the gel matrix (Liu *et al.,* 2006). SDS-PAGE exploits the differences in molecular size to resolve proteins differing by as little as 1% in their electrophoretic mobility through the gel matrix. The technique is also a powerful tool for estimating the molecular weights of proteins. SDS-PAGE is an indispensable tool in protein analysis that has been attributed to three innovations (Marshak *et al.,* 1996). Polyacrylamide gel electrophoresis (PAGE) can be used to separate protein by size, density and purity because they have a more clearer resolving power than

any other biochemical examinations. The widely used method is sodium dodecyl sulphate (SDS, $CH_3(CH_2)_{11}OSO_3Na$) polyacrylamide gel electrophoresis (SDS-PAGE). This is due to its ease, simplicity and speed, relative feasibility and high resolution of the analysis; and importantly a small amount (microgram) sample is required.

The present study utilized polyacrylamide gel electrophoresis as a molecular characterization method to determine genetic similarity or variability in the local and exotic chickens.

MATERIALS AND METHODS

Location and Blood collection

Fifteen male local chicken (MLC) and 15 female (FLC) and thirty exotic Harco breeds of male species (MEC) were obtained from the Department of Animal Sciences, Obafemi Awolowo University, and Sultad Agrobiz Nig. Ltd, Modakeke, Osun State, Nigeria respectively. The study was conducted at the Biotechnology Laboratory, Department of Animal Sciences and Central Science Laboratory of Obafemi Awolowo University, Ile Ife, Nigeria. Blood (1.5 ml) was drawn from the wing veins of the chicken using a 2ml hypodermic syringe and needles. Blood sample was gently released into a serological glass tubes containing 1 ml normal saline (0.9% NaCl), and kept for an hour at ambient temperature. After an hour, the blood cloth that was formed was removed and the samples were centrifuged for 10 minutes at 2,500rpm. The sera were then stored in a refrigerator at 4°C before electrophoretic analysis.

Electrophoresis

10% resolving gel was prepared (10g of SDS/90ml in distill water) at pH of 8.8; and 4% stacking gel at pH of 6.8, were used to cast the gel following Betiku and Omitogun (2006) and (Adeola and Omitogun (2012). The blood samples were denatured in a sample buffer of 7.5% β-mercaptoethanol (SIGMA) by heating at 95°C for 5 min. 10 ml of the mixture was then loaded using micropipette into the wells of the casted gel and proteins were separated at constant 150V for an hour with the aid of Bio-Rad Mini Protean II Cell and its electrophoresis power supply kit (Model 200/2.0). After the electrophoretic separation, the gel was gently removed from the kit and placed into a staining solution made up of 0.1% Coomassie Blue in 1:4 glacial acetic acid: methanol for about 2-3 hours. Thereafter, the gel was then de-stained in a 60% solution containing 1:4 glacial acetic acid: methanol.

The analysis was repeated for constancy of protein pattern. Gels were then scored based on the intensity of the protein bands (Ferrier, 1981; Pasteur *et al.,* 1988). The signs "+" and "–" represent the presence and absence of band, respectively. The sign "++" indicates strong presence of band thickness more than single "+". The data generated from the scoring were subjected to clustering algorithm analysis using the Unweighted Pairgroup average algorithm (UPGMA) of Paleontological Statistics (PAST) software for similarity association between the samples. The relative mobility of the protein band was also evaluated using the following equation: Relative mobility (RM) = Migration distance of a protein band/Migration distance of the fastest migrating band (Caprette, 2005; Diyaware *et al.,* 2011). The separation between proteins is from the differences in their electrophoretic relative mobilities. The electrophoretic mobility of a particular protein is a function of its charges to its frictional coefficient; and that they travel down the gel according to their molecular weight which was calculated using the first visible band as the reference point (Garfin, 2009).

RESULTS AND DISCUSSION

Table 1: Measurement of relative mobility and scoring of the gel

Protein Bands	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	*Total no of bands*
Relative Mobility	0.9	1.6	2.1	2.9	3.5	4.0	4.5	4.8	5.1	5.4	5.8	6.4	6.6	6.9	
1(ML)	+	+	+	++	+	-	-	++	-	-	+	+	+	+	10
2(ML)	+	-	+	++	-	-	-	++	-	-	+	++	++	++	8
3(ML)	+	+	+	++	+	-	+	++	+	+	+	++	++	++	13
4(FL)	+	+	+	++	+	-	-	++	-	-	+	+	+	+	10
5(FL)	+	-	+	++	-	-	-	++	-	-	-	+	+	+	7
6(ME)	+	+	+	++	+	-	-	++	+	-	+	+	+	+	11
7(ME)	+	+	+	++	+	+	+	++	+	+	+	++	++	++	14
8(ME)	+	+	+	++	+	-	-	++	+	+	+	++	++	++	12
9(ME)	+	+	+	++	+	+	+	++	+	+	+	+	+	+	14
10(ME)	+	-	+	++	+	-	-	++	-	-	+	+	+	+	9

The signs "+" and "–" represent the presence and absence of band respectively. "++" indicates strong presence of band thickness more than single "+".ME = Male exotic animal, ML = Male local animal, FLC = Female local animal.

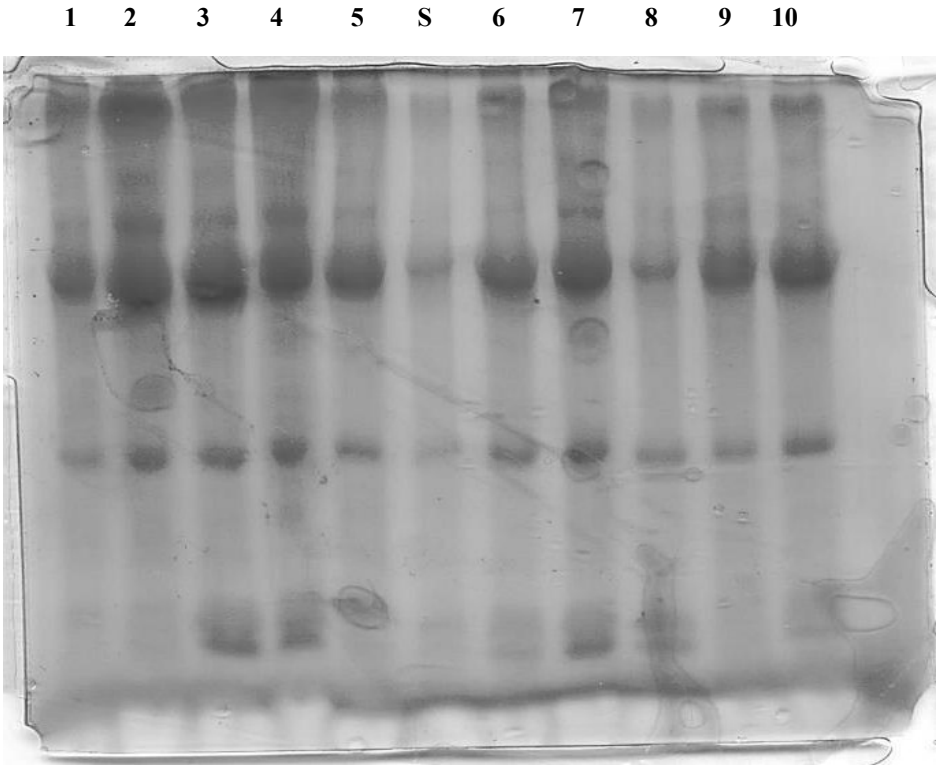


Plate 1. Representative gel of SDS-PAGE serum proteins patterns of local and exotic breeds of chicken. 1-3 depict male local chicken; 4-5 = female local chicken; S= Molecular standard; 6-10 = male exotic chicken.

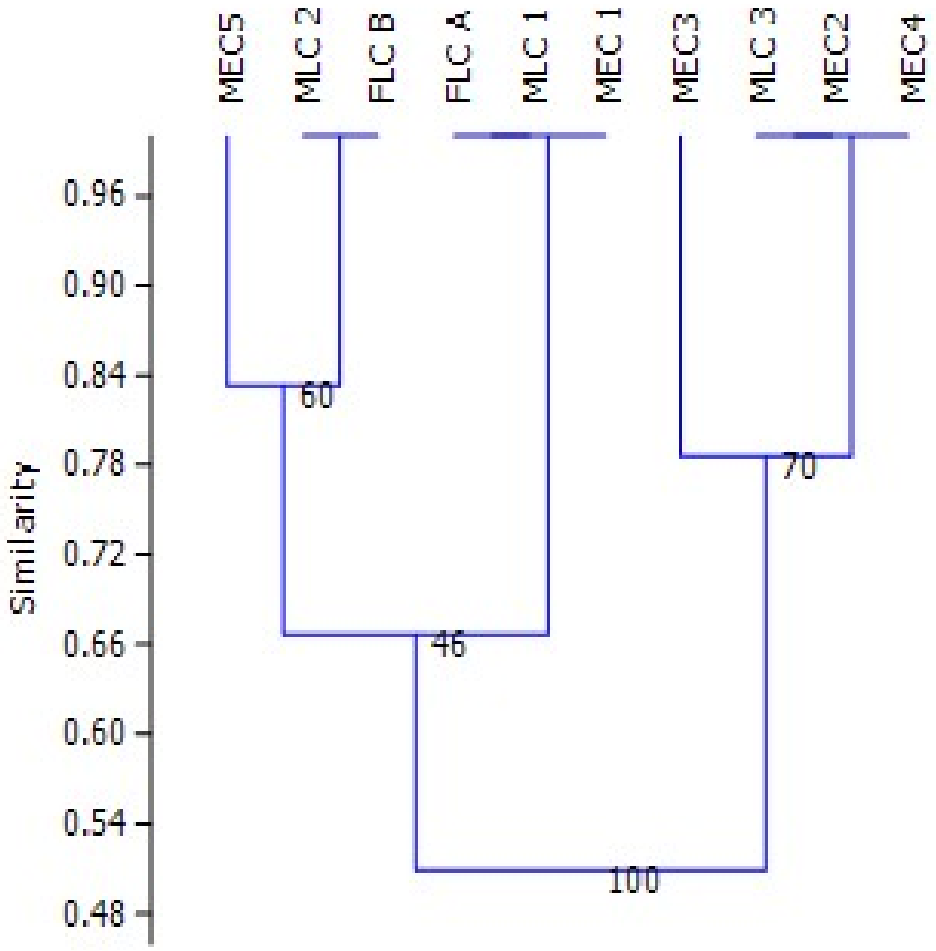


Figure 1. Dendrogram generated by PAST clustering algorithm (UPGMA) for genetic similarity between the local and exotic chicken. 1-5 and A and B, depict individual animal, while MEC = Male exotic chicken, MLC = Male local chicken, FLC = Female local chicken

Plate 1 shows the representative bands intensity and their mobility of serum protein patterns of local and exotic breeds of chicken using the gel of SDS-PAGE. Table 1 shows the relative mobility and scoring of the protein bands. The exotic birds (males) showed no difference except for the absence of seventh protein band (6, 8, and 10) in 65 percent of the samples. There was the strong presence of band thickness at bands 4 and 8 among all the chickens; while bands 12, 13 and 14 that also possess high band intensity are only common in the male samples of both breeds. The local chicken (females) showed the same number of missing protein band (6, 7, 9 and 10); while the fifth sample has additional missing bands (2, 5 and 11). Figure 1 shows the UPGMA-dendrogram generated by PAST clustering algorithm for the genetic similarity between the local and exotic chicken. The dendrogram revealed different sub-clusters based on the degree of similarity between and within chicken types. A very high level (100%) of similarities were observed between MLC 2 and FLC B; FLC A and MLC 1 and between MEC1 and MLC3.

The comparison of the pooled data of the representative samples of local chickens (MLC 2 and FLC B) with the exotic (MEC 5) gave 83% similarity, while comparing all of them with its sub-cluster (FLC A, MLC 1 and MEC 1) revealed 67% similarity. The other separated sub-cluster (MLC 3, MEC 2 and MEC 4) in relation with the standalone sample (MEC 3) which indicated 79% level of similarity; while the mean level of similarity coefficient for all the individuals (local and exotic chickens) was 51%. The level of polymorphism within individuals of each group showed very close relationship and common ancestor; and between the breeds showed good

relationship in their phylogeny. This indicates there will be successful hybridization or crossbreeding programs between the two breeds. Diyaware *et al.* (2011) explain such similarity as one of the pointers to hybridization. The ~50% genetic similarity and/ or genetic variation can explain the differences that may arise in their morphology for within and between each breed; and moreover, necessarily confirming common ancestry in their evolutionary phylogenetic relationship (Hoffmann *et al.,* 2004; Pym *et al.,* 2006, Adeleke et al., 2011; Banerjee, 2012).

CONCLUSION

This study revealed approximately 50% genetic similarity and/ or genetic variation in both the local and exotic breeds of the studied chicken, that may lead to differences in their morphology for within and between each breed; and also confirming common ancestry in their evolutionary trend. The level of relationship between the individual local and exotic breeds serves as indicators for successful hybridization between the two breeds.

REFERENCES

Adebambo, A.O., Adeoye, R.A., Osikomaya, O.A., Durosaro, S.O., Ilori, B.M. Sanda, A.J., Wheto, M. and Adebambo, O.A. (2015). Frequencies of some morphological features in indigenous chickens of South-Western Nigeria. *Nig J Anim Prod* 42(1): 4-10.

Adeleke, M.A., Peters, S.O., Ozoje, M.O., Ikeobi, C.O.N., Adebambo, A.O., Olowofeso, O., Bamgbose, A.M. and Adebambo, O.A. (2011). A preliminary screening of genetic lineage of Nigerian local chickens based on blood protein polymorphisms. *Animal Genetic Resources* 48: 23–28. doi:10.1017/S2078633610000962

Adeola, A.C. and Omitogun, O.G. (2012). Characterization of indigenous pigs in Southwestern Nigeria using blood protein polymorphisms. *Animal Genetic Resources* 51, 125-130.

Ambaye, F.B., Adnoy, T., Gjoen, H.M., Kathle, J., and Abebe G. (2012). Performance of 4-way cross-bred Ethiopian and exotic chicken breeds in two management systems in Southern Ethiopia. *Animal Genetic Resources* 51: 139-148.

Ashraf, M., Mahmood, S., Khan, M. S. and Ahmad F. (2003). Productive behavior of lyallpur Silver black and Rhode Island red breeds of poultry. *Intl J Agric Biol* 3: 384- 387.

Banerjee, S. (2012). Morphological characterization of indigenous chickens of Sikkim and West Bengal, India. *Animal Genetic Resources* 51: 57-71.

Besbes, B. (2009). Genotype evaluation and breeding of poultry for performance under sub-optimal village conditions. *World's Poult. Sci. J.* 65: 260-271.

Betiku, O.C. and Omitogun, O.G. (2006). Genetic diversity of commercial *Clarias gariepinus* population revealed by Sodium dodecyl sulfate-Polyacrylamide gel Electrophoresis (SDS-PAGE). In: *Proceedings of the 31st Annual Conference of the Genetics Society of Nigeria,* 2006. 135-140 pp.

Caprette, D. R. (2005). Measuring relative mobility of protein band. Experimental Bioscience. www.ruf.rice.edu/~bioslabs/studies/sds-page/rf.html

Carbales Jr, J.C., Lambio, A.L., Vega, S.A., Capitan, S.S., and Mendioro, M.S. (2012). Distinct morphological features of traditional chicken (*Gallus gallus domesticus* L.) in Western Visayas, Philippines. *Animal Genetic Resources* 51: 73-87.

Diyaware, M.Y., Haruna, A.B., Abubakar, K.A. and Omitogun, O.G. (2011). Serum protein characterization of intergeneric hybrids between *C. anguillaris* × *H. bidorsalis* and their hybrids from semi-arid zones of Nigeria. *J. Microbiol. Biotech. Res.* 1(4):227-234.

FAO, 2007. The State of the World' Animal Genetic Resources for Food and Agriculture. B. Rischkowsky & D. Pilling (eds), FAO, Rome.

Ferrier, V. (1981). La gynogenese experimentale et son application a l'etude de l'heredite de divers polymorphismes enzymatiques dans le genre Pleuorodeles (Amphibiens Urodeles). These de doctorat d'Etat, Universite Paul-Sabatier, Toulouse.

Garfin, D.E. (2009). Introduction to Biophysical Method for Protein and Nucleic Acid Research, academic press, San Diego, pp. 53.

Hoffmann, I., Siewerdt, F. and Manzella, D. (2004). Research and investment – challenges and options for sustainable use of poultry genetic resources. Paper presented at the *XXII World Poultry Congress, CD Proceedings,* 8–13 August, 2004, Istanbul, Turkey. 10p

Lambio, A.L., Bondoc, O.L., and Grecia, M.C. (1998). Brooding and growing performance of four genetic groups of Philippine native chickens. *Philippine J. Vet. Anim. Sci.* 24(1-2): 1-8.

Lingaya, R.A.I.N., Oliveros, M.C.R. and Magpantay, V.A. (2007). Production performance and marketing of native chicken in the First and Fourth Districts of Iloilo. *Philippine J. Vet. Anim. Sci.* 33(2): 163-171.

Liu, Y. P., Wu, G. S., Yao, Y. G., Miao, Y. W., Luikart, G. F., Baig, M. O., Beja-Pereira, A.Y., Ding, Z. L., Palanichamy, M. G. and Zhang,Y. P. (2006). Multiple maternal origins of chickens: out of the Asian jungle. *Journal of Molecular Phylogenetic Evolution*, 38: 12-19.

Marshak, D. R., Kadonaga, J. T., Burgess, R. R., Knuth, M. W., Brennan, W. A. and Lin, S.H. (1996). *Strategies for protein purification and characterization*. Cold Spring Harbor Laboratory Press, New York, Pp. 37-38.

Nosrollah, V. (2008). Indigenous chicken production in Iran: a review. *Pak. J. Biol. Sci.* 11(22): 2525-2531.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J., and Britton-Davidian, J. (1988). Practical isozyme genetics. Ellis Howood Ltd. Chichester, ISBN 0-7458-0501-9. 215 pp.

Pláteník, J. (2008). Electrophoresis in Biochemistry. Institute of Medical Biochemistry, First Faculty of Medicine, Charles University in Prague. 7p.

Pym, R.A.E., Guerne Bleich, E. and Hoffmann, I. (2006). The relative contribution of indigenous chicken breeds to poultry meat and egg production and consumption in the developing countries of Africa and Asia. In: Proceedings of the XII European Poultry Conference, 10-14 September, 2006, Verona, Italy. CD Rom.

Tadelle, D., Alemu, Y. and Peters, K.J. (2000). Indigenous chicken in Ethiopia: their genetic potential and attempts made at improvement. *World's Poult. Sci. J.* 56: 45-54.

INSULIN-LIKE GROWTH FACTOR-1 (IGF-1) POLYMORPHISM AND ITS ASSOCIATION WITH BODY WEIGHT AND SEMEN QUALITY CHARACTERISTICS IN NIGERIAN INDIGENOUS COCKS

[1]Olutunmogun, A. K., [2]Orunmuyi, M., [3]Kabir, M., [4]Musa, A. A., [5]Afolabi-Balogun, N. B. and [3]Daudu, O. M.

[1]National Animal Production Research Instiute, Shika-Zaria, Kaduna State, Nigeria.
[2]Department of Animal Production and Health, Federal University, Oye-Ekiti, Ekiti State, Nigeria.
[3]Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[4]Department of Animal Production, Kogi State University, Anyigba, Kogi State, Nigeria.
[5]Department of Chemical Sciences, Fountain University, Osogbo, Osun State, Nigeria.
Corresponding Author: olutunmogunkayode@yahoo.com

Abstract: 105 randomly selected Nigerian indigenous cocks of normal feathered, frizzle and naked neck genotypes were used to measure body weight and semen quality characteristics and their association with insulin-like growth factor-1 (IGF-1) polymorphism. Blood samples for DNA analysis were collected into an ETDA bottle. The genotyping and association of the IGF-1 was identified using the polymerase chain reaction (PCR)-RFLP method. From the data obtained, three polymorphic variants (denoted AA, AB and BB) were obtained from the studied gene using the EcoR1 digest enzyme. The genotypic frequencies for AA and BB (homozygote) and AB (heterozygote) were 56.25, 12.5 and 31.25, respectively, while the allelic frequencies for alleles A and B were 72 and 28%, respectively. The frequencies of the chicken IGF-1 alleles conform to the Hardy-Weinberg equilibrium. Body weight and semen quality characteristics were significantly ($P<0.05$) associated with the IGF-1 gene) with the AA genotype being advanced in body weight and semen volume at all ages, although no significant different ($P>0.05$) was observed at 28 weeks for semen volume. The BB genotype had a better semen pH result at all ages than AA and AB. It is concluded from the present results that the IGF-1 could be used as a candidate gene in fast genetic selection for body weight and semen quality in Nigerian indigenous chickens.

Keywords: Bodyweight, IGF-1, indigenous cock and semen quality characteristics.

INTRODUCTION

The Nigerian indigenous chicken constitutes about 90 percent of the total chicken population in Nigeria. Indigenous chickens consist of various unimproved sub-populations of heterogeneous characteristics, not yet classified into breeds and varieties since they do not breed true to type and have no clear plumage colours (Ibe, 2001). There are varying genotypes (such as frizzle, naked neck and dwarf) that plays significant role in the productive adaptability of the Nigerian local chicken (Ozoje and Ikeobi, 1995). Current effort is aimed at determining the links between the physiological, biochemical and metabolic products and the productive efficiencies of farm animals (Isidahomen *et al.*, 2011).

Growth and semen characteristics are critical selection criteria for roosters (McDaniel *et al*., 1998). A growing body of evidence as described by Andreassen *et al.* (2014) and Chandrashekar *et al.* (2004) has pointed to the possibility that growth hormone and its primary downstream mediator IGF-1 may be important for normal male reproductive function.

Insulin-like growth factors-1 (IGF-1) gene is a suitable candidate gene marker for growth, body composition, metabolism, and differentiation of cell types, skeletal characteristics, reproduction and growth of adipose tissue in chickens (Scanes, *et al.,* 1999; Amills *et al.,* 2003; Zhou *et al.,* 2005). It was observed that IGF-1 has effect on egg production of laying hens (McMurtry, *et al.,* 1997) while Nagaraja *et al.* (2000) found association of IGF-1 with egg production and egg shell quality. Since candidate gene approach has become a powerful technique for genetic improvement (Zhu and Zhao, 2007), IGF-1 gene has been reported in several works to influence growth and feed efficiency. In addition, it has also been reported in the reproductive

traits in poultry (Amills *et al.,* 2003; Zhou *et al.,* 2005). Velazquez *et al.* (2005) reported the association of IGF-1 gene with fertility and reproduction in cattle. Association between IGF-1 and IGFBP3 with semen volume, concentration, total motility and total spermatozoa activity in healthy young men was reported by Andreassen *et al.* (2014) However there is a dearth of information on the association of IGF-1 gene with fertility and reproduction in traditional chickens.

There is the need to investigate the effects of IGF-1 gene polymorphism on the male bodyweight and reproductive potential. Therefore, this study was aimed at determining the association of IGF-1 gene polymorphism with bodyweight and semen quality characteristics of the Nigerian indigenous chicken.

MATERIALS AND METHODS

Experimental location: The study was conducted at the Poultry Unit of the Department of Animal Science Research Farm, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria. The geographical location of Zaria is in the Northern Guinea Savannah which lies between latitude 11º 09' 06" N and longitude 7º 38' 35" E (Ovimaps, 2012). The mean relative humidity during the hamattan period, hot season and the wet season are 21%, 37% and 77%, respectively.

Source of Experimental Birds and Management: The 105 birds used for the study were obtained from normal feathered, frizzle and naked neck Nigerian indigenous chickens that were raised on farm station. The background of the birds was as described in an earlier report (Olutunmogun, *et al.,* 2014). The birds were wing tagged at day old and intensively raised on the deep liter management system. The birds were fed chick mash (2652 Kcal/Kg DM and 21% CP), grower mash (2400 Kcal/Kg DM and 16.08% CP) and breeders mash (2520Kcal/Kg DM and18.05% CP). Feed and water were given *ad-libitum.*

Data Collection: Data were collected on bodyweight and semen quality characteristics from the 105 cockerels on a monthly basis starting from 24 to 32 weeks of age. 2mls of blood was collected from each of the cockerels using a disposable syringe and needle, one for each cockerel into ethylene diamine tetra acetic acid (EDTA) sample bottle. The blood samples when collected were put into ice pack flask before taken them for DNA analysis at Centre for Biotechnology Research and Training (CBRT), Ahmadu Bello University, Zaria, Nigeria.

Extraction of Dna And Polymerase Chain Reaction (PCR): Genomic DNA was isolated by using Thermo Scientific GeneJET Genomic DNA Purification kit. The protocol used is as described for DNA purification from nucleated blood. The restriction patterns/bands were visualized on a 0.75% agarose gel which was followed by the investigation of the quality and quantity of the DNA in a spectrometer. The purity and concentration of DNA products was estimated using the UV-visible range spectrometer. The visualized bands were taken and stored in a documentation system.

The IGF-1 gene primer was selected from a previous publication (Nie *et al.,* 2005) for use in amplifying the Nigerian indigenous chicken ortholog. According to Nie *et al.* (2005), the sequences of the candidate gene of the somatotropic axis is from Genbank (http://www.ncbi.nlm.nih.org). The primers had been designed using the GENETOOL program (http://www.biologysoft.com). The primers were synthesized through a commercial service (BioNEER Corp., USA). Information on the primer are given in Table 1. The PCR was performed with total volume of 50 µL in each PCR tube, containing 25 µL of 2 x PCR master mix, 1 µL each of the forward and reverse primers, 1 µL of genomic DNA and 22 µL of nuclease free water. The PCR tube was put in FTGENE5D thermocycler (by TECHNE Cambridge) and the PCR condition was set at 94ºC for 5 minutes for initial denaturing, followed by 35 cycles at 94ºC for 30 seconds for denaturing, 52ºC for 45 seconds for annealing, and 72ºC for 90 seconds extension, and a final extension step at 72ºC for 5 minutes.

Restricted Digest and Restricted Fragment Length Polymorphism: The restricted digest was done using 1 µL of Fast Digest enzyme (EcoRI) according to the manufacturer's (Thermo Scientific) recommendation and at an incubation temperature of 37ºC for 20 minutes. The enzyme was subsequently deactivated by heating for 5 minutes at 80ºC. The digested products were loaded on a gel. Then, the electrophoresis tank was set up and connected to electric source to run for 20 minutes at 75-100 volts. The gel was removed and viewed under UV light to observe the bands. The

restriction patterns were visualized by 0.75% agarose gel electrophoresis; gels were stained with GR Green DNA stainer. Gels were visualized and photographed using a gel documentation system (Uvipro Silver by Uvitec).

Statistical Analysis: General linear model procedure of Statistic Analysis System program (SAS, 2002) was used to test the three genetic groups and age. Duncan's Multiple Range Tests was used to separate the significant means. The following model was used to investigate the effect of the IGF-1 gene on the bodyweight and semen quality characteristics of the cocks at different ages:

$Y_{ijk} = \mu + I_i + G_j + e_{ijk}$

Where; Y_{ijk} = observation on the i^{th} IGF-1 marker on the j^{th} genotype, μ = overall population mean, I_i = effect of the i^{th} IGF-1 marker, G_j = effect of the j^{th} genotype, e_{ijk} = random error.

The gene frequencies were calculated according to Hardy-Weinberg's equation as follows:

$$p = \frac{2(AA) + (AB)}{2N}$$

$$q = \frac{2(BB) + (AB)}{2N}$$

Where; p = the gene frequency of allele A, q = the gene frequency of allele B and N = the total number of birds tested.

A chi-squared test for goodness-of-fit was performed to verify if genotype frequencies agree with Hardy-Weinberg's equilibrium (HWE) expectations using the following formula:

$$\chi^2 = \frac{\sum (O - E)^2}{E}$$

Where; χ^2= Chi square, $\sum$ =Summation, O= Observed frequency, E= Expected frequency.

Table 1: Primer used for the amplification of IGF-1 gene in Nigerian indigenous chickens

Primer	Gene	Sequence of oligonucleotide primers (forward primer 5'-3'/ Reverse primer 5'-3')	Sequence ID[1]	Length (bp)	Annealing temperature for PCR amplification (°C)[2]
309	IGF-1	AGCTGTTCGAATGATGGTGTTTT/ GCCCCAGCATTCTCTTTCCTT	AY253744	583	56.4 58.2

Source: Nie *et al.* (2005). [1]Sequence accession number used for primer designing. [2]Annealing temperature as specified by the manufacturer (BioNEER Corporation, USA).

Table 2: Genotypic distribution and allele frequencies of IGF-1 genes in three Genotypes of Nigeria indigenous cockerels

	Genotypic frequencies (%)			Allelic frequencies (%)		χ^2
Gene	AA	AB	BB	A	B	
IGF-1	56.25	31.25	12.5	72	28	0.82

IGF = Insulin growth factor, χ^2 = Chi-square

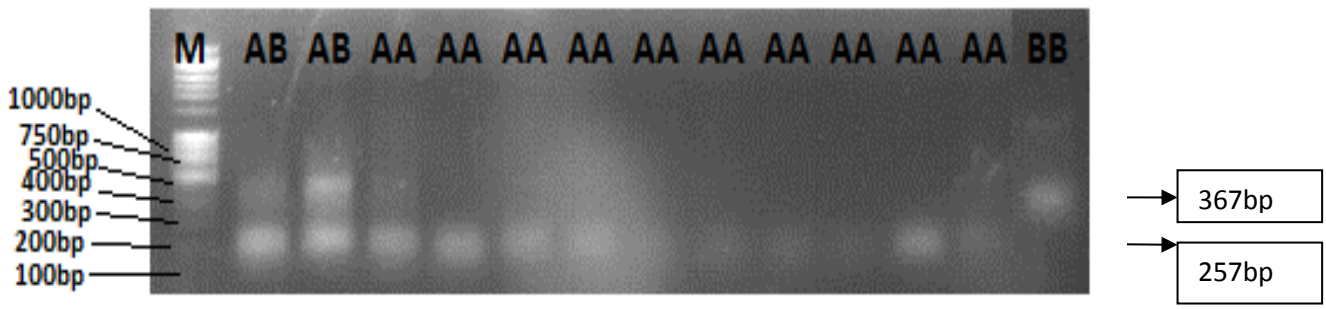


Plate1: Electropherogram showing genotyping profiles of IGF-1 gene detected by PCR method.

Table 3: Association between IGF-1 gene and bodyweight and semen characteristics in the three Nigeria indigenous cocks

Genotype	Week 24						
	Wt (g)	**Vol.(ml)**	**Colour**	**Mot. (%)**	**pH**	**Conc. (10^9)**	**Live (%)**
AA	1107.38±118.02^a	0.29±0.09^a	0.81±0.35^c	88.89±16.55^b	7.11±0.47^b	3.32±17.08^c	70.88±18.02^b
AB	1071.29±77.47^a	0.13±0.04^c	1.37±0.23^b	76.02±10.86^c	7.64±0.31^a	6.37±11.21^b	53.69±11.83^c
BB	976.43±58.53^b	0.18±0.03^b	1.68±0.17^a	91.61±8.21^a	7.20±0.24^b	7.94±8.47^a	77.19±8.90^a
SEM	41.64	0.02	0.12	5.84	0.17	0.60	6.36
LOS	*	*	*	*	*	*	*
	Week 28						
AA	1230.27±68.31^a	0.19±0.04	2.63±0.3^a	93.51±3.60^a	7.01±0.26^b	5.16±6.04^a	92.41±13.19^a
AB	1155.27±44.84^b	0.20±0.03	1.17±0.19^b	80.42±2.36^c	7.35±0.17^a	4.27±3.96^b	72.81±8.66^c
BB	1039.27±33.87^c	0.20±0.02	1.11±0.15^b	87.36±1.78^b	7.02±0.13^b	4.19±2.99^b	77.16±6.54^b
SEM	24.1	0.02	0.11	1.27	0.09	0.21	4.66
LOS	*	NS	*	*	*	*	*
	Week 32						
AA	1386.07±73.07^a	0.20±0.04^a	0.98±0.30^b	94.29±18.11^a	6.74±0.54^b	5.05±5.34^a	88.58±18.19^a
AB	1249.72±47.96^c	0.17±0.03^a	0.92±0.20^b	90.96±11.90^b	7.20±0.35^a	5.16±3.5^a	87.85±11.94^a
BB	1280.28±36.23^b	0.09±0.02^b	1.42±0.15^a	76.68±8.99^c	7.06±0.26^a	4.36±2.64^b	79.02±9.02^b
SEM	25.78	0.02	0.11	7.43	0.19	0.19	6.42
LOS	*	*	*	*	*	*	*

Means within the same column with different superscript are significantly (P<0.05) different, NS= Not Significant, Wt= Weight, Vol. = Volume, Mot. = Motility, Conc. = Concentration, No= Number, SEM= Standard Error of Mean, LOS= Level of significance.

RESULTS AND DISCUSSION

The restricted digest analysis of the PCR products using the EcoR1 indicated the presence of two restriction patterns. The DNA fragment sizes of 257 and 367bp were observed in the restriction patterns and were assigned AA for those with restriction pattern (257bp), BB with restriction pattern (367bp) while those AB with both restriction patterns together were assigned (257/367bp), respectively as shown in plate 1. Earlier, Babayi *et al.* (2014) reported frequencies of 0.5833 and 0.4167 for A and C alleles in West-Azerbaijan native chickens. An earlier report by Kadlec *et al.* (2011) showed a frequency of 0.915 and 0.085 for the A and C alleles (respectively) in Ross 300 broiler chicken. The report of this study is similar both in type of genotype (AA, AB and BB), genotype frequencies and allelic frequency with those of Musa *et al.* (2016) who reported genotype frequencies of 51.02, 38.78 and 10.20 for AA, AB and BB, respectively in their work on Nigerian indigenous chickens. Furthermore, the result reveals that the IGF-1 gene from the population was in Hardy Weinberg's equilibrium. However, the result did not agree with those of Li *et al.* (2010) and Shah *et al.* (2012) who reported that the population of chickens studied was not in Hardy Weinberg equilibrium.

Association between IGF-1 gene and bodyweight and semen characteristics in the three Nigeria indigenous cocks at various ages

The association between the IGF-1 genotypes, bodyweight and semen characteristics at different ages are shown in Table 3. Cocks with the AA genotype had significantly (P<0.05) higher bodyweight at all ages than cocks with genotypes AB and BB. The homozygote AA and BB gene had better semen quality traits than the heterozygous, with AA being superior to other genotypes. Previously, King (2006) reported that there was a physiological connection between bodyweight and growth with reproductive traits in males and the rate of sexual maturation was much more closely associated with genes associated with body weight than with chronological age. However, this study reveals an association of chronological age with bodyweight and sexual traits. Furthermore, association study as suggested by Babayi *et al.* (2014) and Li *et al.* (2008) cannot be used to determine if IGF-1 gene allele and growth hormone receptors are responsible for the variation in a particular trait or whether the variation is as a result of closely linked locus.

CONCLUSION AND RECOMMENDATION

The IGF-1 gene is a potential marker assisted selection technique which can be employed in selection for

bodyweight in Nigerian local chicken. However, there is the need for more studies to confirm the implication of age differences in the association of IGF-1 gene and reproduction traits in the Nigerian indigenous cocks.

REFERENCES

Amills M.N., Jimenez, D., Villalba, M., Tor, E., Molina, D., Cubilo, C., Francesch, A., Sanchez, A. and Estany, J. (2003). Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. *Poultry Science,* 82:1485-1493.

Andreassen, M., Jensen, R.B., Jorgensen, N. and Juul, A. (2014). Association between GH receptor polymorphism (exon 3 deletion), serum IGF1, semen quality and reproductive hormone levels in 838 healthy young men. *European Journal of Endocrinology*, 170: 555–563.

Babayi, M.S., Seyedabadi, H., Gorbani, A. and Zarghami, N. (2014). IGF-1 gene polymorphism and its association with some growth traits in West –Azerbaijan Native chicken using PCR-RFLP techniques.

Chandrashekar V., Zaczek D. and Bartke A. (2004). The consequences of altered somatotropic system on reproduction. *Biology of Reproduction*, 71: 17–27.

Ibe, S.N. (2001). Problems and proposal of developing Nigerian poultry breeds. *Journal of Science, Agriculture, Food Technology and Environment,* 1: 1-3.

Isidahomen, C.E., Ozoje, M.O. and Njidda, A.A. (2011). Haematology and serum biochemical indices of local and exotic chickens in a sub-humid tropical environment. *European Journal of Biological Science,* 3(1): 16-21. ISSN 2079-2085.

Kadlec, J., Hosnedlova, B., Rehout, V., Citek, J., Vecerek, L. and Hanusova, L. (2011). Insulin like growth factor-1 gene polymorphism and its association with growth and slaughter characteristics in broiler chickens. Journal of Agrobiology, 28(2): 157-163.

King, J.C. (2006). Physiology of pregnancy and nutrient metabolism. *American Journal of Clinical Nutrition,* 71: 1218-1225.

Li, H.F., Zhu, W., Chen, K., Song, W., Shu, J. and Han, W. (2010). Effects of the polymorphisms of GHR gene and IGF-1 gene on egg quality in Wenchang chicken. *Research Journal Poultry Science,* 3(2):19-22.

Li, H.F., Zhu, W., Chen, K., Wu, X., Tang, Q. and Gao, Y. (2008). Association between GHR and IGF-1 gene polymorphisms and reproductive traits in Wenchang chickens. Turkey Journal of Veterinary and Animal Science, 32(4): 281-285.

McDaniel, C.D., Hannah, J.L., Parker, H.M., Smith, T.W., Schultz, C.D and Zumwalt.C.D. (1998). Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. *Poultry Science,* 77: 888-893.

McMurtry, J.P., Francis, G.L. and Upton, Z. (1997). Insulin-like growth factors in poultry. *Domestic Animal Endocrinology,* 14: 199-229.

Musa, A.A., Orunmuyi, M., Akpa, G. N., Olutunmogun A.K. and Afolabi-Balogun, N.B. (2016). Effects of insulin-like growth factor I (IGF-I) polymorphism on bodyweight of Nigerian indigenous chickens. *African Journal of Agricultural Research,* 11(14): 1194-1198.

Nagaraja, S.C., Aggrey, S.E., Yao, J., Zadworny, D., Fairfull, R.W. and Kuhnlein, U. (2000). Traits association of a genetic marker near the IGF-1 gene in egg-laying chicken. *Journal of Hereditary,* 91: 150-156.

Nie, Q., Lei, M., Ouyang, J., Zeng, H., Yang, G. and Zhang, X. (2005). Identification and characterization of single nucleotide polymorphisms in 12 chicken growth correlated genes by denaturing high performance liquid chromatography. *Genetic Selection Evolutionary,* 37:339-360.

Olutunmogun, A.K., Orunmuyi, M., Kabir, M., Musa, A.A. and Muhammad, H. (2014). Biochemical and haematological parameters of normal, frizzle and naked neck Nigerian indigenous roosters reared in the northern guinea savannah of Nigeria. *Nigerian Journal of Genetics*, 28: 20-27.

Ovimaps, (2012). Ovilocation map; ovi earth imaginery. Dated July, 2012.

Ozoje, M.O. and Ikeobi, C.O.N. (1995). Productive performance and occurrence of major genes in the Nigeria local chicken. *Nigerian Journal of Genetics*, 10: 67-77.

SAS (2002). Statistical Analysis System, Computer Software, Version 9: Statistics SAS Institute Inc. Cary, Northern Carolina 27513, USA.

Scanes, C.G., Dunnington, E.A., Buonomo, F.C., Donoghue, D.J. and Siegel, P.B. (1999). Plasma concentrations of insulin-like growth factors (IGF-I and IGF-II) in dwarf and normal chickens of high and low weight selected lines. *Growth Developing and Aging,* 53: 151-157.

Shah, T., Deshpande, S., Dasgupta, U., Singh, K.M. and Joshi, G.C. (2012). Genotypic and Allelic frequencies of IGF-I and IGF2 genes in Broilers Analysed by Using PCR-RFLP Technique. *Iran. Journal of Applied Animal Science,* 2(4):357-360.

Velazquez, M.A., Newman, M., Christie, M.F., Cripps, P.J., Crowe, M.A., Smith, R.F. and Dobson, H. (2005). The usefulness of a single measurement of insulin-like growth factor-1 as a predictor of embryo yield and pregnancy rates in a bovine MOET program. *Theriogenology,* 64: 1977–1994.

Zhou, H., Mitchell, A.D., McMurtry, J.P., Ashwell, C.M., Lamont, S.J. (2005): Insulin-like growth factor-1 Gene polymorphism associations with growth, body composition, skeleton integrity, and metabolic traits in chickens. *Poultry Science,* 84: 212−219.

Zhu M. and Zhao, S. (2007). Candidate gene identification approach: progress and challenges. *International Journals of Biological Science,*3:420-427.

ASSOCIATION BETWEEN IGF- 1 GENE POLYMORPHISMS AND BODY WEIGHT IN NIGERIA LOCALLY ADAPTED TURKEYS

Oyewola, K. A*, Akinyemi, M. O, Osaiyuwu, O. H, Fijabi, O. E, Shobanke, I. A., Nwokorie, G.I. and Ewuola, K. M.

Animal Breeding and Genetics Unit, Department of Animal Science, University of Ibadan

Corresponding Author: writekhadijat@yahoo.com; +2348060403412

Abstract: The insulin-like growth factor 1 (IGF-1) gene polymorphism plays important roles in development, growth and reproduction. Genetic intervention for the improvement of Nigeria locally adapted turkeys based on growth rate and higher mature weight is important. This study was aimed at investigating the association between IGF-1 gene polymorphism and body weights in Nigeria locally adapted turkeys using PCR-RFLP method. Fifty poults were randomly selected for DNA analysis at 10 weeks. Zymo Miniprep kit was used for genomic DNA extraction from blood samples and 529bp fragment of intron 2 of IGF-1 gene was amplified. The genetic structure of the population was analysed using POPGENE 32 software. Association of the genotypes with body weight was evaluated using the General linear model of SAS 9.2. The enzyme digested products revealed A and B alleles with frequencies of 0.61 and 0.39 respectively. Two genotypes AA (0.38) and AB (0.62) were detected. Chi-square test (0.001830) for Hardy-Weinberg equilibrium showed that the population sampled was not in equilibrium for the gene investigated. Also, significant association was not observed between IGF-1 polymorphs and body weight at 4, 8 and 12 weeks of age in Nigeria locally adapted turkeys.

Keywords: DNA, Hardy-Weinberg equilibrium, Improvement, PCR-RFLP, Polymorphs.

INTRODUCTION

Nigeria is endowed with an impressive array of indigenous and locally adapted livestock, which have significant roles in socio-economic life of the rural and semi urban populace. These livestock are reservoir of untapped valuable rare genes and alleles which are linked to their adaptability and survival. Locally adapted turkeys are functionally valuable because they play vital roles in human nutrition, and contain genetic materials which may have been lost in the improved gene pool. They possess relic traits or genetic variants that are either absent in modern improved stocks or that exist in their rare ancestors which may be of commercial value (Adebambo, 2003). Selection of Nigerian locally adapted turkeys for improved growth and higher mature weight is imperative. Traditional approach of selection which is based on phenotype is time consuming and very difficult to achieve (Zhang *et al*., 2008). Therefore the use of molecular marker which is a powerful tool in animal breeding for genetic improvement to define the genotype and predict the performance of animal very early in life is important.

Polymorphism in growth-related genes like growth hormone (GH), growth hormone receptor(GHR), insulin-like growth factor-1(IGF-1), insulin-like growth factor-2(IGF2) and Myostatin (MSTN) have been closely linked with economic traits in poultry (Musa *et al.,* 2016). Genetic polymorphisms in several animal species can be identified using Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) among other techniques (Sartika, 2007). PCR-RFLP as a technique used to multiply certain DNA fragment to detect whether restriction site difference exists or not in individuals within the same population (Griffiths *et al*., 2003)

Insulin-like growth factor 1 belongs to the family of polypeptide hormones which is a structural homologues of insulin. Circulating IGF-1 is generated by the liver under the control of growth hormone (Akinfenwa *et al.,* 2011). Hegarty *et al*., (2006), reported that Insulin-like growth factor 1 (IGF-1) is a naturally occurring protein responsible for the stimulation of cellular growth, proliferation and differentiation. IGF-1 stimulates systemic body growth and has growth promoting effects on almost every cell in the body especially skeletal muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs (Yilmaz *et al.,* 2011). IGF-1 has a fundamental role in both prenatal and postnatal development and exerts all of its known physiologic effects by binding to

the insulin-like growth factor 1 receptor (Le Roith *et al.,* 2001). IGF-I gene has been reported to influence growth rate, carcass traits and feed efficiency in poultry (Amills *et al.,* 2003). Also studies have established a link between the concentration of the circulating IGF-1 and growth trait in many livestock species and laboratory animals (Hegarty *et al.,* 2006). IGF-1 is a mediator for many biological effects such as; increase in glucose absorption, stimulation of myogenesis, increase in lipids synthesis, and also stimulates progesterone production during DNA, RNA and protein synthesis (Etherton, 2004). In view of these biological functions, IGF-1 can be considered as a candidate gene for predicting growth, egg and meat quality traits in the animal genetics (Andrade *et al*., 2008). This study aimed at evaluating the polymorphism in IGF-1 gene and its association with body weight in Nigeria locally adapted turkeys.

MATERIAL AND METHODS

Experimental location and management of experimental birds

This study was conducted at Poultry section of Duke Farm, Orogun Express Ibadan Nigeria. The experiment was performed with 300 one-day old poult of Nigeria locally adapted turkeys. The birds were tagged at 1-day old and raised on deep litter system with feed and water supplied *ad-libitum*. Body weight data was collected at day old and weekly basis for 12 weeks.

Extraction of DNA: 50 poults were randomly selected and bled from the jugular vein at 10 weeks. 4 ml of blood were collected into heparinized sample bottles and transferred to -20 °C freezer. Genomic DNA was isolated using Zymo mini prep kit following manufacturer protocol. DNA was also examined by loading samples on 1.5% agarose gel and visualizing the band under gel documentation system.

PCR-RFLP for IGF-1 gene: Intron 2 region of the IGF-1 gene was amplified to a product of 529 bp using Forward (5′-TGTTCTGCATTTGGCCCATAC-3′) and Reverse:
(3′CAGAATGTCAGCTTTTTGTCC-5′) primers according to Nie *et al*., (2005) as follows:
The PCR was performed in a total volume of 25 µL in each PCR tube, containing 10 µL of 2 x PCR master mix, 1 µL each of the forward and reverse primers, 5 µL of genomic DNA and 8 µL of nuclease free water. The PCR tube was put in thermocycler and the PCR condition was set at 94°C for 5 min for initial denaturing, followed by 35 cycles at 94°C for 45 s for denaturing, 60°C for 45 s for annealing, and 72°C for 60 seconds extension, and a final extension step at 72°C for 10 min.
Restriction digestion was done using 1 µL of Msp1 enzyme according to the manufacturer's (Thermo Scientific) recommendation and at an incubation temperature of 37°C for 15 min. The enzyme was subsequently inactivated by heating for 20 min at 80°C. The digested products were electrophoresized on 1.5% agarose gel in 1X TBE and visualized by ethidium bromide staining for 15 min at 100 V. Gels were visualized using a gel documentation system and individual fragment sizes in each sample were determined based on standard DNA molecular weight marker for IGF-1 gene.

Statistical Analysis

POPGENE 32 software package was used to calculate genotypic and allelic frequencies and also to detect the state of population about Hardy-Weinberg equilibrium (HWE). Body weight data obtained was then subjected to analysis of variance following General linear model procedure of Statistical Analysis System (SAS) 2012 and Least Square mean (LSM). The following model was used to investigate effect of IGF-I genotypes on body weight:

$Yij=\mu+Gi+eij$

Where Yij = observed trait (body weight)
µ= the overall mean,
Gi= fixed effect of polymorphic variant,
eij= Random error term

RESULTS AND DISCUSSION

The restriction digest analysis of the PCR products using Msp1 indicated the presence of two restriction patterns as shown in Figure 2 below.

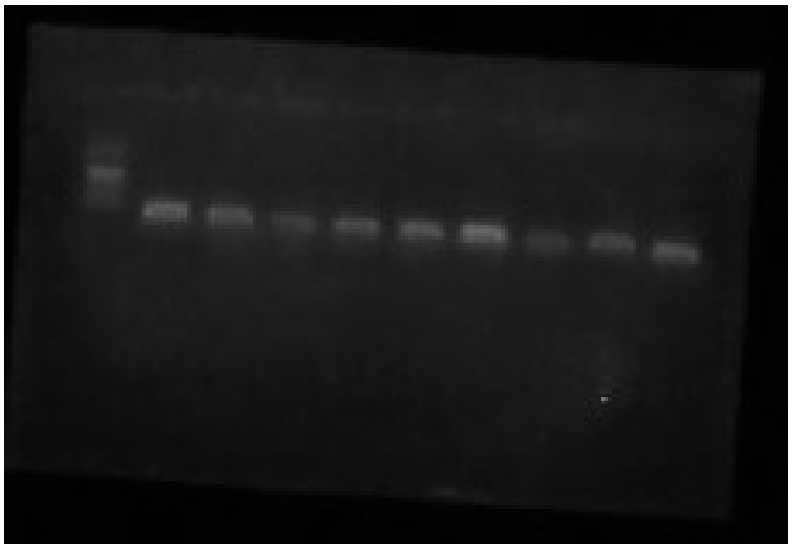


Figure 1: PCR products for IGF-1 gene of Nigeria locally adapted turkeys using 1.5 % agarose gel stained with ethidium bromide and100bp ladder

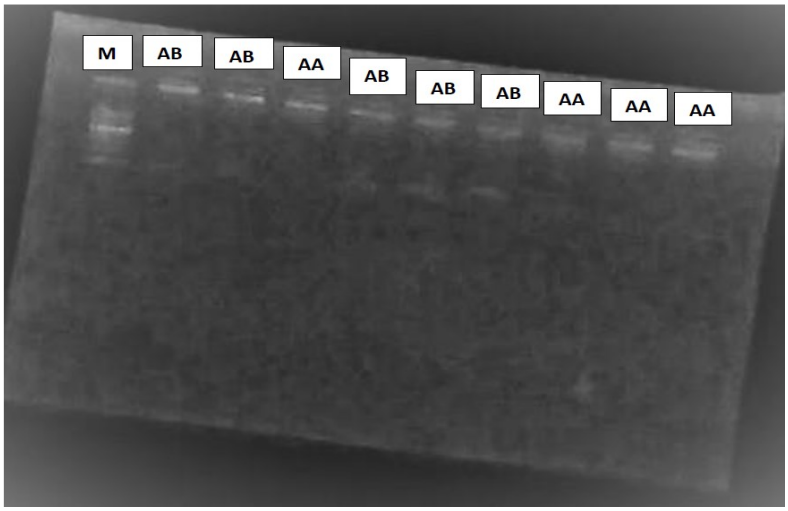


Figure 2: Results of analysis PCR-RFLP for IGF-1 gene in Nigeria locally adapted turkeys by MSP1 restriction enzyme on 1.5% agarose gel and 100bp ladder

M – Molecular weight marker
AA and AB- IGF-1 gene genotypes

Genotypic and Allelic frequencies of the IGF-I gene in Nigeria locally adapted turkeys

Intron 2 of IGF-1 gene in this study was identified to be polymorphic with only two genotypes; AA and AB. The BB homozygous genotype was not identified at all in the Nigeria locally adapted turkey sampled. However, the observed results in this study agreed with that of Babayi *et al.,* (2014) that reported the absence of CC homozygote genotype in west Azerbaijan native poultry. Moe *et al.,* (2009) and Kadlec *et al.,* (2012), also reported 2 polymorphic forms (AA and AC) of the IGF-1 gene with no CC genotype identified in Cobb and Ross broiler chickens. The results of Allelic and Genotypic frequencies are as shown in Table 1. Allele A(0.69) is significantly higher*(0.0018)* than allele B(0.31) in the studied population. Also the frequency of AA genotype was lower (0.38) than the frequency of AB genotype (0.62). In addition, these observed genotype frequencies are in agreement with the results of Abbasi and Kazemi (2011), that reported that allele A(0.51) was predominant over allele B(0.49), and genotype frequency for AA(0.26) was lower than that of AB(0.50) in Mazandaran Native Chicken. Kadlec *et al.,* (2011) reported, in Ross 300 strain frequency of A and C alleles in the UTR of IGF-1 gene were 0.915 and 0.085, respectively, while the genotypic frequencies of AA and AC were 0.83 and 0.17, respectively. Babayi *et al.,* (2014) reported allele A(0.58), and C allele (0.42) with genotype frequencies of AA(51.04), AC(14.58) and CC(34.37) in west Azerbaijan native poultry. The analysis of the turkey IGF-I gene revealed that the population deviated from Hardy Weinberg's equilibrium as evidenced by the significant Chi square values ($P<0.05$), and this is in line with the findings of Wang *et al.,* (2011), and Li *et al.,* (2010), who reported that the population of goats and chickens studied for IGF-1 gene were not in Hardy Weinberg equilibrium. However, Musa *et al.,* (2015), and Abbasi and Kazemi (2011), reported that Nigerian indigenous chicken and Mazandaran Native Chicken population studied were in Hardy-Weinberg equilibrium.

Table 1: Allele and Genotype frequencies at intron 2 locus of IGF-1 gene in Nigeria locally adapted turkeys

Allele frequency	Genotype frequency	X^2	G^2
A(0.69)	AA(0.38)	0.00183	0.000184
B(0.31)	AB(0.62)		

X^2: Chi-square test for Hardy-Weinberg equilibrium
G^2: Likelihood ratio test for Hardy-Weinberg equilibrium.

Effects of IGF-I genotypes on body weight of Nigeria locally adapted turkeys at various ages

The effects of IGF-I genotypes on body weight of Nigeria locally adapted turkeys at ages 4,8 and 12 weeks is presented in Table 2. The results showed that, there were no significant effect ($p>0.05$) of AA and AB genotypes with the body weight at 4, 8 and 12 weeks of age. The results obtained is consistent with that of Nagaraja *et al.,* (2000) who identified that different genotypes had no significant association for 140, 265 and 365 days weight in chickens. Promwatee *et al.,* (2013), reported higher body weights at 4, 8, 12 and 14 weeks of age in the AA genotype than in AB and BB genotype in the Khai Mook Esarn and Soi Pet population of chickens. Amills *et al.,* (2003) identified suggestive associations between IGF1-SNP and average daily gain at 107 days in black Penedesenea chicken. Fang *et al.* (2008), identified a significant correlation between IGF1 polymorphism and egg production in wenchang chickens. Gouda and Essawy, (2010) analyzed the polymorphism of IGF-I gene among Egypt chicken breeds and indicated that their effects on the growth traits of chicken was significant. Wang *et al.,* (2011), reported that a novel of SNP at IGF-1-P1 locus was significantly associated with cashmere production traits in exon 4 of Nanjiang Cashmere goat population in China

.

Table 2: Least Square Means (LSM±SD) of IGF-I genotypes on body weight (g) of Nigeria Locally Adapted Turkeys at Different Ages

WEEKS	AA (19)	AB (31)	SEM	P-VALUE
BW4	317.52±48.9	308.71±45.68	0.61	0.52
BW8	593.84±103.47	596.77±97.84	0.20	0.92
BW12	854.00±205.74	852.29±140.86	0.12	0.97

BW4, BW8 and BW12: Body weight at 4, 8 and 12 weeks of age respectively.
SEM: standard error of the means.
AA and AB: observed genotypes.

CONCLUSION

The results obtained in this study indicates that the population analysed deviated from Hardy- Weinberg equilibrium. Allele A in the IGF-1 gene of Nigeria locally adapted turkey is the predominant allele and there was no significant association ($p>0.05$) of IGF-1 genotypes on body weight of the sampled population.

RECOMMENDATION

There is need for further analysis to be performed to validate the association of polymorphic variant (IGF-1 gene) at intron 2 with the body weight of Nigeria locally adapted turkeys using large population sample size from different unrelated farms in other to increase precision rate and accommodate all the assumptions of Hardy–Weinberg principle. Also studies on association of IGF1-SNP (Single Nucleotide Polymorphism) with body weight traits of Nigeria locally adapted turkey should be carried out.

REFERENCES

Abbasi HA, Kazemi M (2011). Detection of polymorphism of the insulin-like growth factor-I (IGF-I) gene in Mazandaran native chicken using PCR-RFLP method. *African Journal of Biotechnology,* 10(61):13351-13354.

Adebambo, O. A. (2003). Animal Breeds: A National Heritage. *UNAAB inaugural lecture series16*: 47-77. Herman, L. (2004). Species Identification on Poultry Eggs Products. *Poultry Science* 83: 2083-2085.

Akinfenwa, M. O., Peter, S. O., Adenaike, A.S., Obetoh, J.O. and Ikeobi, C.O.N. (2011). Bioinformatics analysis of insulin-like growth factor-1 (IGFI) in five mammalian species. *Proceedings of the 16th Annual Conference of the Animal Science Association (ASAAN)* September 12th, 2011, K.S.U, Anyigba, Nigeria. P 19-21.

Amills M. N, Jimenez D, Villalba M, Tor E, Molina D, Cubilo C, Francesch A, Sanchez A, Estany J (2003). Identification of three single nucleotide polymorphisms in the chicken insulin- like growth factor 1 and 2 genes and their associations with growth and feeding traits. *Poultry Science* 82:1485-1493.

Andrade, P.C., Gross, D.A., Paz, C.C. and Alenca, M.M. (2008). Association of an insulin-like growth factor-1 gene Micro satellite with phenotypic variation and estimated breeding values of growth traits in canchim cattle. *Animal Genetics* 39: 480-485.

Babayi, M.S., H. Seyedabadi, A. Gorbani and N. Zarghami, (2014). IGF-I gene polymorphism and its associations with some growth traits in west-Azerbaijan native chicken

using PCR-RFLP Techniques. *Bulletin of Environment, Pharmacology and Life Science* 3: 29-32.

Etherton, T. D., (2004). Somatotropic Function: The somatomedion hypothesis revisited. *Journal of Animal Science* (E. Supply), 8: E239-E214.

Fang, L. H., W. Zhu, K. Chen, X. Wu, Q. Tang and Y. Gao, (2008). Associations between GHR and IGF-1 gene polymorphisms and reproductive traits in wenchang chickens. *Turkish Journal of Veterinary and Animal Science*, 32: 281-285.

Gouda, E.M. and G.S. Essawy, (2010). Polymorphism of insulin-like growth factor I gene among chicken breeds in Egypt. *Zeitschrift fur Naturforschung*, 65: 284-288.

Griffiths, A.J.E., J.H. Miller, D.T. Suzuki, R.C. Lewontin and W.M. Gilbart. (2003). Intoduction to Genetic Analysis. 7th Edition. W.H. Freeman and Company, USA. Department of Agriculture, Washington D.C, pp. 26

Hegarty, R., Macfarlene, S., Banks, R. and Harden, S. (2006) Association of Plasma metabolites and hormone with the growth and composition of lambs as affected by nutrition and size genetics. *Austrian Journal of Agricultural Resource* 57: 683-690.

Kadlec, J., B. Hosnedlova, V. Rehout, J. Citek, L. Vecerek and L. Hanusova, 2011. Insulin-like growth factor-I gene polymorphism and its association with growth and slaughter characteristics in broiler chickens. *Journal of Agrobiology*, 28: 157-163.

LeRoith, D., Scavo, L., Butler, A. (2001). What is the role of circulating IGF1? Trends *Endocrinology Metabolism,* 12, 48–52

Li H, Zhu W, Chen K, Song W, Shu J, Han W (2010). Effects of the polymorphisms of GHR gene and IGF-1 gene on egg quality in Wenchang chicken. *Research Journal of Poultry Science,* 3(2):19-22

Moe HH, Shimogiri T, Kawabe K, Nishibori M, Okamoto S, Hashiguchi T, Maeda Y (2009): Genotypic frequency in Asian native chicken populations and gene expression using Insulin-like Growth Factor I (IGF1) gene promoter polymorphism. *Journal of Poultry Science,* 46: 1−5.

Musa A A, Orunmuyi M, Akpa GN, Olutunmogun AK, Muhammad H, Adedibu II (2015). Diallel analysis for body weight involving three genotypes of Nigerian indigenous chickens. *South African Journal of Animal Science* 45(2):188-197.

Musa, A.A.; Orunmuyi, M.; Akpa, G.N.; Olutunmogun, A.K. and Afolabi-Balogun, N.B. (2016). Effects of insulin-like growth factor I (IGF-I) polymorphism on bodyweight of Nigerian indigenous chickens. *African Journal of Agricultural Research*, Vol. 11(14), pp. 1194-1198,

Nagaraja SC, Aggrey SE, Yao J, Zadworny D, Fairfull RW, Kühnlein U (2000): Trait association of a genetic marker near the IGF-I gene in egg-laying chickens. *Journal of Heredity* 91: 150–156.

Nie Q, Lei M, Ouyang J, Zeng H, Yang G, Zhang X (2005). Identification and characterization of single nucleotide polymorphisms in 12 chicken growth correlated genes by denaturing high performance liquid chromatography. *Genetic Sel. Evolution.* 37:339-360.

Promwatee N, Laopaiboon B, Vongpralub T, Phasuk Y, Kunhareang S, Boonkum W, Duangjinda M (2013). Insulin-like growth factor I gene polymorphism associated with growth and carcass traits in Thai synthetic chickens. *Genetic Molecular Resources,* 12(4):4332-4341.

Sartika, T. (2007). Pembibitan and Peningkatan Mutu Genetik Ayam Lokal. Taksonomi and Asal-Usul Ayam Domestikasi.Keanekaragaman Sumber Daya Hayati Ayam Lokal Indonesia*: Manfaat and Potensi*. LIPI Press. Jakarta, pp: 105-125

Wang Qiong, Fang Chao, Liu Wu-Jun, Fang Yi and Yu Shi-Gang, (2011). A Novel Mutation at Exon 4 of IGF-1 Gene in Three Indigenous Goat Breeds in China. *Asian Journal of Animal and Veterinary Advances*, 6: 627-635.

Yilmaz, A., Daus, M.E. and Simmen R.C.M. (2011). Reproductive Performance of bulls divergently selected on the basis of blood serum LAF-1 concentration. *Animal Science Journal,* 77: 835-839.

Zhang Y (2008). "Progress and challenges in protein structure prediction". *Curr Opin Struct Biology,* 18 (3): 342–348. PMC 2680823. PMID 18436442.