Assessment of Genetic Diversity based on RAPD Analysis in Cultivars of Peppers (*Capsicum annuum*, *Capsicum chinense* and *Capsicum frutescens*) Grown in Nigeria

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Abstract
Randomly amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity among 24 *Capsicum* cultivars collected from some states in Nigeria. These cultivars differ in their fruit morphology. 18 RAPD markers detected a total of 176 alleles (mean, 9.78; range, 5–15). Unique alleles (22) were found in 10 *Capsicum* cultivars. Genetic distance based on Jaccard coefficient range from 0.21 to 0.88, with an average of 0.61. Line pairs in *Capsicum annum* (rodo) ranged from 0.21 to 0.63. A dendrogram based on RAPD markers divided the lines into 4 main groups and 1 line, which separated from other lines. The first group included 9 CR lines (*Capsicum annum*) while the second group included 7 CS lines (*Capsicum frutescens*). The third group contained 1 CT (*Capsicum annum*) and 2 CA lines (*Capsicum frutescens*). Lastly, the fourth group was separated into 2 subgroups; the first subgroup included the closely related cultivars CG (CG22 and CG23) lines (*Capsicum annum*) whereas the second subgroup included CS17 and CA21 (*Capsicum frutescens*).
Principal component analysis (PCA) also grouped the lines and these were consistent with the dendrogram groupings. The grouping of these lines reflected their genetic similarity at the species level. These results define the existence of genetic diversity in the *Capsicum* species grown in some Nigerian states, which might be useful for future decisions in their conservation and management strategies.

Keywords: *Capsicum*, genetic diversity, germplasm conservation, RAPD markers, unique allele

Introduction

The genus *Capsicum* L. is the most widely grown of the family Solanaceae. It consists of 27 species and 5 of which were domesticated as far back as 6000 B.C. by Native Americans (DeWitt and Bosland, 1993; Onus and Pickersgill, 2004). Cultivated *Capsicum* species that are used as vegetables and spices are generally called peppers, which include: *Capsicum annuum* L., *Capsicum frutescens* L., *Capsicum chinense* J., *Capsicum pubescens* R. and P., and *Capsicum baccatum* L.

Among the peppers, they vary most in fruit size, shape (globose to cylindrical, elongate or ovoid, wrinkled and smooth) and colour (red, orange, cream, yellow, purple or white). Peppers have medicinal values, such as: vitamins A, B, C, E and K, which promote human health (Votava *et al*., 2000; Djian-Caporalino *et al*, 2007).

The *Capsicum* species is an important economic food crop commonly grown in northern Nigeria, where they are well adapted. The soil and climate favour the cultivation of a large number of cultivars (Ado, 1990). They are also grown in limited areas in southern Nigeria. Sweet pepper (*Capsicum annuum*), aromatic hot pepper (*Capsicum chinense*) and bird pepper (*Capsicum frutescens*) are widely cultivated and consumed in large amounts as fresh or dried by the people of Nigeria (Erinle, 1989).

The manipulation of wild progenitors during crop domestication and selection processes produced the present plant cultivars leading to the loss of some important traits and reduction in genetic variability attributed to population genetic bottleneck (Tanksley and McCouch, 1997; Zohary and Hopf, 2000; Buckler *et al.,* 2001; Wright *et al.,* 2005; Doebley *et al.,* 2006). Modern intense plant breeding methods have also contributed to the loss of genetic diversity (Tanksley and McCouch, 1997) and diversity among the *Capsicum* species has been of great concern (González-Pérez *et al.,* 2014).

The analysis of genetic diversity in cultivated crops is vital to provide insight into the level of variations resulting from the effects of continuous human selection processes upon the domestication of traits (Doebley *et al*., 2006). Also important is the genetic characterisation of the *Capsicum* diversity in breeding programmes for effective crop improvement and to facilitate the collection, maintenance and utilisation of genetic resources in gene banks. Thus, preventing genetic loss, which would be later explored for the breeding of traits.

Genetic diversity analyses in *Capsicum* genus have utilised methods such as seed proteins, isoenzyme, RAPDs, RFLPs, AFLPs, SSRs, LTR-retrotransposon, gene-specific markers and Unigene Pepper GeneChip (Prince *et al*., 1992; Odeigah *et al*., 1999; Rodriguez *et al*., 1999; Hernández-Verdugo *et al*., 2001; Guzmán *et al*., 2005; Stágel *et al*., 2009; Tam *et al*., 2009; Aguilar-Meléndez *et al*., 2009; Pacheco-Olvera *et al*., 2012; Hill *et al*., 2013). Although, the random amplified polymorphic DNA (RAPD) marker has been used since the beginning of molecular studies for estimating genetic diversity in plant populations, it still remains very efficient and reliable in distinguishing cultivars. RAPD allows polymorphisms in the whole genome to be analysed. It can be used for classification of varieties, identification of cultivars and to understand the extent of genetic variation among cultivated lines of the *Capsicum* genus.

The *Capsicum* species constitute a rich source of genetic diversity. Molecular markers have been used to assess the extent of genetic diversity among the cultivars of crop species. A number of studies have been done on genetic diversity assessment among the cultivars of *Capsicum* via RAPD markers (Rodriguez *et al.*, 1999; Baral and Bosland, 2002).

(A) *C. annum* **(B)** *C. annum* **(C)** *C. chinense*
(D) *C. frutescens* **(E)** *C. frutescens* **(F)** *C. annum*

Figure 1: Types of *Capsicum* Cultivars used

In a previous study, the genetic diversity of *Capsicum* cultivars grown in Nigeria was analysed using the RAPD marker technique (Adetula, 2006). To our knowledge, the present study is another attempt to assess the level of genetic variations among the present cultivars grown in some states of Nigeria using molecular data (RAPD). A knowledge of the variability among cultivated varieties is useful for the development of efficient germplasm collections and management in gene bank conservation to prevent genetic erosion. Also, it will be of value for germplasm improvement.

Materials and Methods

Plant Materials

The plant materials for this study were composed of 24 morphologically distinct *Capsicum* lines. These represented 3 cultivated species: *Capsicum annuum*, *Capsicum chinense* and *Capsicum frutescens* (Figure 1). They were collected from 7 States (Delta, Ebonyi, Kano, Kaduna, Kastina, Plateau and Sokoto) where they are predominantly grown in Nigeria in the months of January to April, 2015 (Table 1). The seeds were germinated for 3 weeks in a greenhouse under standard conditions in the University of Lagos Botanical Garden, Akoka. Lagos.

Table 1: Cultivars used for the Diversity Assessment

	Code	Location[a]	Local Names	Scientific Names
1	CR01	Kano	Rodo	*C. annum*
2	CR02	Kano	Rodo	*C. annum*
3	CR03	Kano	Rodo	*C. annum*
4	CR04	Sokoto	Rodo	*C. annum*
5	CR05	Sokoto	Rodo	*C. annum*
6	CR06	Sokoto	Rodo	*C. annum*
7	CR07	Kaduna	Rodo	*C. annum*
8	CR08	Kaduna	Rodo	*C. annum*
9	CR09	Kaduna	Rodo	*C. annum*
10	CS10	Sokoto	Shombo	*C. frutescens*
11	CS11	Sokoto	Shombo	*C. frutescens*
12	CS12	Sokoto	Shombo	*C. frutescens*
13	CS13	Kaduna	Shombo	*C. frutescens*
14	CS14	Kaduna	Shombo	*C. frutescens*
15	CS15	Kaduna	Shombo	*C. frutescens*
16	CS16	Kano	Shombo	*C. frutescens*
17	CS17	Kano	Shombo	*C. frutescens*
18	CT18	Katsina	Tatase	*C. annum*
19	CA19	Ebonyi	Atawewe	*C. frutescens*
20	CA20	Kano	Atawewe	*C. frutescens*
21	CA21	Kano	Atawewe	*C. frutescens*
22	CG22	Kano	Green pepper	*C. annum*
23	CG23	Plateau	Green pepper	*C. annum*
24	CY24	Delta	Yellow pepper	*C. chinense*

[a] State in Nigeria where it is grown

DNA Extraction and Quantification

Leaf samples were pooled from 2–6 plants from each cultivar, put in an Eppendorf tube and then stored in a freezer at –80 °C. 200 mg of fresh leaves was ground in liquid nitrogen per cultivar. A modified procedure was used for the total genomic DNA extraction (Dellaporta *et al.*, 1993). The extracted genomic

DNA was quantified using Nanodrop (Thermo Scientific, Wilmington, DE, USA). The quality of DNA samples was checked on 1% agarose gel.

RAPD Analysis

As shown in Table 2, 18 RAPD primers were used for the DNA amplification of *Capsicum* species. Polymerase chain reaction (PCR) was carried out in 10 µL reaction volume containing 1 µL 10x buffer [10 mM Tris HCl (pH 8.0)] with 2.5 mM $MgCl_2$, 2.5 mM dNTP mixture, 1 unit of Taq polymerase, 1%, dimethyl sulphoxide (DMSO), 2 µL of 10 ng of DNA, 5 pMol of random primer; 10 ng of genomic DNA template and ultrapure water. The RAPD amplifications were performed in a 96-well PCR thermal cycler Applied Biosystems Veriti (AB, USA) with the following conditions: 94 °C for 3 min, 94 °C for 20 secs, 40 secs at 38 °C, 1 min at 72 °C; repeated 44 times, followed by a final extension period at 72 °C for 5 min. PCR products were resolved on 1.5% (w/v) agarose gels in 1x TBE and stained with ethidium bromide (EtBr). The bands were visualised with a UV transilluminator and photographed. Distinct and polymorphic bands were scored as present (1) or absent (0) using 100 bp DNA ladder.

Table 2: RAPD Marker Profiles used for Genotyping 24 *Capsicum* Cultivars Grown in Nigeria

	Primer Codes	Polymorphic Alleles	Allele Size (bp)
1	OPA-05	12	500–900
2	OPH-09	13	300–900
3	OPB-10	11	300–900
4	OPK-06	7	400–820
5	OPC-08	9	350–850
6	OPA-12	14	200–850
7	OPB-14	11	200–880
8	OPT-04	15	250–750
9	OPB-04	9	300–800
10	OPB-12	5	400–620
11	OPH-05	13	150–820
12	OPT-06	10	400–850
13	OPT-07	6	400–630
14	OPH-02	8	500–900
15	OPB-04	8	450–850
16	OPT-01	8	420–820
17	OPD-06	11	400–800
18	OPT-04	6	300–800
	Total	**176**	
	Mean	**9.78**	

Data Analysis

The number of alleles per RAPD marker, the total number of alleles in all polymorphic loci and average number of alleles were estimated. The RAPD data was used for the estimation of the genetic distance (GD) with the Jaccard coefficient (Sneath and Sokal, 1973). The resulting estimates of GD were used to generate an unweighted pair group method using arithmetic means (UPGMA) method and principal component analysis (PCA). Bootstrap resampling of 1000 replicates was performed to test the robustness of the topology. Computing was performed using the NTSYS-pc software version 2.01 (Rohlf, 1997).

Results

Genetic diversity among 24 cultivated *Capsicum* cultivars differing morphologically in fruit shape and colour was assessed using 18 RAPD markers generating a total of 176 polymorphic alleles, with a mean of 9.78 per marker in the lines of *C. annuum*, *C. chinense* and *C. frutescens* (Table 2). The RAPD allele numbers varied from 5–15 across the 24 lines. Figure 2 shows the alleles amplified using RAPD marker OPT-01 among the 24 *Capsicum* cultivars.

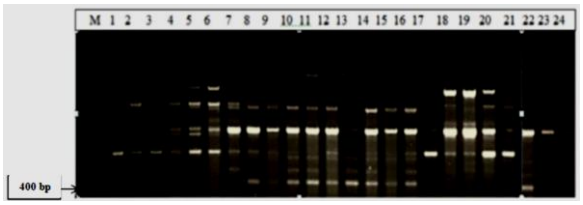


Figure 2: Agarose Gel Electrophoresis showing Alleles Amplified with RAPD Marker OPT-01

Within these lines, the RAPD allele size obtained using 18 markers varied from 150 bp to 900 bp. The RAPD markers used in this study produced unique alleles and were found to be present in a few lines in the 3 species (Table 2). 9 of the 18 RAPD markers produced unique alleles across 10 *Capsicum* lines whereas of the 176 polymorphic alleles among the 24 lines, 22 unique alleles were found in the *Capsicum* species (Table 3). The largest number (5) of unique alleles was identified in CS17, followed by CR02 and CG23 (with 3 each), and (2 each in) CR01, CR05, CS16 and CA21 lines. A unique allele was also identified among 3 lines (CR03, CR07 and CS14). The overall distribution was highest in *C. annum*.

Genetic distance matrix based on Jaccard coefficient ranged from 0.21–0.88 with an average of 0.61 at the RAPD loci. The genetic distance between line pairs in *C. annum* (rodo) ranged from 0.21–0.63. The lowest was between CR02 and CR04 while the highest was for CR01 and CR07. The genetic distance between line pairs in *C. frutescens* (shombo) ranged from 0.25–0.87. Lines CS10 and CS11 had the least genetic distance while the largest distance was between lines CS13 and CS17. The cluster

analysis results using 1574 alleles found within all lines were used for the construction of a dendrogram by the unweighted pair group method using arithmetic means (UPGMA) method and principal component analysis (PCA) (see Figures 3 and 4). The cluster analysis separated the 24 cultivated *Capsicum* species into their various genetic relationships.

Table 3: Unique Alleles-Produceing RAPD Primers

Primers	Number of Alleles	Cultivars
OPA-05	1	CS17
	3	CG23
OPH-09	1	CR05
	1	CS17
	1	CA21
OPB-10	1	CR01
OPC-08	1	CR01
	1	CR02
	1	CA21
OPA-12	1	CR05
	2	CS17
OPB-14	1	CR03
	1	CR07
OPT-04	1	CS14
	2	CS16
OPH-05	2	CR02
OPD-06	1	CS17
Total	**22**	

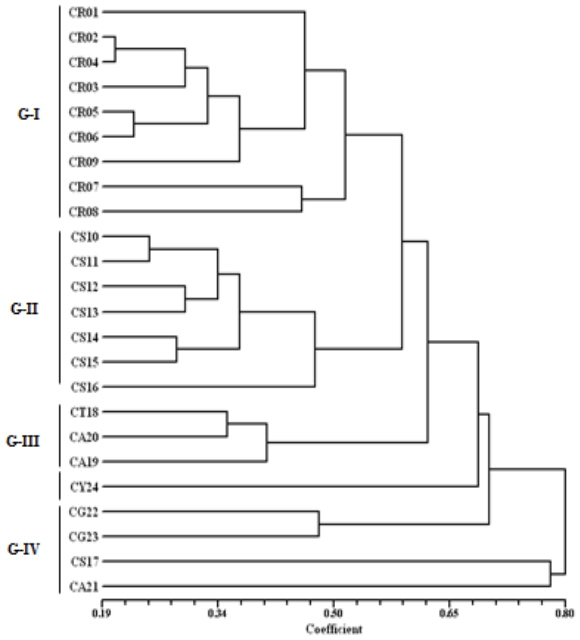
The dendrogram is divided into four main groups. The first group (*C. annum*) includes all the 9 CR lines whereas the second group (*C. frutescens*; shombo) includes 7 CS lines. The third group contains 1 CT (*C. annum*) and 2 CA lines (*C. frutescens*) but the fourth group is separated into 2 sub-groups; the closely related cultivars CG (CG22 and CG23) lines (*C. annum*) and CS17 and CA21 lines (*C. frutescens*). CY24 (*C. chinense*) stood alone in the dendrogram.

Within the CR, all the lines (rodo) from Kano, Sokoto and Kaduna States were closely related to each other than they were to the CG (green peppers) from Kano and Plateau States. CT18 (tatase) was grouped closely with CA20 (atawewe) and both of them were from Katsina State. 2 lines from Kano State (CS17 and CA21) were grouped together.
PCA using the RAPD alleles also grouped the lines and it was consistent with the UPGMA genetic similarity and grouping (Figure 4).

Discussion

The set of RAPD markers used for this genetic study in *Capsicum* species allowed for the detection of polymorphic alleles. The molecular study showed that a total of 176 polymorphic alleles were amplified using 18 RAPD markers. These results indicated that the genetic variations among the cultivated *Capsicum* species were high at the molecular level. This is consistent with other findings (Lanteri, 2003; Lee *et al.*, 2004; Thul *et al.*, 2012).

An average allele of 9.78 was obtained in *C. annuum*, *C. chinense* and *C. frutescens*. This revealed a high level of polymorphisms among the species. On the other hand, another study detected 1.6 alleles among 34 *C. annum* using 12 polymorphic RAPD primers (Paran *et al.*, 1998). Some alleles amplified by 9 RAPD markers were unique among 10 *Capsicum* cultivars in this study. This showed that the domesticated *Capsicum* species may possess traits of economic importance and thus can be reservoirs of genetic uniqueness for crop improvement. The same finding was observed in a previous study with other molecular markers (Gonzalez-Perez *et al*., 2014).



Genetic relationships were obtained from RAPD-based Jaccard coefficient genetic distances. The groupings of the cultivars are represented by G-I, G-II, G-III and G-IV.

In the present study, the RAPD data was capable of separating the 24 lines assessed. The *Capsicum* species formed distinct groups and this result suggested that the RAPD markers used in the present study were useful for estimating the genetic diversity of the *Capsicum* species. The study also supports the fact that RAPD analysis can be used as an efficient molecular marker for differentiating cultivars in genetic studies (Votava *et al*., 2002; Baral and Bosland, 2002).

Figure 3: **Dendrogram of 24 *Capsicum* Cultivars**

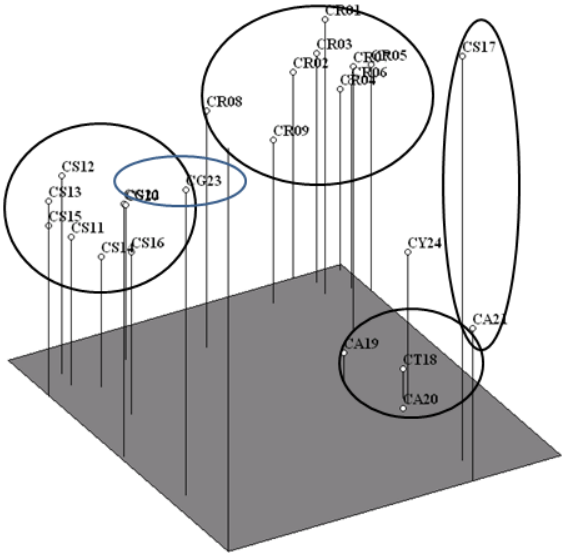


Figure 4: 3-D Plot of 24 *Capsicum* Cultivars (PCA)

The mean of the genetic distance between line pairs in *Capsicum annum* was 0.61. The genetic distance that exists in the present lines further indicated considerable genetic variability among the lines. In addition, the result revealed that the present *Capsicum* lines might be a representative of an originally genetically diverse germplasm since their domestication and selection or could have arisen as a result of cross pollination (Pickergill, 1997).

A previous study using 12 RAPD markers to assess the genetic diversity in 13 genotypes of chilli and paprika collected from different places in India found that the genetic distance was between 0.36 and 0.91 thus discovering high polymorphisms among species. This indicated that the nature of RAPD primers, sample size and sample strategy can determine RAPD data in any study (Tilahun *et al*., 2013). All these suggested that a wide genetic diversity is present among the *Capsicum* species. Two (2) *C. frutescens* cultivars that were morphologically different (CS17 and CA21) belonged to the same group. Similarly, CT18 grouped closely with CA20, showing that they might possess a common ancestry despite their morphological differences.

Genetic diversity in economic crops is limited due to domestication, cultivation and selection during breeding (Votava *et al*., 2002; Aguilar Mele'ndez *et al*., 2009). To prevent this, conservation of various Nigerian cultivars through germplasm gene banks would help preserve the genetic diversity. In northern Nigeria, where *Capsicum* species are mostly grown, measures to conserve and manage the cultivars should be implemented to preserve the genetic variation as well as establish genetic improvement programmes for new improved varieties like other countries. Consequently, it is suggested that the integration of crop conservation into policies and that the implementation of such policies should also be recognised.

Conclusion

These results defined the existence of genetic diversity in the *Capsicum* species grown in some states in Nigeria. These lines could be targeted for the collection of seeds for the gene bank storage of the *Capsicum* species. Thus, this will facilitate future decision in the conservation and management strategies of the genetic resources of *Capsicum*. Furthermore, other domesticated *Capsicum* species grown in other states of Nigeria should be investigated to have a better understanding of the genetic relationships among all the different species using molecular data generated from other PCR-based markers, such as Inter-Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR), and markers linked to resistance and pungency genes in *Capsicum* germplasm.

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