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Original articles

Effect of seed size on *in vitro* seed germination, seedling growth, embryogenic callus induction and plantlet regeneration from embryo of maize (*Zea mays* L.) seed

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Abstract

Immature embryo-derived callus is more efficient for plant regeneration in maize but appears difficult to obtain in all seasons of the year compared to mature embryos from dry seeds which are readily available throughout the year. This study investigated the effect of seed size on *in vitro* seed germination, seedling growth, callus induction and plantlet regeneration, as well as the relationships between these parameters in five maize varieties. Seeds were designated either as large or small for each variety based on its 100-seed weights, while seed germination were obtained in petri-dishes placed between two sheets of pre-wetted filter paper. Seeds were disinfected, and mature embryos were excised from the maize endosperm and inoculated on the Murashige and Skoog salt (MS medium) supplemented with 30 g/l sucrose, 8 g/l agar, 0.1 g/l myo-inositol and 3 mg/l 2,4-D for callus induction, while embryogenic calli were transferred to medium containing 0.5 mg/l Benzylaminopurine (BAP) and 0.5 mg/l Kinetin for plant regeneration. The study showed that large seed size had significant effect on almost all the traits studied, while positive and significant correlations were observed between *in vitro* germination, seedling growth, callus induction and plantlet regeneration. It can be concluded that callus fresh weight may be used as a marker for improving regeneration efficiency in maize. The results from this study suggest that genetic control of *in vitro* regeneration from maize mature embryo can be utilized to determine inherent genotypic potentials of maize varieties with tissue culture traits for maize improvement.

Keywords: Maize; Seed size; Callus induction; Germination; Plant regeneration; MS medium

1. Introduction

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice in terms of production in the world [9]. It is a major cereal crop for livestock feed, human nutrition and important raw material for several agro-based industries in Nigeria [1]. But under the pressures exerted by limited land, expanding population, plant diseases and insect pest stresses, traditional breeding methods alone have not incorporated the great demand for maize of both quality and quantity. Consequently, several biotechnology approaches have received more emphasis. Among such are particle bombardment [14] and Agrobacterium-mediated [23]. However, success or failure of maize genetic transformation largely depends on the ability of transformed tissues to proliferate and subsequently to regenerate into whole plants.

Immature embryo-derived calli are more efficient for plant regeneration but its production is a time-dependent procedure and difficult to obtain all seasons of the year, while mature embryos from dry seeds are available any time throughout the year. As explants, mature embryos have been used to induce callus and regenerate plants [8]. It has been established that large seeds had higher germination rate, seedling emergence success and more rapid growth than small seeds [21]. The

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higher seedling emergence and growth of large seeds were attributed to large storage reserves in their endosperm or cotyledons and also to their biochemical compositions [13].

Seed size has a special role in crop production. There have been immense studies on seed size in various plant species. Seed size is one of the most important characteristics of seeds that can affect the seed development [19]. Larger seed size indicates a higher protein synthesizing ability, which is probably attributed to more available substrate and energy (ATP), active enzymes and machinery for protein synthesis [6]. Therefore, in endosperm-supported mature embryo culture, seed size, which is proportionately reflected in the endosperm size, may also affect callus induction and plant regeneration [17].

Seed size has also been reported to have effect on tissue culture response of callus from endosperm-supported mature embryos in barley [17], wheat [20] and rye [24] while little information is available on the effect of seed size on *in vitro* seed germination, seedlings growth, embryogenic callus induction and plantlets regeneration from mature embryos in maize. This study therefore sought to determine the effect of seed size on *in vitro* seed germination, seedling growth, callus formation, plantlet regeneration as well as the relationship among these parameters.

2. Materials and method

The experiment was conducted at Biotechnology laboratory of Institute of Agricultural Research and Training (I.A.R&T), Ibadan, Oyo State in 2015. Physiologically matured and well dried cobs of five maize varieties (Table 1) were obtained from seed production field of I.A.R&T., Ibadan. The cobs were shelled and seeds were designated as large or small according to seed weight (g). Seeds weighing less than 25 g were grouped as small seed, while seed weighing above 25 g were classified as large seed (Fig. 1). Most large seeds were shelled from the bottom of the cobs while small seeds were shelled from top of the cobs. One hundred small and large seeds of each variety in four replicates were weighed to determine the 100 seed weight. Mean of the four replicates were recorded (Table 1). Ten seeds were randomly selected from each category to determine seed morphometric parameters through the use of digital Vernier calliper (Table 2).

2.1. In vitro seed germination

The seeds were washed with Tween20 (detergent) under running tap water. They were then disinfected in 70% methylated spirit for 5 min and rinsed in three changes of sterile

Table 1
Grouping of maize seeds based on their 100-seed weight (g).

Maize varieties	Small seed size	Large seed size
DMR-LSR-Y	23.93	27.77
BR9943DMR	24.09	29.75
ART/98/SW6-OB	24.19	28.86
SUWAN-1-SR-Y	23.69	29.69
DMR-ESR-Y	22.29	28.36

Fig. 1. Seed sizes of five maize varieties; **A**: Small seed size; **B**: Large seed size; **1**: DMR-LSR-Y; **2**: BR9943DMR; **3**: ART/98/SW6-OB; **4**: SUWAN-1-SR-Y; **5**: DMR-ESR-Y.

Table 2
Morphometric parameters of maize seed.

Genotypes	Whole seed (Cotyledon + Embryo + Endosperm)					
	Large seed size			Small seed size		
	Seed length(mm)	Seed width(mm)	Seed thickness(mm)	Seed length(mm)	Seed width(mm)	Seed thickness(mm)
DMR-LSR-Y	10.11 ± 0.06	8.71 ± 0.04	4.06 ± 0.02	8.71 ± 0.06	7.51 ± 0.04	3.98 ± 0.02
BR9943DMR	10.91 ± 0.06	8.43 ± 0.04	4.00 ± 0.02	9.17 ± 0.06	7.49 ± 0.04	4.23 ± 0.02
ART/98/SW6-OB	9.83 ± 0.04	8.69 ± 0.04	4.20 ± 0.02	9.21 ± 0.06	7.41 ± 0.04	4.74 ± 0.02
SUWAN-1-SR-Y	10.11 ± 0.06	8.87 ± 0.04	4.27 ± 0.02	8.78 ± 0.06	8.17 ± 0.04	4.39 ± 0.02
DMR-ESR-Y	9.39 ± 0.06	8.58 ± 0.04	4.92 ± 0.02	8.29 ± 0.06	7.50 ± 0.04	4.03 ± 0.02

Embryo fraction of the seed

Genotypes	Large seed size		Small seed size	
	length(mm)	weight(mg)	length(mm)	weight(mg)
DMR-LSR-Y	6.00 ± 0.00	7.78 ± 0.16	5.67 ± 0.00	5.50 ± 0.16
BR9943DMR	6.60 ± 0.00	8.74 ± 0.16	5.50 ± 0.00	6.54 ± 0.16
ART/98/SW6-OB	6.50 ± 0.00	8.00 ± 0.12	6.30 ± 0.00	7.42 ± 0.16
SUWAN-1-SR-Y	6.50 ± 0.00	8.76 ± 0.16	6.00 ± 0.00	6.14 ± 0.16
DMR-ESR-Y	6.00 ± 0.00	6.68 ± 0.16	6.00 ± 0.00	6.65 ± 0.16

Values are means of the ten seeds/embryos per seed size from each variety (± values are standard error).

distilled water to reduce microbial load at early stages of germination. Twenty (20) disinfected seeds from each seed size of each maize variety were germinated between two sheets of filter papers (Whatman 1) laid in 100 × 10 mm Petri dishes moistened with 10 ml of distilled water. The filter papers were regularly moistened to ensure water saturation throughout the seedling germination period. The petri dishes were laid out in a completely randomized design (CRD) with five replicates per seed size. Data were taken on the followings:

- Percentage germination (Germ %) evaluated as

$$\frac{\text{No of germinated seed}}{\text{Total number of seeds cultured}} \times 100$$

- The shootlength (SL) and root lengths (RL) (cm) were measured on seedlings after 10 days of germination with the use of transparent meter rule
- Seedling fresh weight (SFW) measured on a sensitive electronic weighing balance immediately after removal from test tubes and wiped dry with paper towel.
- Dry seedling weight (SDW) was calculated after oven-drying of the seedlings at 70 °C and the average seedling dry weight was then calculated [3,12].

2.2. Callus induction

Seeds were washed with Tween20 under running tap water. They were then disinfected in 70% methylated spirit, 0.1% and 0.2% mercuric chloride respectively and rinsed in three changes of sterile distilled water. Disinfected seeds were soaked in sterile distilled water overnight to soften the seed coat. Callus was induced using [7] protocol with modifications. Mature embryos were excised from seeds aseptically and cultured on Murashige and Skoog basal medium (1962) supplemented with 30 g/l sucrose, 3 mg/l 2,4-D and 8 g/l agar. The pH of culture medium was adjusted to 5.8. Culture was incubated at 25 ± 2 °c in complete darkness for 14 days. Two embryos were inoculated per plate and experiment was laid out in a completely randomised design with twenty (20) replicates. Data were collected on callus length and width (i.e. the longest distance between one end of the callus and the other end in the petri plate was taken as the length, while the shortest distance was taken as the width in millimetre) [25], Percentage of callus formation [(number of calli formed from explant/total number of explants cultured) × 100], percentage of shoot formation [(number of shoot formed from explant/total number of explants cultured) × 100], and percentage root formation[(number of root formed from explant/total number of explants cultured) × 100].

2.3. Plantlet regeneration

After two weeks of culture, embryogenic calli were transferred to regenerating medium containing MS medium [15] supplemented with 0.5 mg/l BAP and in combination with 0.5 mg/l kinetin [7]. Regenerated plantlets were then transferred to rooting medium containing half strength MS basal medium supplemented with 1 mg/l IBA [7] with modification. Percentage plants regeneration was determined as

$$\frac{\text{number of plantlets regenerated}}{\text{number of calli formed}} \times 100$$

2.4. Data analysis

Analysis of variance (ANOVA) was performed on data collected using IBM SPSS version 21.0 software. Difference between means was separated by the Duncan Multiple Range test (DMRT) at 5% and 1% levels of significance. Relationships between *in vitro* seed germination and *in vitro* callus

induction parameters were determined by Pearson correlation analysis using Statistical Tool for Agricultural Research (STAR, version: 2.0.1).

3. Results and discussion

3.1. In vitro germination and seedling growth

There were no significant differences between the percentage seed germination and root length of the varieties (Table 3) while the SL, SFW, SDW and Seedling length of the varieties were significantly different from each other (Table 3). However, there were significant differences observed in shoot length, seedling fresh weight, seedling dry weight and seedling length among the varieties (Table 3). Seed size had no significant effect on percentage seed germination, SL, RL and seedling length, but it has significant effect on seedling fresh weight (SFW) and seedling dry weight (SDW). There was no significant difference in the interaction between seed size and variety for all parameters measured in this study. This result is in agreement with the work of [11] that seed size had no effect on seed germination of soybean and the interaction between seed size and cultivar.

Although there were no significant difference between mean of germination %, shoot length and root length of large and small seed. Large seed size had the highest mean value for all the parameters in this experiment while the mean value recorded for seedling fresh weight (652.48), seedling dry weight (58.32) and seedling length (29.44) were significantly higher than that of the small seed (Table 4).

This result is in concurrence with the findings of [12] who reported that seedling growth of large seed size were higher than small seeds in Triticale. The difference between fresh and dry weights is equal to the water content of the tissues as water is important for photosynthesis because it is the source of hydrogen for the sugars formation through photosynthesis. Water content in large seed size was higher than small seed size in this study. This agrees with the findings of [17] who reported that higher water content was observed in large seed size of Barley genotype [18] reported that there was a close correlation between seed size and seed nutritional resources; large seeds produced larger seedling compared with small seeds and it can cause an increase in the crop production in the field. The differences between seedlings grown from different seed sizes in the early stage could be a good indicator of the success of plants in later phases of their life cycle [17]. Refs. [17,22] reported that the large food reserves in seeds could allow for better photosynthetic activity, which could contribute to better growth and seedling survival.

3.2. In vitro callus induction and plantlet regeneration

Callus (type II) has good genetic potential to de-differentiate and re-differentiate to form a whole plantlet after passing through series of physiological and biochemical changes (Fig. 2). Calli were initiated two days after

Table 3
Mean square values of *in vitro* seed germination and growth of different maize varieties as affected by seed size.

SV	Df	Germ (%)	SL(cm)	RL(cm)	SFW(mg)	SDW(mg)	Sln(cm)
Variety (V).	4	203.67 ns	48.00**	3.58 ns	59,671.71**	629.85**	178.22**
Seed size (SS)	1	66.26 ns	2.31 ns	3.02 ns	4,67,782.90**	2505.93**	37.82 ns
V × SS	4	133.21 ns	1.39 ns	4.00 ns	22,542.24 ns	80.19 ns	11.79 ns
Error	33	5090.97	56.90	218.68	4,74,533.45	4625.80	701.49

*, ** Significant at $p < 0.05$ and $p < 0.01$ respectively.
df: degree of freedom, SV: source of variation Var.: Variety, MSE: error mean squares, Germ(%): percentage germination, SL(cm): shoot length, RL(cm): root length, SFW(mg): seedling fresh weight, SDW(mg): seedling dry weight, Sln(cm): seedling length(cm).

Table 4
The effect of seed size on *in vitro* seed germination and seedling growth in five maize genotype.

Parameters	Seed size	Varieties					
		DMR-LSR-Y	BR9943DMR	ART/98/SW6-OB	SUWAN-1-SR-Y	DMR -ESR-Y	Mean
Germination (%)	LSS	95.00 ± 5.56	90.00 ± 6.21	94.40 ± 5.56	82.50 ± 6.21	85.75 ± 6.21	89.53a
	SSS	92.25 ± 6.21	94.80 ± 5.56	81.50 ± 6.21	88.33 ± 7.17	78.20 ± 5.56	87.02a
Shoot length(cm)	LSS	4.77 ± 0.59	9.22 ± 0.66	11.40 ± 0.59	10.42 ± 0.66	10.82 ± 0.66	9.33a
	SSS	5.37 ± 0.66	9.05 ± 0.59	10.51 ± 0.66	10.04 ± 0.76	9.31 ± 0.59	8.86a
Root length(cm)	LSS	8.89 ± 1.15	10.65 ± 1.29	11.95 ± 1.15	9.82 ± 1.29	11.85 ± 1.29	10.63a
	SSS	10.25 ± 1.29	10.20 ± 1.15	10.07 ± 1.29	9.88 ± 1.49	10.07 ± 1.15	10.09a
Seedling fresh weight(mg)	LSS	538.38 ± 53.63	567.10 ± 59.96	781.76 ± 53.63	643.68 ± 59.96	741.48 ± 59.96	652.48a
	SSS	419.85 ± 59.96	460.72 ± 53.63	493.60 ± 59.96	301.00 ± 69.23	531.24 ± 53.63	441.30b
Seedling dry weight(mg)	LSS	47.34 ± 5.30	63.90 ± 5.92	67.70 ± 5.30	53.93 ± 5.92	58.73 ± 5.92	58.32a
	SSS	33.28 ± 5.92	45.60 ± 5.30	51.70 ± 5.92	30.93 ± 6.84	52.80 ± 5.30	42.86b
Seedling length(cm)	LSS	20.08 ± 2.06	29.50 ± 2.31	34.14 ± 2.06	30.05 ± 2.31	33.39 ± 2.31	29.44a
	SSS	22.01 ± 2.31	27.90 ± 2.06	30.15 ± 2.31	27.34 ± 2.66	30.30 ± 2.06	27.54a

LSS: Large seed size, SSS: Small seed size. Means with the same letter(s) in the same column are not significantly different from each other at 5% level of probability (±values are standard error).

inoculation on MS medium in complete darkness (Fig. 2B). This could be attributed to the presence of meristematic cells in the scutellum. Ref. [2] has reported the presence of the meristematic cells in the scutellum of maize embryos from which callus was induced. Mean squares of the varieties were significantly different for callus formation, shoot formation, root formation and plant regeneration (Table 5). Seed sizes did not significantly affect callus length and callus width while callus formation, callus fresh weight, shoot formation, root formation and plant regeneration were significantly affected by seed size. Variety by seed size interaction was significant for callus formation, shoot formation, root formation and plant regeneration (Table 5). Ref. [17] reported statistically significant differences in weight of callus, number of shoots regenerated and number of plants regenerated in seed sizes of wheat genotypes. Genotypic differences observed might due to effect of endogenous hormone.

Highest callus fresh weight (366 mg) and plantlet regeneration (76%) were observed in the large seed of SUWAN-1-SR-Y while highest callus formation (97%) and root formation (20%) were observed from large seed of BR9943DMR. Similarly, highest shoot formation (78%) was obtained from large seed of ART/98/SW6-OB while the lowest values in all parameters were obtained from small seed size (Table 6). Result obtained from this study, showed that callus induction and plantlet regenerating ability is majorly dependent on seed size. Ref. [10] reported that in embryogenesis, related genes are involved during somatic embryogenesis in some plants; this could be responsible for the varietal differences observed in the response of these maize varieties to callus induction in this study. Ref. [4] reported that factors influencing the expression of totipotency in cell culture are genotype, composition of plant culture medium, growth regulators, and embryo size. Ref. [20] stated that callus weight might be used as an indicator for regeneration capacity in wheat.

3.3. Correlation between in vitro seedling germination and in vitro callus induction

Significant and positive correlation was detected among some of the *in vitro* seed germination parameters; Shoot length was positively and significantly correlated with seedling

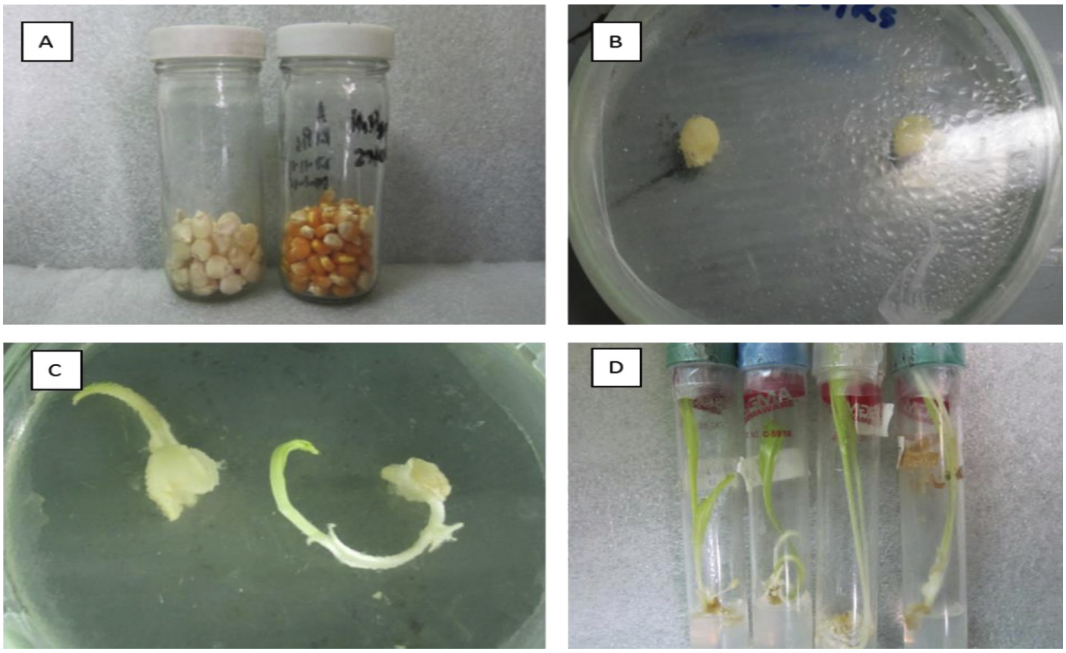

Fig. 2. Somatic embryogenesis from mature embryos of 'SUWAN-1-SR-Y' maize variety, (A) Fresh maize seeds, (B) Callus initiation after 2 days of culture, (C) Shoot formation from callus (type 11) within two weeks of culture, (D) Plantlet regeneration from large-seed-derived callus cultures.

Table 5
Mean square values of *in vitro* callus induction of different maize genotypes, seed size and their interaction.

SOV	CF(%)	CFW(mg)	CL(mm)	CW(mm)	SF(%)	RF(%)	Regr(%)
Variety(V)	547.45**	5739.12 ns	5.71 ns	2.73 ns	979.94**	139.67**	851.88**
Seed size(SS)	803.31**	96109.45**	0.04 ns	8.81 ns	1888.37**	231.17**	2184.53*
V × SS	1716.61**	5315.81 ns	7.62 ns	4.25 ns	809.08**	168.69**	1512.78**
Error	0.50	6246.46	4.25	5.21	15.78	34.38	0.13

*, ** Significant at $p < 0.05$ and $p < 0.01$ respectively.
SOV: source of variation, CF: callus formation, CFW: callus fresh weight, CL: callus length, CW: callus width, SF: shoot formation, RF: root formation, Regr: plantlet regeneration.

Table 6
The effect of seed size on *in vitro* callus induction parameters and plantlet regeneration in five maize genotypes.

		Varieties					
Parameters	Seed size	DMR-LSR-Y	BR9943DMR	ART/98/SW6-OB	SUWAN-1-SR	DMR -ESR-Y	Mean
Callus formation (%)	LSS	93.00 ± 0.50	97.00 ± 0.50	95.00 ± 0.41	92.00 ± 0.41	92.00 ± 0.41	93.80a
	SSS	89.00 ± 0.41	90.00 ± 0.41	91.00 ± 0.41	91.00 ± 0.41	49.00 ± 0.41	82.00b
Callus fresh weight(mg)	LSS	273.63 ± 21.92	329.91 ± 22.82	317.77 ± 20.41	366.55 ± 55.89	301.98 ± 24.99	317.97a
	SSS	232.55 ± 32.27	289.50 ± 45.63	218.51 ± 23.83	216.13 ± 20.41	230.78 ± 35.35	237.49b
Callus length(mm)	LSS	11.11 ± 0.57	10.42 ± 0.60	11.63 ± 0.53	11.25 ± 1.46	10.10 ± 0.65	10.90a
	SSS	12.08 ± 0.84	12.33 ± 1.19	10.05 ± 0.62	10.00 ± 0.53	9.80 ± 0.92	10.85a
Callus width(mm)	LSS	9.00 ± 0.63	8.55 ± 0.66	7.87 ± 0.59	9.25 ± 1.61	7.60 ± 0.72	8.45a
	SSS	7.67 ± 0.93	7.50 ± 1.32	8.68 ± 0.69	7.57 ± 0.59	7.00 ± 1.02	7.68a
Shoot formation (%)	LSS	65.00 ± 1.10	67.00 ± 1.15	78.00 ± 1.03	74.00 ± 2.81	74.00 ± 1.26	71.60a
	SSS	60.00 ± 1.62	61.00 ± 2.29	67.00 ± 1.20	77.00 ± 1.03	36.00 ± 1.78	60.32b
Root formation (%)	LSS	14.00 ± 1.63	20.00 ± 1.69	19.00 ± 1.51	6.00 ± 4.15	9.00 ± 1.85	13.60a
	SSS	13.00 ± 2.39	6.00 ± 3.39	10.00 ± 1.77	14.27 ± 1.51	5.00 ± 2.62	9.65b
Plant regeneration (%)	LSS	27.00 ± 0.11	24.00 ± 0.11	20.00 ± 0.11	76.00 ± 0.13	21.00 ± 0.11	33.60a
	SSS	29.00 ± 0.11	37.00 ± 0.11	6.00 ± 0.10	6.00 ± 0.10	6.00 ± 0.10	16.80b

LSS: Large seed size, SSS: Small seed size.
Means with the same letter(s) in the same column are not significantly different from each other at 5% level of probability (±values are in standard error).

length (0.96). Root length was significantly and positively correlated with seedling fresh weight (0.95), seedling dry weight (0.97), and seedling length (0.88). Significant and positive relationship was also obtained between seedling fresh weight and seedling dry weight (0.86) (Table 7). Positive relationship was also detected among the *in vitro* callus induction parameters and plantlet regeneration; plantlet regeneration was positively correlated with shoot formation (0.48), callus formation (0.51) and callus fresh weight (0.52). Callus formation was significantly and positively correlated with root formation (0.86) (Table 7). Correlation between *in vitro* germination and *in vitro* callus induction parameters showed that there was positive relationship between percentage seed germination and callus induction parameters. Also, positive association was detected between callus fresh weight and most of *in vitro* seed germination parameters (Table 7). This result corroborates the work of [17] who reported that there was positive relationship between seed size and *in vitro* germination parameters, callus induction and also plant regeneration parameters in mature embryos of Barley. Positive and significant relationship between *in vitro* seed germination and *in vitro* callus induction parameters suggests that these traits may either be controlled by the same or similar genes or may be controlled by closely linked genes [5].

4. Conclusion

The study showed that large seeds had significant effect on almost all the traits and positive correlations were observed between *in vitro* germination, seedling growth, callus

Table 7
Pearson coefficient of correlation between different characters in seed germination, seedling growth, callus induction and plantlet regeneration from mature embryo of five maize genotypes.

	CF	CFW	Reg	CL	CW	SF	RF	Germ	SL	RL	SFW	SDW	SDL
CF	–	0.34	0.51	0.79	0.92*	0.71	0.86*	0.73	−0.17	−0.43	−0.54	−0.24	−0.35
CFW		–	0.52	0.10	0.08	0.28	0.01	0.08	0.41	0.03	−0.29	0.17	0.30
Reg			–	0.39	0.57	0.48	0.05	0.30	−0.24	−0.81	−0.92*	−0.74	−0.47
CL				–	0.67	0.17	0.83	0.99**	−0.68	−0.59	−0.63	−0.37	−0.74
CW					–	0.82	0.74	0.58	−0.25	−0.61	−0.61	−0.50	−0.46
SF						–	0.43	0.06	0.33	−0.24	−0.31	−0.21	0.08
RF							–	0.82	−0.29	−0.19	−0.22	0.01	−0.35
Germ								–	−0.69	−0.52	−0.56	−0.30	−0.71
SL									–	0.73	0.59	0.67	0.96**
RL										–	0.95*	0.97**	0.88*
SFW											–	0.86*	0.77
SDW												–	0.82
SDL													–

*, ** Significant at $p < 0.05$ and $p < 0.01$ respectively.
CF: callus formation(%), CFW: callus fresh weight(mg), CL: callus length(mm), CW: callus width(mm), SF: shoot formation(%), RF: root formation(%), Reg: plantlet regeneration(%), Germ: seed germination(%), SL: shoot length(cm), SFW: seedling fresh weight(mg), SDW: seedling dry weight(mg), SDL: seedling length(cm).

induction and plantlet regeneration. These results therefore, suggest that these traits may either be controlled by the same or similar genes or may be controlled by closely linked genes. It can be concluded that callus fresh weight can be used as a marker for improving regeneration efficiency in maize. Findings from this study are good information that can assist plant breeders to improve maize using biotechnology for efficient and rapid incorporation of relevant seed traits on desirable maize genotypes.

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References

[1] S.R. Akande, G.O. Lamidi, Afr. J. Biotech. 5 (19) (2006) 1744−1748.
[2] D. Al-Abed, S. Redrabhatla, R. Talla, S. Goldman, Planta 223 (2006) 1355−1360.
[3] A. Bibi, H.A. Sadaqat, M.H.N. Tahir, H.M. Akram, J. Animal Plant Sci. 22 (3) (2012) 671−678.
[4] N.E. Bohorova, B. Luna, R.M. Briton, L.D. Huerta, D.A. Hoisington, Maydica 40 (1995) 275−281.
[5] J. Brown, P. Caligari, An Introduction to Plant Breeding, Blackwell Publishing Ltd, Oxford, UK, 2008.
[6] T.M. Ching, L. Rynd, Plant Physiol. 62 (1978) 866−870.
[7] M. Guruprasad, S.K. Jaffar, P. Rajakumar, E. Muralinath, D. Sandeepraja, Int. J. Res. Rev. Pharm. Appl. Sci. 1 (3) (2011) 192−196.
[8] X.Q. Huang, Z.M. Wei, Plant Cell. Rep. 22 (2004) 793−800.
[9] IITA (International Institute of Tropical Agriculture), Cereals and Legumes Systems (21st September, 2009) 1. http://old.iita.org/cms/details/maize_project_details.aspx?zoneid=63&articleid=273.
[10] M. Ikeda, M. Umehara, H. Kamanda, Plant Biotechnol. 23 (2006) 153−161.
[11] D.R. Johnson, V.D. Luedders, Agron. J. 66 (1974) 117−118.
[12] D. Kaydan, M. Yagmur, Afr. J. Biotechnol. 7 (2008) 2862−2868.
[13] E. Khurana, U.S. Singh, Environ. Conserv. 28 (2001) 39−52.
[14] T.M. Klein, L. Kornstein, J.C. Sanford, M.E. Fromm, Plant Physiol. 91 (1989) 440−444.
[15] T. Murashige, F.A. Skoog, Physiol. Plant 15 (1962) 473−497.
[17] M. Ozgen, M. Yildiz, N. Koyuncu, S. Onde, Cereal Res. Commun. 35 (3) (2007) 1415−1425.
[18] D.A. Perry, Adv. Res. Technol. Seeds 5 (1980) 25−40.
[19] R. Rezapour, H. Kazemi-arbat, H. Yarnia, P. Zafarani-Moattar, Int. Res. J. Appl. Basic Sci. 4 (11) (2013) 3396−3401.
[20] M.T. Sayar, M.A. Bitsin, H. Ulukan, M. Ozgen, Wheat Inf. Serv. 89 (1999) 1−6.
[21] K. Seiwa, K. Kikuzawa, Can. J. Bot. 69 (1991) 532−538.
[22] R.S. Tripathi, M.L. Khan, Oikos 57 (1990) 289−296.
[23] A. Valdez-ortiz, S. Medina-godoy, M. EvCerde, O. Paredes- lópez, Plant Cell. Tissue Organ Cult. 91 (2007) 201−214.
[24] K.A. Ward, M.C. Jordan, Invit. Cell. Dev. Biol. Plant 37 (2001) 361−368.
[25] S.R. Akande, M.O. Balogun, B.A. Ogunbodede, Effects of plant growth regulators and explant types on Callus Formation in African Yam Bean (Sphenostylis stenocarpa (Hochst. Ex A. Rich) Harms). Kasetsart J. (Nat. Sci.) 43 (2009) 442−448.