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In vitro Regeneration and Proliferation of Maize (*Zea mays* L.) Genotypes through Direct Organogenesis

Odunayo Joseph, Olawuyi[1], Olugbenga, Dalamu[1], Olumayowa Mary, Olowe[2*]
1. Department of Botany, University of Ibadan, Nigeria
2. Department of Crop Sciences, College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda, Rwanda.

Abstract

The variability of *in vitro* regeneration and proliferation of maize genotypes POOLISSRQPMX, DTSR-WCO, TZLCOMP4C3, TZE COMP 3C2, EV99QPM, POP66 SR/ACR94-YQPM, DTSR-WC, SAMMAZ 19S-14DT and TZEE-YPOPSTRC4 was investigated. Explants were regenerated through direct organogenesis using 0.1 mg/l NAA + 2.0 mg/l BAP, 0.1 mg/l NAA + 2.0 mg/l KIN, 0.3 mg/l NAA + 3.0 mg/l BAP, 0.3 mg/l NAA + 3.0 mg/l KIN, 0.5 mg/l NAA + 4.0 mg/l BAP, 0.5 mg/l NAA + 4.0 mg/l KIN and MS medium only. The regenerated maize yielded the highest number of multiple shoots (1.70 cm) and shoot length (3.35 cm) on MS medium supplemented with 0.3 mg/l NAA + 3.0 mg/l BAP within 31 days. Genotype EV99QPM and DTSR-WCO had the highest number of shoots, rooting traits respectively.

Keywords: Maize, regeneration, hormones, variability, survival, character

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1. Introduction

Maize (*Zea mays* L.) is an important monocotyledonous plant cultivated over a wide range of diverse climates as feed crop, fodder and industrial products for global food security and poverty alleviation (Olakojo et al., 2001; Bello and Olaiya, 2009; olawuyi et al., 2010; Gorji et al., 2011; Olawuyi et al., 2011; Olawuyi et al., 2013; Olawuyi et al., 2014). Genetic variation depends on plant species, genotype, type of explant, culture media and conditions (Shuangxia et al., 2008). The tissue culture and transformation are important components for successful generation of transgenic crops conferring resistance or tolerance against biotic and abiotic stresses. Micropropagation using meristem and shoot culture produce large numbers of identical individuals (Harding, 2010; Olowe et al., 2014; Feyisola et al. 2015). Rapid clonal propagation of crops is accomplished through multiplication of auxillary shoots, adventitious shoot production or somatic embryogenesis (Bouquet and Terregrosa, 2003). A combination of cytokinin at a lower concentration with auxins especially 2,4-D had been reported to induce embryogenic calli from maize and other cereal crops (Chang et al. 2003; Al-Abed et al. 2006). Maize regeneration from immature embryos (Green and Phillips, 1975; Aguado-Santacruz et al., 2007:Ombori et al., 2008), mature embryos (Huang and Wei, 2004; Al-Abed et al., 2006), nodal regions (Vladmir et al., 2006), anthers (Barloy and Beckert, 1993), and shoot meristems (Sairam, 2003) had been reported. The regeneration method using immature embryo in maize is laborious and time consuming compared with direct regeneration from explants which is faster and safe time in obtaining the whole plant without callus interphase that can cause somaclonal variation (Zapata et al., 1999). Plant regeneration through somatic embryogenesis was achieved using leaves as explants (Steinmacher et al., 2007). Akula et al. (1999) reported the use of mature embryo of maize to induce callus, but plantlets were not regenerated. Huang and Wei (2004) also observed regeneration of temperate maize lines from mature embryos. Direct shoot induction and regeneration had also been reported for some cereals including Sorghum (Baskaran and Jayabalan, 2005), barley (Sharma et al., 2004), wheat (Sharma et al., 2005) and maize (Zhang et al., 2002). There is need to develop a reliable, simple and time saving *in vitro* approach using direct organogenesis for regenerating multiple transgenic plants of both clonal propagation and successful genetic engineering of plants which may lead to the development of new resistant varieties against abiotic and biotic stresses. Therefore, this study investigated direct organogenesis method in *in vitro* regeneration and multiple shoot proliferation of maize genotypes using auxin and cytokinin concentrations.

2. Materials and Methods

2.1 Experimental Location and Sources of Plant Materials

The experiment was carried out in the Tissue Culture Laboratory of National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor plantation, Ibadan, Nigeria. Ten (10) genotypes of maize seeds evaluated in this study were collected from the seed germplasm unit of (NACGRAB), Moor plantation, Ibadan (Table 1).

2.2 Sterilization of Zea may L. Explants

Equal amount of matured seeds were initially surface sterilized with 70% ethanol for 5 minutes, and rinsed with distilled water. The seeds were then treated with 4% w/v sodium hypochlorite (Chlorox) with the addition of few drops of tween 20 for 20 minutes and rinsed with sterile distilled water 6 to 8 times. The seeds were transferred into 250ml conical flasks containing 150 ml of sterile distilled water and kept for 24 hours in the refrigerator at -4°C. To remove the surfactants, sterilized seeds were rinsed five (5) times in 200 ml of sterile de-ionised distilled water and blotted onto a sterile Whatman No1 filter paper. Matured embryos were excised from seeds with a scalpel and cultured on hormone free MS media (Murashige and Skoog, 1962). All the steps were performed under the laminar flow according to the procedure described by (Krishna el., 2013).

2.3 Experimental Design, Regeneration and Proliferation Medium

The germinated embryos were proliferated in five replications each on MS basal medium supplemented with 0.1 mg/l NAA + 2.0 mg/l BAP, 0.1 mg/l NAA + 2.0 mg/l KIN, 0.3 mg/l NAA + 3.0 mg/l BAP, 0.3 mg/l NAA + 3.0 mg/l KIN, 0.5 mg/l NAA + 4.0 mg/l BAP, 0.5 mg/l NAA + 4.0 mg/l KIN and MS medium only. Plantlets in culture tubes were arranged in a completely randomized design (CRD) on the culture shelves in the growth room with alternating light of 16 hours (18 ± 2 °C with 50 µmol m^{-2} s^{-1}) and dark cycle of 8 hours for regeneration of new shoots and induction of roots directly from the mature embryos without callus interphase stage.

2.4 Acclimatization

The regenerated plantlets were transferred to small pots containing a mixture of vermiculite, sand and peat moss in 1:1:1 ratio (compost mixture). Each pot was covered with a polythene bag to maintain high humidity initially for few days. Subsequently, the humidity was reduced by making holes in the polythene bags to harden the plants. After 7 days, the plantlets were transferred to the screen house (Figure 2).

2.5 Data Collection and Statistical Analysis

Data collection on growth parameters of the genotypes commenced a day after the embryo culture were; number of germinated embryos, number of leaves, number of shoots, shoots length, number of roots, root length and number of survived plantlets. The data obtained were subjected to analysis of variance (ANOVA) and principal component analysis (PCA) using SAS generalised linear model (GLM) software, while means with significant differences were separated by Duncan Multiple Range Test (DMRT) (P=0.05). Also, the relationships among the quantitative and qualitative traits were established using Pearson correlation coefficient.

3. Results and Discussion

3.1 Percentage Germination and Genotypic Variation of Hormone Concentrations on Survival Rate of Maize Genotype

After 2-4 days, the excised matured embryo of maize seeds of the ten genotypes produced multiple shoots in MS media with hormones compared with MS media without hormones (control). The genotypes responded differently in the media in which DTSR-WCO, SAMMAZ 19S-14DT, TZE COMP 3C2, POP66 SR/ACR94-YQPM and DTSR-WC showed the highest germination percentage of 100%, but not significantly different ($P<0.05$) from other genotypes. This is followed by genotypes EV99 QPM and POOL ISSR QPMX which are statistically similar, but different from other genotypes. TZEE-YPOP STR C4 had higher germination percentage of 94.29% than TZL COMP4C3 (85.71%), while the least germination percentage of 37.14% was recorded for NG/SA/07/153 genotype. The result of genotypic variation on *in vitro* hormonal concentration on survival rate of maize genotypes shows that the genotypes; POOL ISSR QPMX, DTSR-WCO, TZL COMP4C3, SAMMAZ 19S-14DT and TZEE-YPOP STR C4 produced highest significant ($p<0.05$) effect of hormonal treatments on survival rate than other genotypes. EV99 QPM and POP66 SR/ACR94-YQPM did not differ from each other, while NG/SA/07/153 produced the least survival rate of 0.00% (Table 1). The establishment of rooted plantlets acclimatized facilitated the survival rate in consonance with (Krishna et al., 2013).

3.2 Mean Square and Genotypic Effects of In vitro Treatments of Auxin (NAA), Cytokinin (BAP and Kinetin) and Growth Stages on Shoot Proliferation and Rooting traits of Maize

The effect of hormone concentrations, genotypes and growth stages were significantly ($P<0.01$) higher for number of shoots, shoot length, number of root, root length and the number of leaves of maize. The first order interactions, (Genotype x Replicate), (Genotype x Treatment), (Genotype x Week) and (Treatment x Replicate) produced highly significant effect on shoot proliferation and rooting of maize. The second order of interactions also showed highly significant effect for most of the traits with the exception of (Genotype x Week x Replicate) for number of leaves, (Genotype x Week x Treatment) for shoot length, number of root, root length, number of leaves and (Week x

Treatment x Replicate) for all the traits were not significant (Table 2). These were in accordance with the findings of (Huang and Wei, 2006).

The effect of the genotypes on shoot proliferation and rooting traits in in vitro regeneration of maize showed that DTSR-WCO produced the highest number of roots and leaves, as well as highest values of shoot and root lengths, which are significantly different ($P<0.05$) from other genotypes. On the other hand, EV99 QPM had significant higher number of shoots than other genotypes, but not significantly different from DTSR-WCO as similarly reported by (Bohorovaet et al., 1995; Webin et al., 2002; Garcia-Moya et al., 2007). POOL ISSR QPMX and TZE COMP 3C2 were not significantly different for number of shoot while, POP66 SR/ACR94-YQPM and EV99 QPM, TZEE-YPOP STR C4 and TZE COMP 3C2, POOL ISSR QPMX, TZL COMP4C3 and SAMMAZ 19S-14DT were significantly similar for shoot length. EV99 QPM and POOL ISSR QPMX, TZEE-YPOP STR C4, TZE COMP 3C2 and POP66 SR/ACR94-YQPM had similar values not significantly different from each other for number of roots while EV99 QPM and TZL COMP4C3 are significantly similar for root length. TZE COMP 3C2 and POP66 SR/ACR94-YQPM, TZE COMP 3C2, TZL COMP4C3 and SAMMAZ 19S-14DT are not significantly different from each other for number of leaves while NG/SA/07/153 performed least for all the shoot proliferation and rooting traits of maize (Table 3). Thus, the success of regeneration procedure is affected predominantly by genotype, the type of explants materials employed and media composition (Lindsay and Jones, 1989; Binott et al., 2008). Moreover, the genotypic difference based on regeneration response could be related to variation in endogenous hormones levels in accordance with the findings of (Bhaskaran and Smith, 1990; Odour et al., 2006).
The result of the treatment combinations of hormonal concentration on shoot proliferation and rooting traits in *in vitro* regeneration of maize showed that 0.3mg/l NAA + 3.0mg/l BAP produced the highest number of shoots and leaves, as well as the highest value of shoot length, which are significantly different ($P<0.05$) from other treatments (Table 4, Figure 1). The control (MS medium with no hormone supplements) produced the highest number of roots and root lengths, which are significantly different ($P<0.05$) from other genotypes. The hormone treatments of 0.5 mg/l NAA+ 4.0 mg/l BAP and 0.5 mg/l NAA+ 4.0 mg/l KIN were not significantly different for number of shoots and roots, shoot lengths and root lengths while 0.5 mg/l NAA+ 4.0 mg/l BAP and 0.1 mg/l NAA+ 2.0 mg/l KIN were similar for number of shoots and leaves.

3.3 Pearson Correlation Coefficient of In vitro Treatment Combinations of Auxin (NAA), Cytokinin (BAP and Kinetin), Growth Stages on Shoot Proliferation and Rooting Traits of Maize Genotypes

The result of correlation coefficient of growth characters of in vitro regenerated maize genotypes shows that the shoot length was positive and strongly associated with number of roots, root length, leaf number and week at $P< 0.01$; $r =0.85, 0.82, 0.83, 0.50$ respectively. The number of root was positive and strongly correlated with root length and leaf number at $P< 0.01$; 0.85 and 0.77 respectively, but negatively related with the genotype at $P< 0.05$; $r = -0.43$. The root length is highly significant and positively correlated with the leaf number at $P< 0.01$; $r =0.73$ but negatively associated with the genotype ($r =-0.42$) while, the leaf number was positively correlated with weeks ($r =0.43$) (Table 6).

Again, 0.1 mg/l NAA+ 2.0 mg/l BAP, 0.5 mg/l NAA+ 4.0 mg/l BAP, 0.1 mg/l NAA+ 2.0 mg/l KIN, 0.5 mg/l NAA+ 4.0 mg/l KIN and control were not significantly different from each other for the number of shoots produced while 0.5 mg/l NAA+ 4.0 mg/l BAP and 0.5 mg/l NAA+ 4.0 mg/l KIN were significantly the same for the shoot length. The number of roots for 0.1 mg/l NAA+ 2.0 mg/l BAP and 0.1 mg/l NAA+ 2.0 mg/l KIN, 0.5 mg/l NAA+ 4.0 mg/l BAP and 0.5 mg/l NAA+ 4.0 mg/l KIN were not significantly ($P>0.05$) different, while 0.1 mg/l NAA+ 2.0 mg/l BAP and 0.3 mg/l NAA + 3.0 mg/l BAP, 0.1 mg/l NAA+ 2.0 mg/l KIN and 0.3 mg/l NAA + 3.0 mg/l KIN, 0.5 mg/l NAA+ 4.0 mg/l BAP and 0.5 mg/l NAA+ 4.0 mg/l KIN were not significantly different for root lengths. Also, the number of leaves in treatment combinations of 0.5 mg/l NAA+ 4.0 mg/l BAP and 0.1 mg/l NAA+ 2.0 mg/l KIN were not significantly different (Table 4). This implies that different combinations of hormone concentrations of Naphthalene acetic acid (NAA), 6-Benzylaminopurine (BAP) and Kinetin (KIN) could enhance direct multiple shoot induction and proliferation in maize genotypes. This conforms to the observations made by (Farahani et al., 2008; Krishna et al., 2013; Feyisola et al., 2015). The performance of shoot proliferation and rooting traits at different growth stages of in vitro regeneration of maize revealed that two, three and four weeks are not significantly different from each other for number of shoots, but significantly different ($P<0.05$) from first week. The number of roots and leaves as well as shoot lengths and root lengths on the first week are not significantly different from each other, while week two were significantly similar for number of shoots and leaves as well as shoot lengths and root lengths. On the third week, the number of roots and leaves as well as shoot lengths and root lengths were not significantly different, while the number of shoots and leaves as well as shoot lengths and root lengths on the fourth week were significantly similar (Table 5).

3.4 Contribution of Principal Component Axis to the Variation of the Shoot proliferation and Rooting Traits of In vitro Regenerated Maize Genotypes

The contribution of Principal Component Axis (PCA) to the variation of the shoot proliferation and rooting traits in in vitro regenerated maize is presented in Table 7. Prin 1 and Prin 5 accounted for the highest and least variations with proportion and Eigen values of (67.34%) 3.37 and (3.51%) 0.18 respectively. The variations were shown across five PCA as; Prin 1 had 3.37 (67.34%), Prin 2 had 0.86 (17.51%), Prin 3 had 0.37 (7.33%), Prin 4 had 0.22 (4.31%) and Prin 5 had 0.18 (3.51) eigenvalues and proportion respectively. The first PCA shows that the number of root (0.49), root length (0.48) and leaf number (0.47) were highly related compared to number of shoots (0.23) and shoot length (0.50), while shoot length (-0.11) and number of roots (-0.11) were closely related compared to other characters in Prin 2. The fourth PCA shows that shoot length and leaf number are closely related than other characters, while in Prin 3 and Prin 5, character association did not exist. The principal component axis between the growth characters shows the extent of variations in the genotypes which could invariably improve yield (Olawuyi et al., 2015).

***Table 1**: Genotype Sources and Effect of Hormone Concentrations on Percentage Germination and Survival rate of In vitro Regenerated Maize*

Genotype	Source	Germination% Survival		Survival rate %
		In Vitro	Control	*In Vitro*
EV99 QPM	IITA	97.14^{ab}	93.33^{ab}	70.00^{d}
POOL ISSR QPMX	IITA	97.14^{ab}	93.33^{ab}	100.00^{a}
DTSR-WCO	IITA	100.00^{a}	90.00^{b}	100.00^{a}
TZL COMP4C3	IITA	85.71^{c}	86.67^{c}	100.00^{a}
SAMMAZ 19S-14DT	KANO	100.00^{a}	100.00^{a}	100.00^{a}
TZEE-YPOP STR C4	IITA	94.29^{b}	100.00^{a}	100.00^{a}
TZE COMP 3C2	IITA	100.00^{a}	100.00^{a}	80.00^{c}
NG/SA/07/153	GOMBE	37.14^{d}	6.67^{d}	0.00^{e}
POP66 SR/ACR94-YQPM	IITA	100.00^{a}	100.00^{a}	70.00^{d}
DTSR-WC	IITA	100.00^{a}	100.00^{a}	90.00^{b}

Values are the mean of 5 replicates. Mean values with the same letter(s) in a column are not significantly different from each other using Duncan Multiple Range Test at P ≥ 0.05.

***Table 2:** In vitro* Treatment Combinations of Auxin (NAA), Cytokinin (BAP and kinetin) and Growth Stages on Shoot and Rooting of Maize Genotypes

Source of Variation	Df	Number of Shoot	Shoot Length	Number of Root	Root Length	Number of Leaves
Gen	9	10.16**	100.53**	181.48**	59.98**	35.52**
Rep	4	1.72	26.36	56.05	15.44	5.89
Trt	6	19.89**	28.65**	66.03**	10.94**	10.40**
Week	3	1.45**	421.97**	343.53**	98.69**	128.37**
Gen x Rep	36	0.72**	6.04**	7.32**	4.33**	2.12**
Gen x Trt	54	1.13**	7.75**	9.05**	3.87**	3.08**
Gen x Week	27	0.10**	2.57**	2.42**	0.82**	1.52**
Trt x Rep	24	1.21**	4.08**	4.28**	1.93**	0.99**
Week x Rep	12	0.07*	2.16**	3.08**	0.90**	0.85**
Week x Trt	18	0.52**	1.33**	4.31**	0.48**	0.45*
Gen x Trt x Rep	216	0.62**	7.34**	7.67**	3.44**	3.04**
Gen x Week x Rep	108	0.05*	0.28**	0.74**	0.15**	0.22
Gen x Week x Trt	162	0.06**	0.18	0.46	0.07	0.20
Week x Trt x Rep	72	0.02	0.16	0.40	0.08	0.24
Error	648	0.04	0.17	0.45	0.08	0.24
Corrected Total	1399					

P<0.05, *= Significant, **= Highly Significant, values without * are not significant, Gen = Genotypes of maize, Trt = Treatment combinations of NAA, BAP and Kinetin, Rep = Replicate

Table 3: *Genotypic Effect on Shoot Proliferation and Rooting Traits of In vitro Regeneration of Maize*

Genotype	Number of Shoot	Shoot Length (cm)	Number of Root	Root Length (cm)	Number of Leaves
EV99 QPM	1.31[a]	3.10[c]	3.68[b]	1.98[c]	1.69[c]
POOL ISSR QPMX	1.19[b]	3.45[b]	3.81[b]	2.05[b]	1.86[b]
DTSR-WCO	1.29[a]	3.92[a]	4.39[a]	2.41[a]	2.27[a]
TZL COMP4C3	0.96[f]	3.48[b]	3.36[c]	1.93[c]	1.72[c]
SAMMAZ 19S-14DT	1.08[d]	3.39[b]	2.86[d]	1.70[d]	1.73[c]
TZEE-YPOP STR C4	1.14[c]	2.78[d]	2.22[e]	1.32[e]	1.35[e]
TZE COMP 3C2	1.22[b]	2.78[d]	2.24[e]	1.09[g]	1.54[d]
NG/SA/07/153	0.37[g]	0.97[f]	0.72[g]	0.35[i]	0.48[g]
POP66 SR/ACR94-YQPM	1.09[cd]	3.11[c]	2.31[e]	1.21[f]	1.54[d]
DTSR-WC	1.03[e]	2.07[e]	1.41[f]	0.68[h]	0.91[f]

Values are the mean of 5 replicates. Mean values with the same letter(s) in a column are not significantly different from each other using Duncan Multiple Range Test at $P \geq 0.05$

Table 4: *Treatment Combinations of Hormonal Concentration on Shoot Proliferation and Rooting Traits of In vitro Regeneration of Maize*

Concentration of Hormones (Treatment) (mg/l)	Number of Shoot	Shoot Length (cm)	Number of Root	Root Length (cm)	Number of Leaves
MS medium + 0.1 NAA + 2.0 BAP	0.94[c]	3.13[c]	2.64[d]	1.62[b]	1.65[b]
MS medium + 0.3 NAA + 3.0 BAP	1.70[a]	3.35[a]	3.08[b]	1.58[b]	1.87[a]
MS medium + 0.5 NAA + 4.0 BAP	0.92[c]	2.52[f]	2.12[e]	1.18[d]	1.33[d]
MS medium + 0.1 NAA + 2.0 KIN	0.90[c]	2.83[e]	2.52[d]	1.47[c]	1.41[d]
MS medium + 0.3 NAA + 3.0 KIN	1.30[b]	2.97[d]	2.81[c]	1.50[c]	1.51[c]
MS medium + 0.5 NAA + 4.0 KIN	0.84[c]	2.31[f]	2.04[e]	1.15[d]	1.19[e]
MS medium only (control)	0.90[c]	3.22[b]	3.71[a]	1.80[a]	1.63[b]

Values are the mean of 5 replicates. Mean values with the same letter(s) in a column are not significantly different from each other using Duncan Multiple Range Test at $P \geq 0.05$

Table 5*: Growth Stages on Shoot Proliferation and Rooting Traits during In vitro Regeneration of Maize*

Week	Number of Shoot	Shoot Length (cm)	Number of Root	Root Length (cm)	Number of Leaves
One	0.97[b]	1.66[d]	1.55[d]	0.81[d]	0.71[d]
Two	1.10[a]	2.44[c]	2.30[c]	1.33[c]	1.41[c]
Three	1.10[a]	3.33[b]	3.12[b]	1.69[b]	1.79[b]
Four	1.10[a]	4.19[a]	3.83[a]	2.05[a]	2.12[a]

Values are the mean of 5 replicates. Mean values with the same letter(s) in a column are not significantly different from each other using Duncan Multiple Range Test at $P \geq 0.05$

Table 6: *Pearson Correlation Coefficient of In vitro Treatment Combinations of Auxin (NAA), Cytokinin (BAP and Kinetin), Growth Stages, Shoot Proliferation and Rooting Traits of Maize Genotypes*

Correlation	Number of shoot	Shoot length (cm)	Number of root	Root length (cm)	Leaf number (cm)	Genotype	Week	Treatment (mg/l)
Number of shoot								
Shoot length	0.34							
Number of root	0.34	0.85**						
Root length	0.30	0.82**	0.85**					
Leaf number	0.35	0.83**	0.77**	0.73**				
Genotype	-0.20	-0.27	-0.43*	-0.42*	-0.28			
Week	0.07	0.50**	0.40	0.36	0.43*	0.00		
Treatment	-0.17	-0.05	0.06	-0.00	-0.07	0.00	0.00	
Replicates	-0.11	-0.08	-0.09	-0.11	0.00	0.00	0.00	0.00

**P<0.05, *= Significant, **= Highly Significant, values without * are not significant

Table 7: *Contribution of Principal Component Axis to the Variation of Shoot Proliferation and Rooting Traits of In vitro Regenerated Maize Genotypes*

Traits	Prin 1	Prin 2	Prin 3	Prin 4	Prin 5
Number of shoot	0.23	0.96	0.12	0.06	-0.01
Shoot length	0.50	-0.11	-0.07	-0.26	-0.81
Number of root	0.49	-0.11	0.32	-0.63	0.49
Root length	0.48	-0.21	0.48	0.70	0.05
Leaf number	0.47	-0.02	-0.81	0.20	0.30
Proportion (%)	67.34	17.51	7.33	4.31	3.51
Eigen value	3.37	0.86	0.37	0.22	0.18

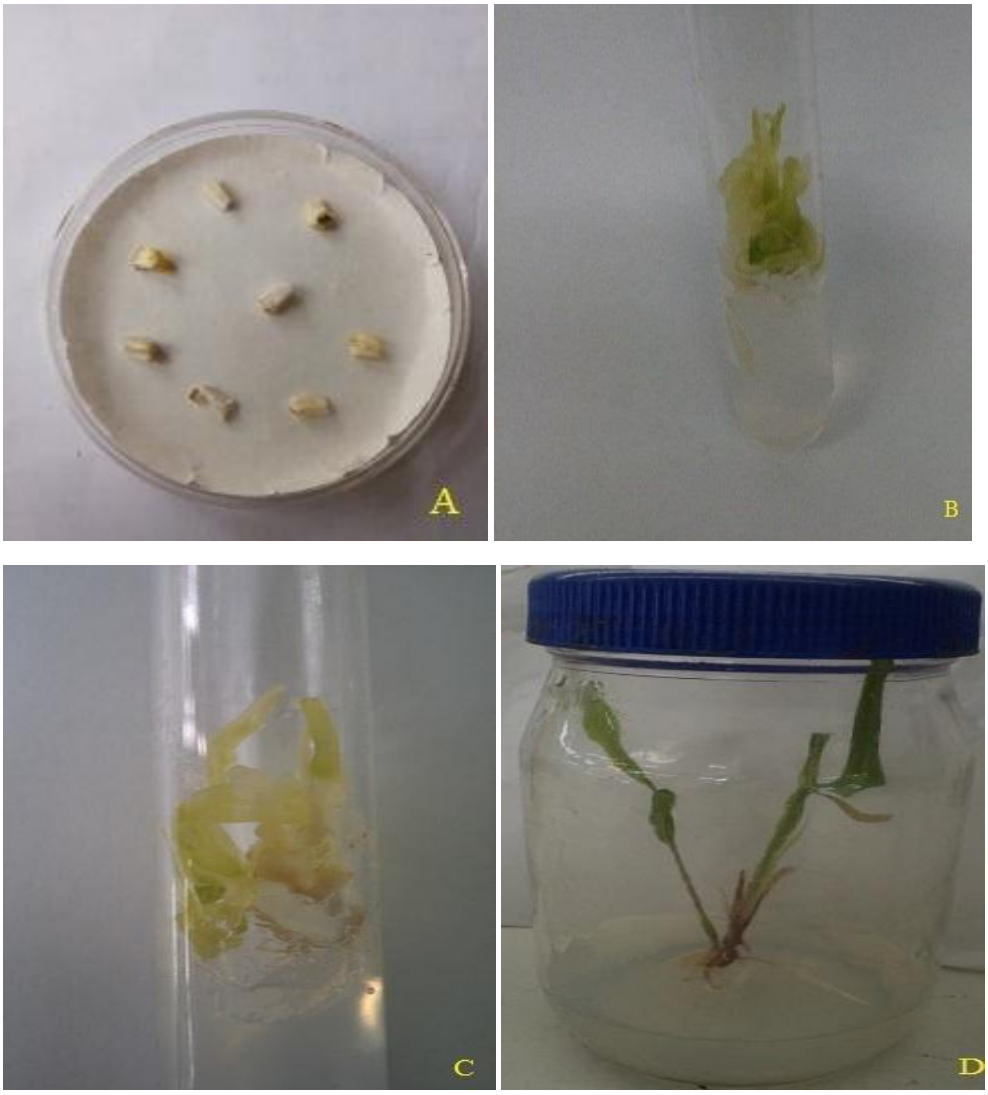

Figure 1: Developmental Stages of *In vitro* Regeneration in Maize.

A= Excised matured embryo of TZL COMP4C3,
B= Multiple Shoots Induction of TZE COMP 3C2 on MS Medium with 0.3 NAA + 3.0 BAP,
C= Multiple Shoots Proliferation of SAMMAZ 19S-14DT on MS Medium with 0.3 NAA + 3.0 KIN,
D= Subcultured Genotype of SAMMAZ 19S-14DT,

Figure 2: Developmental Stages of *In vitro* Regeneration in Maize.
E= Acclimatized Maize Plantlets,
F= Regenerated Maize Plantlets.

4. Conclusion and Recommendation

The explants of mature maize seeds produced regeneration through direct organogenesis. DTSR- WCO and EV99QPM genotypes performed best on shoot proliferation and rooting traits. Thus, they could be considered in future genetic conservation and improvement of maize. The regenerated plants yielded higher number of multiple shoots on MS medium supplemented with 0.3 mg/l NAA + 3.0 mg/l BAP within 31 days with average survival rate of 81%. The development of multiple self-growing shoot indicates several independent transgenic events which could be useful to screen for the performance of transgenic *in vitro* plants. Therefore, the regeneration method in this study is efficient, quick, highly reproducible, and could be useful for conservation, genetic improvement and transformation of plants. It can also complement the conventional breeding approach in management of abiotic and biotic stresses in maize.

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