EVALUATION OF *IN-VITRO* REGENERATED MAIZE GENOTYPES

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ABSTRACT

Field evaluation of ten *in vitro* regenerated maize genotypes collected from NACGRAB and conventional maize was conducted to investigate maize streak severity, root lodging, morpho-agronomic and yield related traits. The *in vitro* regenerated maize genotypes evolved using MS medium with 0.3mg/l NAA + 3.0mg/l BAP while the control was planted conventionally. The mean square effects of the ten maize genotypes and age of plants on growth and yield-related characters for both *in vitro* regenerated maize and control plants were significantly ($P < 0.05$) higher except for the fresh and dry root weight of control. POP66 SR/ACR94-YQPM was the best resistant genotype to root lodging. TZE COMP 3C2 genotypes were earliest in maturing and agronomic traits. The Prin 1 axis had highest contribution to variation in growth and yield related characters with 71.09% and Eigen value of 7.82, while Prin 11 had the least contribution with 0.23% and Eigen value of 0.03. DTSR-WC, POP66 SR/ACR94-YQPM, TZL COMP4C3 and SAMMAZ 19S-14DT were the best dry matter contents. POP66 SR/ACR94-YQP produced the best dry shoot (39.16g) and root weight (3.81g), and was resistant to root lodging and maize streak severity rating. Therefore, these genotypes could be recommended for use in further breeding experiments.

Keywords**:** evaluation, morpho-agronomic, correlation, i*n vitro*, maize, resistant

INTRODUCTION

Maize (*Zea mays* L.) is an important member of the grass family (Poaceae) which was introduced to West Africa by the Portuguese in the 10th century (Olakojo *et al.,* 2001; Akinbode, 2010). Its grain is rich in starch, vitamins, fats, proteins and minerals, as well as in riboflavin, phosphorus and potash (Khawar *et al*., 2007; IITA, 2009). The grain, leaves, stalk, tassel and cob can be used to produce large variety of food and non-food products (IITA, 2001; Olakojo*,* 2004; Olawuyi *et al.,* 2010; 2013). Generally, maize production is constrained by a number of stress factors including: field losses to pests and diseases, root and stem lodging, unreliable and erratic rainfall amount and distribution as well as land degradation; and low soil fertility (Olakojo *et al.,* 2005; Akande and Lamidi, 2006; Olawuyi *et al.,* 2011).

Maize streak virus (MSV) disease is one of the major problems facing maize cultivation in Nigeria and other African nations (Magenya *et al.,* 2008; Martin and Shepherd, 2009). Under field conditions, they cause up to100% yield loss in susceptible varieties (Barrow, 2000; Magenya *et al*., 2008). Plant characteristics and weather conditions are important in determining the susceptibility of a crop to root lodging. Apart from the role of water and nutrient uptake, roots are essential for lodging resistance of the plants (Hetz *et al.*, 1996; Hébert *et al*., 2001) while MSV disease incidence and severity with symptoms characterized by chlorotic stripes centered initially on the tertiary leaf veins varies according to maize genotype and plant age at the time of infection (Adipala *et al*., 1993). This reduces the photosynthetic area, growth and grain yield of the plant (Agrios, 2005). Existing pest management practices such as chemical and biological control had little impact in ameliorating storage losses (Kibata *et al*., 2003). The continuous use of pesticides in agriculture has resulted in the build-up of insect pest resistance, environmental pollution, and secondary pest out breaks which had caused poisonings of 3 million people a year (Chadwick and Marsh, 1993; Kortenhoff, 1993; Pimentel, 2002).

The quest for improved grain yield and disease tolerant/ resistant maize varieties, therefore, becomes imperative for profitable maize production. The use of host plant resistance however, remains the most economically viable and practical means of controlling disease epidemics (Bua and Chelimo, 2010; Olawuyi *et al.,* 2011). Efficiency of tissue culture and transformation protocol is one of the most important components for the successful generation of transgenic crops conferring resistance or tolerance to these biotic and abiotic stresses. Therefore, this study was conducted to evaluate the morpho-agronomic, maize streak severity assessment and yield related traits evaluation on *in vitro* regenerated maize cultivars.

MATERIALS AND METHODS

Study Location, Germplasm Collection and Experimental Design

The evaluation of *in vitro* regenerated maize genotypes from MS medium supplemented with 0.3mg/l NAA + 3.0mg/l BAP and the control experiment were conducted in the screen house of Nigerian Agricultural Quarantine Service (NAQS), Moor Plantation, Ibadan, Nigeria. The seeds of maize collected from the Germplasm Unit of the National Centre for Genetic Resources and Biotechnology (NACGRAB; Table 1) were planted in polythene bags. The polythene bags were arranged in the screen house in factorial design but laid out in a completely randomized design (CRD) spaced out at a distance of 30cm x 40 cm and replicated five times.

Establishment of Maize *In Vitro* Cultures

Sterilization procedures were performed under the laminar flow according to the procedure described by Krishna *et al.* (2013). The glassware and dissecting tools were washed with tap water and detergent, and then rinsed in distilled water. These instruments were later dried in an oven at 250°C. Dissecting tools (forceps and scalpel holders) and Petri dishes were autoclaved at 121°C (1.06kgcm^{-2}) for 30 minutes. The soil was air-dried and screened through a 2 mm sieve to remove large particles, debris, and stones and also sterilized at 121°C for 2hours using electric soil sterilizer. Ten kilograms of soil was later bagged into polythene bags. Matured embryos were excised from the grains with a scalpel and cultured on media supplemented with 0.3mg/l NAA + 3.0mg/l BAP while the untreated plants served as control. The *in vitro* regenerated plantlets with hardened roots were acclimatized for one week (7 days) before transferring to the screen house for evaluation.

Method of Planting

Three well acclimatized plantlets from each genotype were transplanted into each polythene bag and three viable seeds from each genotype was planted into each polythene bag for *in vitro* and control experiment respectively. Crop management practices included weed control with pre-emergence application of herbicide (Attrazine of 80% powder). The herbicide was applied at the rate of 100ml to 20liters of water. The polythene bags were adequately maintained by regular watering, weeding and thinning to one stand after one week of germination.

Assessment of Maize Streak Severity

The root lodging and maize streak severity were scored using scale 1-5, where 1= Normal plant without damage; 2= slight infection, 3= moderate infection; 4 poor and stunted growth and 5= Very poor and heavy infection. Similarly root lodging was rated using scale 1-

5 where 1=Excellent, 2= Very good, 3= good, 4=fair, 5= poor as well as growth and yield characters according to Kim (1994).

Determination of Morpho-Agronomic and Yield Related Characters

Collection of data on growth parameters of genotypes commenced at one week after acclimatizing and sowing for the *in vitro* regenerated and control experiments respectively. Data collected included; plant height (cm), number of leaves, leaf lengths (cm), leaf widths (cm), stem lengths (cm) and stem girth (cm) for the first six weeks with the aid of a metre rule, micro metre screw gauge and manual counting. Agronomic characters such as; plant stand, husk cover, ear aspect, tassel length, days to anthesis and days to silking were carried out for the *in vitro* regenerated cultivars and control. Yield parameters; shoot biomass (g) and root biomass (g) were taken at the end of the experiment from the harvested plants with the use of a weighing scale.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using SAS 9.1 (2003) statistical software, while significant means were separated at 95% confidence interval by New Duncan's multiple Range Test (Duncan, 1955). The data were subsequently subjected to principal component analysis (PCA) and Pearson Correlation Coefficient.

RESULTS AND DISCUSSION

Effects of Growth Characters in *In vitro* Regenerated Maize Genotypes

The mean square variance for genotypes and growth stages effects on maize characters of the *in vitro* regenerated and control experiments shown in Table 2. Plant height, leaf number, leaf area, stem length and stem girth had highly significant ($P<0.01$) effects on the genotypes and age of plants However, plant height, leaf number, leaf area, stem length and stem girth were significantly increased as the age of plant for both *in vitro* regenerated and control maize plant increased (Table 2). A similar observation was made by Olakojo *et al.* (2001), Ihsan *et al.* (2005) and Olawuyi *et al.* (2014b). The highly significant level of both the *in vitro* regenerated and control maize plants experiment recorded in these traits are considered to have contributed to yield components in all the genotypes according to Olakojo *et al.* (2001) and Olakojo (2004). Significant differences in genotypic interactions could be an indication of high genetic diversity in their backgrounds.

The result of growth characters in *in vitro* regenerated maize showed that TZEE-YPOP STR C4 genotype produced the highest mean of leaves (7.43) plant height (49.04cm), leaf area (77.7cm) and stem girth (39.7cm) (Table 2). The plant heights in POOL-ISSR-

QPMSX, DTSR-WCO, TZL-COMP4C3, POP-66SR/ACR 94-YQPM and DTSR-WC genotypes were not significantly ($P < 0.05$) different from one another. Also, the mean number of leaves per plant stand in EV99 QPM, TZE COMP3C2, DTSR-WC and DTSR-WCO as well as SAMMAZ 19S-14DT and TZEE-YPOP STR C4 and POP66SR/ACR94-YQPM were not significantly different. The leaf areas of these pairs of genotypes were not also significantly different: EV99QPM and TZECOMP3C2, POOL-ISSR-QPMSX and DTSR-WCO as well as TZLCOMP4C3, SAMMAZ 19S-14DT, TZEE-YPOPSTRC4, and POP66R/ACR94-YQPM genotypes. Genotypes EV99QPM, POOL-ISSR-QPMSX, DTSR-WCO, TZL-COMP4C3, TZECOMP3C2 and DTSR-WC were not statistically the same for stem length.

Variance on severity of Maize Streak and Agronomic characters of *In vitro* Regenerated genotypes

The result of the mean square variance showed that severity of maize streak virus disease was high and had significant effects ($P< 0.01$) on the root lodging and agronomic characters of *in vitro* regenerated maize cultivars (Table 3). The yield of the plant is dependent on the level of water, nutrient, diseases and pest resistant of the plant (Craig *et al.,* 2000; Golbashy *et al.,* 2010). The effect of maize streak and root lodging on *in vitro* regenerated maize showed that EV99 QPM, SAMMAZ 19S-14DT and TZE COMP 3C2 genotypes were the most resistant to maize streak virus (1.00), and were significantly different ($P< 0.05$) from other genotypes, while EV 99 QPM genotype (1.80) was observed as excellent for the control experiment. Other genotypes were with resistance abilities to maize streak virus except NG/SA/07/153. On the other hand, the *in vitro* regenerated POP66SR/ACR94-YQPM was the most resistant to root lodging which is significantly different ($P< 0.05$) from other genotypes. Although, other genotypes also exhibited good resistant abilities to root lodging with the exception of NG/SA/07/153 (Table 4). The root lodging and maize streak severity ratings six weeks after planting showed negative effects on the agronomic and yield related traits of the maize plants. This correlates with the reports of Olawuyi *et al.* (2011). Disease symptoms such as necrosis, stunted growth, vein clearing, wilting leaf defoliation and stem impairment were observed from the control maize experiment compared to the *in vitro* regenerated plants earlier observed by Welter, (1993) and Craig *et al.*, (2000). The plant height and ear aspect of the control maize were lowered and this negatively affected the grain yield as similarly observed by Ali *et al.* (2012) who opined that plant height and ear height poor lodging resistance could be due to physiological stresses. Moreover, most of the genotypes regenerated through plant tissue culture were resistant to the lodging effect compared to the control.

Mean Square Variance of Agronomic Traits both the Control Experiment and *In Vitro* Regenerated Maize Genotypes

The result in Table 5 showed that days to anthesis and silking, plant stand, husk cover, ear aspect and tassel length had highly significant ($P<0.01$) effect on the maize genotypes (Table 5) SAMMAZ 19S-14DT produced an excellent performance in terms of plant stand, was significantly different ($P< 0.05$) from other genotypes but not for TZL COMP4C3 genotype. POOL ISSR QPMX, TZEE-YPOP STR C4 and TZE COMP 3C2 genotypes showed fair performance for the plant stand. The husk cover in SAMMAZ 19S-14DT genotype was not significantly different from TZEE-YPOP STR C4, DTSR-WCO and TZL COMP4C3 genotypes in terms of good performance, but, EV99 QPM and POOL ISSR QPMX were rated poor for husk cover. TZL COMP 4C3 genotype showed good performance for ear aspect, and was significantly different ($P<0.05$) from other genotypes while, EV99 QPM and POOL ISSR QPMX genotypes had the least performance for ear aspect rating. The longest tassel was found in TZL COMP4C3 genotype (31.26cm), and was significantly different from other genotypes, while NG/SA/07/153 recorded 6.52cm as the poorest (Table 5). TZE COMP 3C2 was the best for days to anthesis and silking (Table 5, Plate 2). This agrees with the earlier reports of Appunu *et al.,* 2007; Farzana *et al*., 2007; Ram *et al.,* 2011; Jawaharlal *et al*., 2012; Ajay *et al.,* 2013; Olawuyi *et al.,* 2013; Kumar *et al.,* 2014. TZE COMP 3C2, TZEE-YPOP STR C4, SAMMAZ 19S-14DT and POOL ISSR QPMX in this study were considered desirable genotypes as early maturing, with the agronomic traits closely similar associated with earliness to anthesis and silking.

Mean Square Variance on Yield-Related Characters of *In vitro* Regenerated Maize Genotypes

The mean square variance was significantly ($P<0.01$) higher for fresh shoot, root weight, dry shoot and dry root weight in *in vitro* regenerated plantlet while the genotypes showed high significant ($P<0.01$) effects on fresh and dry shoot in control experiment. Fresh root was significant ($P< 0.05$) among genotypes while dry root was not significant for the control experiment (Table 6). Meanwhile, there were variations in yield-related characters of *in vitro* regenerated maize.

POP66 SR/ACR94-YQPM had the highest value of fresh shoot weight, fresh and dry root weight, which were significantly different ($P< 0.05$) from other genotypes in *in vitro* regenerated maize, while TZL COMP 4C3 genotype recorded highest value for fresh shoot and root, as well as dry shoot for the control. Also, TZL COMP 4C3 genotype had the highest value for dry shoot and was significantly different ($P < 0.05$) from other genotypes. However, NG/SA/07/153 produced the lowest performance across all the yield-related characters for both the control and *in vitro* regenerated maize plants (Table 7).

Pearson Correlation Coefficient of Growth and Yield-Related Characters of Both *In vitro* Regenerated Maize and Control Experiment

The association of traits among maize genotypes is presented as correlation matrix in Table 8. Plant height had strong positive and significant association with leaf number, leaf length, leaf width, stem length, stem girth and leaf area, fresh shoot, fresh root, dry shoot ($p < 0.01$; $r = 0.79$, 0.90, 0.83, 0.91, 0.83, 0.80, 0.56, 0.54, 0.52 respectively). This showed the positive influence and contribution to other growth and yield related traits. The plant height and number of leaves produced, had direct correlation with other growth characters which is similar to the report of Haq *et al.* (2005). This suggests that selection process based on plant height could favour all the growth and yield related traits, and could invariably enhance seed production. Also, the leaf number was positive and strongly correlated with leaf length, leaf width, stem length, stem girth and leaf area ($p < 0.01$; $r = 0.84$, 0.81, 0.79, 0.83, 0.69 respectively) This may positively influence the photosynthetic ability of the leaves, which in turn enhances plant growth as reported by Skingh and Tsunoda (1978) and Olowe *et al.* (2013). The leaf length was positive and strongly related to leaf width, stem length, stem girth, leaf area, fresh shoot, fresh root, dry shoot and dry root at $p < 0.01$; $r = 0.90$, 0.82, 0.88, 0.88, 0.56, 0.58, 0.52, 0.51 respectively. Brown and Caligari (2008) earlier reported that high and positive association that were observed between characters such as plant height, ear height, number of leaves, yield related characters could be controlled by closely linked genes, same or similar genes on the characters. Furthermore, leaf width was positive and strongly associated with stem length, stem girth, leaf area, fresh shoot, fresh root and dry root at $p < 0.01$; $r = 0.80$, 0.84, 0.95, 0.51, 0.54, 0.52 respectively. Stem length was positively associated with stem girth, leaf area, fresh shoot and fresh root at $p < 0.01$; $r = 0.77$, 0.74, 0.53, 0.51 respectively. Stem girth was highly significant and positively correlated with leaf area, fresh root and dry root at $p < 0.01$; $r = 0.77$, 0.52, 0.50 respectively. Fresh shoot was highly significant and positively linked with fresh root, dry shoot and dry root at $p < 0.01$; $r = 0.84$, 0.94, 0.64 respectively while fresh root is highly associated with dry shoot and dry root at $p < 0.01$; $r = 0.81$, 0.79 respectively. Dry shoot was also highly significant and positively correlated with dry root ($p < 0.01$; $r = 0.63$). These positive and significant association will no doubt makes selection of yeild related characters easy for maize breeder working on improvement of these traits using in vitro regenerated system.

Contribution of Principal Component Axis (PCA) to the Variation of Growth and Yield Related Traits in Maize Genotypes

The contribution of Principal Component Axis (PCA) to the variation of the growth and yield related traits in maize genotypes is presented in Table 9. Prin 1 and Prin 11 accounted for the highest and least variations with proportion and Eigen values of (71.09%) 7.82 and (0.23%) 0.03 respectively. The first PCA showed that the plant height (0.32), leaf number (0.32), leaf length (0.34), leaf width (0.33), stem length (0.31), stem girth (0.32) and leaf area (0.32) were closely related, while fresh shoot (0.27), fresh root (0.28), dry shoot (0.25) and dry roots (0.25) were highly associated. In Prin 2, the plant height (-0.20), leaf number (-0.23), leaf width (-0.19), stem length (-0.24), stem girth (-0.18) and leaf area (-0.21) were closely associated, whereas fresh shoot (0.44), fresh root (0.42) and dry shoot (0.48) were strongly linked than leaf length (-0.14) and dry root (0.35). In Prin 3, leaf number (0.03) and stem girth (0.05), leaf width (0.16) and fresh root (0.16), fresh shoot (-0.37) and dry shoot (-0.38) were highly related compared to stem length (-0.29) and dry root (0.71). In Prin 11, the plant height (0.09), stem girth (0.11) and fresh shoot (0.12) were more associated, while the stem length (-0.04) was strongly related to fresh root (-0.05) compared to other characters. Significant positive correlation and the principal component axis (Prin I and Prin 2) between the growth and yield related characters among the genotypes could improve yield. This observation is in agreement with Olowe *et al.*, and Olawuyi *et al.,* 2015.

Conclusion and Recommendation

The development of multiple self-growing shoots brings about several independent transgenic events which can be potentially useful to screen out the performance of transgenic *in vitro* plants. It can also complement the conventional breeding approach in management of abiotic and biotic stresses in maize. TZE COMP 3C2, TZEE-YPOP STR C4, SAMMAZ 19S-14DT and POOL ISSR QPMX are desirable early maturing genotypes. DTSR-WC, POP66 SR/ACR94-YQPM, TZL COMP4C3, SAMMAZ 19S-14DT genotypes were the best for yield related characters. POP66 SR/ACR94-YQPM genotype produced the best dry shoot and root weight, highly resistant to root lodging and maize streak severity. Therefore, these genotypes could be recommended for cultivation in different breeding purposes.

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Table 1: Genotype Name and Sources

Genotype	Source
EV99 QPM	IITA
POOL ISSR QPMX	IITA
DTSR-WCO	IITA
TZL COMP4C3	IITA
SAMMAZ 19S-14DT DRD	SAMMARU (KANO)
TZEE-YPOP STR C4	IITA
TZE COMP3C2	IITA
NG/SA/07/153	GOMBE
POP66 SR/ACR 94	IITA
DTSR-WC	IITA

Plate 1: *In vitro* Regenerated and Control Maize Cultivars in the Screen House

A=Weaned *in vitro* plant of EV99 QPM, **B**= Lodged control trial plant of DTSR-WC, **C**= Maize streak virus symptom on control experiment of TZE COMP 3C2, **D**= Sterile and resistant *in vitro* plant of TZE COMP 3C2.

Table 2: Growth Characters of *In Vitro* Regenerated Maize Genotypes

Genotype	Plant Height (cm)	Leaf Number	Leaf Area (cm^{2})	Stem Length (cm)	Stem Girth (cm)
EV99 QPM	40.98^{c}	6.37^{b}	48.93^{bc}	20.35^{bc}	2.45^{c}
POOL ISSR QPMX	41.85^{bc}	6.93^{ab}	43.47^{c}	20.06^{bc}	1.98^{de}
DTSR-WCO	42.43^{bc}	6.23^{b}	44.62^{c}	18.71^{c}	1.84^{e}
TZL COMP4C3	41.79^{bc}	6.83^{ab}	64.56^{a}	19.12^{c}	2.15^{d}
SAMMAZ 19S-14DT	45.46^{b}	7.40^{a}	70.00^{a}	23.80^{a}	2.76^{b}
TZEE-YPOP STR C4	49.01^{a}	7.43^{a}	67.71^{a}	22.59^{ab}	3.17^{a}
TZE COMP 3C2	37.42^{d}	6.40^{b}	49.06^{bc}	21.49^{abc}	1.80^{e}
NG/SA/07/153	0.00^{e}	0.00^{c}	0.00^{d}	0.00^{d}	0.00^{f}
POP66 SR/ACR94-YQPM	44.45^{bc}	7.23^{a}	70.30^{a}	22.82^{ab}	2.95^{ab}
DTSR-WC	43.85^{bc}	6.43^{b}	60.70^{ab}	21.29^{abc}	2.91^{ab}

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 3: Mean Square Variance on Maize Streak Severity, Agronomic and Early Maturing Characters of Maize Genotypes

Source of Variation	Df	Plant Stand	Husk cover	Ear Aspect	Tassel Length	Days to Anthesis	Days to Silking	Root Lodging	Maize streak disease severity
Genotype	9	4.98^{**}	7.55^{**}	8.49^{**}	236.74^{**}	1046.82^{**}	1499.03^{**}	1.07^{**}	1.11^{**}
Replicate	4	0.25	0.18	1.61	122.70	112.67	78.17	0.35	0.13
Error	36	1.45	1.28	1.83	61.82	144.30	91.67	0.31	0.15
Corrected Total	49								

** = significant at 0.05 and 0.01 levels of probability, respectively

Table 4: Severity of Maize Streak and Root Lodging of *In Vitro* Regenerated Maize Genotypes

Genotype	Maize Streak Severity		Root lodging	
	In vitro	Control	*In vitro*	Control
EV99 QPM	1.00^{b}	1.80^{de}	1.40^{ab}	1.60^{abc}
POOL ISSR QPMX	1.60^{a}	3.80^{ab}	1.20^{b}	1.80^{abc}
DTSR-WCO	1.60^{a}	2.60^{bcd}	1.60^{a}	2.80^{a}
TZL COMP4C3	1.20^{ab}	3.00^{abcd}	1.20^{b}	1.80^{abc}
SAMMAZ 19S-14DT	1.00^{b}	3.20^{abcd}	1.20^{b}	2.20^{ab}
TZEE-YPOP STR C4	1.60^{a}	4.40^{a}	1.40^{ab}	2.00^{ab}
TZE COMP 3C2	1.00^{b}	3.80^{ab}	1.40^{ab}	1.40^{abc}
NG/SA/07/153	0.00^{c}	0.80^{e}	0.00^{d}	0.40^{c}
POP66SR/ACR94-YQPM	1.20^{ab}	2.00^{cde}	1.00^{bc}	1.00^{bc}
DTSR-WC	1.20^{ab}	3.60^{abc}	1.60^{a}	2.80^{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 $P \geq 0.05$. Kims Ratings (1994):1= excellent, 2= good, 3= fairly good, 4= fair, 5= poor

Table 5: Agronomic and Early Maturing Characters of Maize Genotypes

Genotype	Plant Stand	Husk cover	Ear Aspect	Tassel Length (cm)	Days to Anthesis	Days to Silk
EV99 QPM	3.20^{ab}	4.60^{a}	4.80^{a}	19.82^{b}	62.20^{a}	70.60^{a}
POOL ISSR QPMX	3.60^{a}	4.80^{a}	5.00^{a}	14.00^{bc}	48.60^{ab}	75.20^{a}
DTSR-WCO	2.60^{ab}	2.80^{bc}	2.80^{bc}	17.90^{b}	52.00^{ab}	62.40^{ab}
TZL COMP4C3	1.80^{bc}	2.80^{bc}	2.20^{cd}	31.26^{a}	56.20^{a}	65.60^{ab}
SAMMAZ 19S-14DT	1.60^{bc}	2.20^{bc}	2.80^{bc}	24.28^{ab}	52.20^{ab}	61.80^{ab}
TZEE-YPOP STR C4	3.60^{a}	2.80^{bc}	3.80^{abc}	18.52^{b}	48.40^{ab}	61.40^{ab}
TZE COMP 3C2	3.60^{a}	3.40^{abc}	3.20^{abc}	24.76^{ab}	47.80^{ab}	61.20^{ab}
NG/SA/07/153	0.80^{c}	0.60^{d}	0.80^{d}	6.52^{c}	10.00^{c}	12.20^{c}
POP66SR/ACR94-YQPM	2.00^{abc}	3.60^{abc}	4.40^{ab}	23.68^{ab}	58.20^{a}	64.80^{ab}
DTSR-WC	3.20^{ab}	4.00^{ab}	4.20^{ab}	24.86^{ab}	54.60^{ab}	62.00^{ab}

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 $p \geq 0.05$. Kims Ratings: 1= excellent, 2= good, 3= fairly good, 4= fair, 5= poor

Table 6: Mean Square Variance of Yield-Related Characters on *In Vitro* Regenerated Maize Genotypes and Control Experiment

Source of variation	Df	Fresh Shoot (g)		Fresh root (g)		Dry Shoot (g)		Dry Root (g)	
		In Vitro	Control	*In Vitro*	Control	*In Vitro*	Control	*In Vitro*	Control
Genotype	9	6254.46**	5100.16**	264.17**	127.51*	769.75**	568.09**	8.30**	4.34
Replicate	4	1861.20	4521.68	59.78	203.38	405.95	1026.26	0.38	2.76
Error	36	794.31	952.94	49.36	67.56	145.88	183.05	1.79	2.23
Corrected Total	49								

*, ** = significant at 0.05 and 0.01 levels of probability, respectively.

Table 7: Yield-Related Characters of *In Vitro* Regenerated Maize Genotypes

Genotype	Fresh Shoot (g)		Fresh root (g)		Dry Shoot (g)		Dry Root (g)	
	In Vitro	Control	*In Vitro*	Control	*In Vitro*	Control	*In Vitro*	Control
EV99 QPM	74.17^{bc}	69.05^{ab}	16.94^{ab}	14.83^{ab}	30.99^{ab}	29.95^{abc}	2.45^{b}	2.14^{abc}
POOL ISSR QPMX	71.65^{bc}	70.03^{ab}	10.93^{b}	11.81^{ab}	29.70^{ab}	28.32^{abc}	2.04^{b}	1.67^{bc}
DTSR-WCO	42.73^{c}	35.89^{bc}	14.25^{b}	10.92^{ab}	19.34^{b}	16.96^{bcd}	2.16^{b}	2.01^{bc}
TZL COMP4C3	110.56^{ab}	113.65^{a}	20.56^{ab}	21.18^{a}	39.03^{a}	40.32^{a}	3.91^{ab}	4.25^{a}
SAMMAZ 19S-14DT	97.07^{ab}	91.30^{a}	21.25^{ab}	20.71^{a}	32.99^{ab}	31.59^{abc}	3.09^{ab}	2.65^{abc}
TZEE-YPOP STR C4	72.64^{bc}	40.44^{bc}	21.10^{ab}	11.74^{ab}	24.20^{ab}	15.22^{cd}	4.59^{a}	3.32^{ab}
TZE COMP 3C2	56.06^{c}	75.79^{ab}	11.47^{b}	12.86^{ab}	21.41^{b}	28.06^{abc}	2.04^{b}	2.10^{abc}
NG/SA/07/153	0.00^{d}	16.04^{c}	0.00^{c}	6.53^{b}	0.00^{c}	7.47^{d}	0.00^{c}	0.78^{c}
POP66 SR/ACR94-YQPM	114.87^{a}	104.03^{a}	25.29^{a}	20.97^{a}	39.16^{a}	36.11^{ab}	3.81^{ab}	2.54^{abc}
DTSR-WC	104.62^{ab}	96.36^{a}	18.96^{ab}	18.05^{ab}	41.63^{a}	37.43^{a}	2.84^{ab}	2.44^{abc}

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 8: Pearson Correlation Coefficient (r) of Growth and Yield-Related Characters of Both *In vitro* Regenerated Maize and Control Experiment

Correlation	(cm)	er	(cm)	(cm)	(cm)	(cm)	(cm^2)	(g)	(g)	(g)	(g)	Genotype	Type	Replicate
number	0.79**													
Leaf length	0.90**	0.84**												
Leaf width	0.83**	0.81**	0.90**											
Stem length	0.91**	0.79**	0.82**	0.80**										
Stem girth	0.83**	0.83**	0.88**	0.84**	0.77**									
Leaf area	0.80**	0.69**	0.88**	0.95**	0.74**	0.77**								
Fresh shoot	0.56	0.41	0.56	0.51	0.53	0.49	0.45							
Fresh root	0.54	0.49	0.58	0.54	0.51	0.52	0.49	0.84						
Dry shoot	0.52	0.40	0.52	0.45	0.48	0.44	0.39	0.94	0.81					
Dry root	0.47	0.47	0.51	0.52	0.41	0.50	0.45	0.64	0.79	0.63				
Genotype	-0.02	-0.22	-0.07	0.05	-0.01	-0.07	0.13	0.08	0.05	0.01	0.01			
Type	-0.14	-0.01	-0.28	-0.25	-0.02	-0.15	-0.37	0.04	0.06	0.02	0.09	0.00		
Replicate	0.17	-0.04	0.06	0.04	0.15	0.04	0.06	0.32	0.24	0.39	0.10	0.00	0.00	

*, **= significant at 0.05 and 0.01 levels of probability, respectively.

Table 9: Contribution of Principal Component Axis (PCA) to the Variation of Growth and Yield-Related Traits in Maize Genotypes

Trait	Prin 1	Prin 2	Prin 3	Prin 4	Prin 5	Prin 6	Prin 7	Prin 8	Prin 9	Prin 10	Prin 11
Plant height	0.32	-0.20	-0.21	0.15	0.41	0.26	-0.12	-0.37	-0.59	0.23	0.09
Leaf number	0.32	-0.23	0.03	0.37	-0.27	-0.64	0.35	-0.09	-0.16	-0.13	0.25
Leaf length	0.34	-0.14	-0.04	-0.02	-0.08	0.06	-0.10	-0.62	0.44	-0.30	-0.41
Leaf width	0.33	-0.19	0.16	-0.40	-0.16	-0.06	0.18	0.32	-0.34	0.13	-0.62
Stem length	0.31	-0.24	-0.29	0.13	0.57	-0.10	0.02	0.49	0.41	-0.06	-0.04
Stem girth	0.32	-0.18	0.05	0.40	-0.52	0.48	-0.28	0.30	0.09	0.11	0.11
Leaf area	0.32	-0.21	0.20	-0.65	-0.02	0.04	-0.03	-0.06	0.17	0.06	0.60
Fresh shoot	0.27	0.44	-0.37	-0.15	-0.10	0.15	0.04	0.15	-0.26	-0.66	0.12
Fresh root	0.28	0.42	0.16	0.01	0.06	-0.45	-0.70	0.04	-0.03	0.16	-0.05
Dry shoot	0.25	0.48	-0.38	-0.03	-0.14	0.03	0.38	-0.10	0.23	0.58	0.00
Dry root	0.25	0.35	0.71	0.23	0.30	0.23	0.33	0.00	0.04	-0.10	0.01
Proportion (%)	71.09	15.00	4.59	2.34	2.12	1.48	1.30	0.91	0.49	0.46	0.23
Eigen value	7.82	1.65	0.51	0.26	0.23	0.16	0.14	0.10	0.05	0.05	0.03