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Evaluation of somatic embryogenesis and plant regeneration in tissue culture of ten sorghum (*Sorghum bicolor* L.) genotypes

Shireen Kamal Assem[1]*, Mohamed Mahmoud Zamzam[1], Basita Abbas Hussein[2] and Ebtissam Hussein Aly Hussein[2]

[1]Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt.
[2]Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt.

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Optimization of tissue culture conditions for *Sorghum bicolor* L. through somatic embryogenesis from immature embryos is important for the genetic manipulation and improvement of this agronomically valuable crop. In an attempt to develop a successfully reproducible *in vitro* regeneration protocol for a group of diverse sorghum genotypes, 10 sorghum lines including locally adapted and commercially important elite genotypes were assessed for their regeneration potential on different culture media–containing adequate growth regulators combinations. The maximum response of embryogenic callus induction was obtained from explants cultured on Murashige and Skoog (MS) medium supplemented with 1.5 mgL^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.7 mgL^{-1} L-proline. The addition of kinetin to the MS-based culture media had a negative effect on the formation of embryogenic calli. The results reveal that embryogenic callus formation and regeneration were highly genotype dependent. The line LG3 revealed the highest mean number of embryogenic callus (47.5 ± 6.0%) across the media tested. On the other hand, SPP462 was the least responsive (13.4 ± 3.3%) to embryogenic callus induction. The regeneration percentage of the different genotypes ranged from zero to 22.1%. The lines LG3, LG4, Dorado, SPGM94021 and SPMD94001 succeeded to form shoots on the three tested regeneration media. Nevertheless, the genotypes LG8 and TX2794 produced shoots on only two out of the three media. Three lines failed to regenerate on any of the tested media. Adding 0.5 mgL^{-1} naphthalene acetic acid and 0.5 mgL^{-1} of indole-3-butyric acid (IBA) did not enhance the root induction. Regenerated shoots developed into normal mature plants. Regeneration of sorghum genotypes could be improved through the use of different auxins and cytokinins in callus induction and shoot formation media. The auxin, 2,4-D was critical for the induction of embryogenic calli. However, the addition of the cytokinin (kinetin) adversely affected the formation of embryogenic callus. On the other hand, the shoot induction was more influenced by the addition of indole-3-acetic acid (IAA), 6-benzylaminopurine (BA) and thidiazuron (TDZ).

Key words: Sorghum, immature embryos, callus induction, regeneration, auxin, cytokinin.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench], a tropical plant belonging to the family Poaceae, is one of the most significant crops in Asia, Africa and Latin America. *Sorghum* is the fifth most cultivated and consumed grain

in the world after maize, rice, wheat and barley (FAO STAT, 2010). The crop is well adapted to tropical and subtropical areas throughout the world. In addition to its principal uses as flour, in the preparation of porridge and unleavened bread, sorghum species are sources of fiber, fuel and secondary products and are also used in the alcohol industry (sweet sorghum) as they contain high amounts of starch. In addition, sorghum grain has enhanced protein quality that could contribute significantly to the nutritional value of the diets of people and livestock (Ignacimuthu and Premkumar, 2014). Sorghum has been considered as one of the most difficult plant species to manipulate through tissue culture (Manjula et al., 2000; Chandrakanth et al., 2002; Hagio, 2002; Harshavardhan et al., 2002; Jeoung et al., 2002; Visarada et al., 2003; Kishore et al., 2006; Gupta et al., 2006; Maheswari et al., 2006). Protocols have been established for *in vitro* plant regeneration of sorghum from different types of explants such as immature embryos (Brar et al., 1979; Sairam et al., 2000; Nguyen et al., 2007; Grootboom et al., 2008; Muhumuza and Okori, 2013), immature inflorescences (Boyes and Vasil, 1984; Eapen and George, 1990; Zarif et al., 2013) shoot tips (Bhaskaran et al., 1988; Bhaskaran and Smith, 1988, 1989; Nahdi and de Wet, 1995; Seetharama et al., 2000; Kingsley and Ignacimuthu, 2014), leaf base (Mishra and Khurana, 2003), leaf segments (Pola and Mani, 2006) and also from cultured mesophyll protoplasts (Seetharama and Sairam, 1997; Sairam et al., 1999). However, the frequency of plant regeneration reported so far does not seem to be high enough. Rapid and highly uniform regeneration system with high regeneration efficiency is a prerequisite for successful genetic transformation.

Most of the studies were carried out on previously assessed, tissue culture amendable sorghum genotypes. In the present study, 10 sorghum genotypes have been selected on the basis that they represent genotypes of diverse origins and backgrounds. The criteria of selection were based on the importance of these genotypes for commercial hybrids formation. Our study was carried out with the main objective to identify a suitable tissue culture medium for callus induction and to determine the ability of these genotypes for plant regeneration through somatic embryogenesis. Establishing a reliable regeneration protocol will pave the way for successful use of immature zygotic embryos as target tissues for DNA delivery of important genes, to produce genetically modified sorghum lines with enhanced characteristics adapted with the climate changes and thus, cover the needs for food and feed.

MATERIALS AND METHODS

Sorghum genotypes and preparation of explants

Seeds of 10 elite sorghum genotypes (*S. bicolor* L.) of locally adapted and commercially important elite germplasms of different origins were obtained from the Sorghum Department, Field Crops Research Institute, ARC, Egypt. Sorghum genotypes included: three local lines (that is, LG3, LG4 and LG8), one cultivar “Dorado” from central America and 6 lines with exotic background (that is SPP462, SPGM94021 and SPMD94001 from ICRISAT, TX2794 and TX631 from USA and Keyman from Zimbabwe).

Sorghum seeds were sown in the field at equal intervals (every three weeks). The plants were carefully cultivated. Their panicles were harvested after 9 to 11 days since pollination, or when immature embryos were 1.0 to 2.0 mm in length. Immature embryos were aseptically isolated from freshly harvested sorghum panicles or sorghum heads stored at +4°C for no longer than two days prior to isolation. Panicles were surface sterilized by submerging each head in 50% Clorox bleach (5.25% sodium hypochlorite) supplemented with two drops of Tween-20, with constant stirring for 10 min. The panicles were subsequently rinsed three times with sterile distilled water and allowed to air dry in a laminar flow hood. Individual seeds were picked from the heads and subsequently sterilized in 70% ethanol for 1 min followed by one rinse with sterile distilled water and 20 min in 50% Clorox followed by three rinses with sterile distilled water. The seeds were then placed in an open Petri plate and allowed to briefly air dry in a laminar flow hood. The immature embryos were excised and used as explants.

Callus induction media

Aseptically excised immature embryos were cultured with scutellum side up on seven callus induction media (Table 1). All the media were MS-based (Murashige and Skoog, 1962) except medium CI3 which was an N6- based medium (Chu et al., 1975). The main difference among the six media (CI2, CI3, CI4, CI5, CI6 and CI7) was in the concentrations of 2,4-D and kinetin. In contrast, medium CI1 was supplemented with 0.5 mg L^{-1} nicotinic acid, 0.5 mgL^{-1} pyridoxine-HCl, 10 mgL^{-1} thiamine-HCl, 1.5 mgL^{-1} 2,4-D, 10 mgL^{-1} ascorbic acid and 2% sucrose. All callus induction media were supplemented with 0.5 gL^{-1} 2-(N-morpholino) ethanesulfonic acid (MES), 10 gL^{-1} polyvinylpyrrolidone (PVP) and 3% sucrose. Immature zygotic embryos were cultured for six weeks on callus induction media, and sub-cultured biweekly onto fresh media. Intervals between subcultures were reduced to one week when excess phenolic secretions were found. All callus cultures were maintained in dark at 25°C.

*Corresponding author. E-mail: shireen_assem@yahoo.com, sassem@ageri.sci.eg.

Abbreviations: ABA, Abscisic acid; **ANOVA,** analysis of variance; **BA,** 6-benzylaminopurine; **2,4-D,** 2,4-dichlorophenoxyacetic acid; **IAA,** indole-3-acetic acid; **IBA,** indole-3-butyric acid; **MES,** 2-(N-morpholino) ethanesulfonic acid; **MS,** Murashige and Skoog; **NAA,** α-naphthaleneacetic acid; **PVP,** polyvinylpyrrolidone; **TDZ**, thidiazuron.

Table 1. Callus induction media used in optimization of regeneration conditions. (amounts of ingredients per 1 liter medium).

Ingredient	Callus induction media						
	CI1	CI2	CI3	CI4	CI5	CI6	CI7
MS salts with vitamins	-	4.4 g	-	4.4 g	4.4 g	4.4 g	4.4 g
MS salts without vitamins	4.4 g	-	-	-	-	-	-
N6 salts with vitamins	-	-	4 g	-	-	-	-
Nicotinc acid	0.5 mg	-	-	-	-	-	-
Pyridoxine HCl	0.5 mg	-	-	-	-	-	-
Thiamine HCl	2.5 mg	-	-	-	-	-	-
2,4-D	1.5 mg	1.5 mg	1.5 mg	0.5 mg	2 mg	1 mg	0.5 mg
L-proline	0.7 g	0.7 g	0.7 g	0.7	0.7 g	0.7 g	0.7
Kinetin	-	-	-	0.5 mg	0.1 mg	0.2 mg	1 mg
Myo inositol	0.1 g	-	-	-	-	-	-
MES	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
PVP	10 g	10 g	10 g	10 g	10 g	10 g	10 g
Ascorbic acid	10 mg	-	-	-	-	-	-
Sucrose	2%	3%	3%	3%	3%	3%	3%

Table 2. Media used for the regeneration of sorghum lines (amounts of ingredients per 1 liter medium).

Ingredient	Shoot induction media			Rooting media	
	R_O	STR1M1	STR1M4	$Rooting_0$	$Rooting_1$
MS salts with vitamins		4.4 g	4.4 g	2.2	2.2
MS salts without vitamins	4.4 g	-	-		
Benzyl adenine	-	-	2 mg		
Kinetin	-	1.5 mg	-		
Myoinositol	0.1 g	-	-		
L-proline	0.7 g	0.7 g	0.7 g		
Nicotinic acid	0.5 mg	-	-		
Pyridoxine HCl	0.5 mg	-	-		
ThimanineHCl	10 mg	-	-		
Sucrose	6%	3%	3%	2%	2%
PVP	10 g	10 g	10 g		
IAA	1 mg	-	0.5 mg		
IBA				0.5 mg	-
NAA				0.5 mg	-
Zeatin	0.5 mg	-	-		
TDZ	0.1 mg	-	-		
ABA	0.25 mg	-	-		

Shoot regeneration and root formation

Three different shoot induction media and two rooting media (Table 2) were used for the all tested sorghum genotypes. All tissue culture media were adjusted to pH 5.8 and solidified with 0.3% phytagel.

Six weeks later, embryogenic calli were cultured for two to six weeks on shoot induction media. Cultures were incubated at 25 ± 2°C with a 16 h photoperiod. Light was provided by cool white fluorescent lamps at a photon flux density of 30 µmol m^{-2} s^{-1}. Regenerated shoots were transferred onto two different rooting media (Table 2) for root development.

Acclimatization and plant recovery

The regenerated plantlets were acclimatized in the greenhouse in an aquarium containing a modified Hoagland solution as recommended by Johnson et al. (1957). Healthy rooted plantlets were then transferred from Hoagland solution to pots containing a

mixture of peatmoss: soil: sand (1:1:1) under 16 h photoperiod in greenhouse adjusted at 22-25°C and 50% humidity.

Data collection and statistical analysis

For callus induction, each experiment was repeated three times, each consisting of 30-70 immature embryos. After six weeks on callus induction media, all embryogenic calli were equally distributed among the three regeneration media and the regeneration frequency was calculated as percentage of callus producing at least one shoot. Statistical analysis was performed according to Silva and Azevedo (2009) using ASSISTAT software, version 7.7. Experiments were designed as factorial experiments in a completely randomized design with three repetitions. Means of percentage were compared by t-test at a level of 5% of probability. The values of the standard errors were obtained using MSTAT-C software, version 4.

RESULTS AND DISCUSSION

Somatic embryogenesis and plant regeneration of sorghum have been significantly improved by screening the response of different genotypes on different growth regulators containing media. The effects of different media components on sorghum regeneration have been determined. The identification of regenerable sorghum genotypes other than those commonly used in the previous studies has been achieved.

Embryogenic callus induction

Sorghum has been suggested as one of the most recalcitrant plant species for regeneration and transformation (Zhao et al., 2000). Sorghum tissue culture recalcitrance is mainly due to genotype dependence, phenolic production, lacking of regeneration in long term *in vitro* culture, low frequency and prolonged phase of somatic embryos conversion into plantlets (Maheswari et al., 2006; Jogeswar et al., 2007; Raghuwamshi and Birch, 2010). Nowadays, crop agronomical and quality characteristics amelioration could not be achieved if without efficient regeneration and transformation systems. In the present study, we investigated callus initiation and regeneration potential of 10 sorghum genotypes on different nutrient media. Callus initiation was observed after few days of culturing on callus induction media showing two types of embryogenic calli: white compact embryogenic callus and creamy to yellowish embryogenic callus (Figure 1).

Effect of callus induction media

Seven different media were investigated for their potential to induce embryogenic callus from the 10 sorghum genotypes. Out of the seven investigated callus induction media, two media, that is, CI4 and CI7, failed to induce any embryogenic calli from any genotype under investigation. The results (Table 3) reveal that the media C11, C12 and CI3 had higher potentiality to induce embryogenic calli from the 10 genotypes as compared to media CI5 and CI6. The mean numbers of calli induced from the ten sorghum genotypes on media CI1, CI2 and CI3 were insignificantly different, that is 42.4, 41.3 and 40.8%, respectively although, they were significantly different from their respective values for media CI5 and CI6 (16.8 and 12.9%, respectively). The main difference between these two groups of media was that media CI5 and CI6 contain kinetin (0.1 and 0.2 mgL^{-1}, respectively), while medium CI1, CI2 and CI3 were devoid of kinetin. This could suggest that the addition of kinetin to the medium exerts a suppressing effect on the induction of embryogenic calli. Moreover, the results reveal that this suppressing effect was accentuated by increasing the concentration of kinetin in the callus induction medium as the mean numbers of calli on medium CI5 (0.1 mgL^{-1} kinetin) was higher than on medium CI6 (0.2 mgL^{-1}). Meanwhile, media CI4 (0.5 mgL^{-1} kinetin) and CI7 (1 mgL^{-1} kinetin) failed to produce any embryogenic calli. The negative effect of kinetin on callus culture is in consistence with the findings of Mastellar and Holden (1970), Wernicke and Brettell (1982), Zhao et al. (2010) and Muhumuza and Okori (2013) in sorghum. Similar effect of kinetin was noted by Lazar et al. (1983) and Rashid et al. (2009) in wheat. In addition, Li et al. (2009) reported that caryopses of indiangrass cultured on media supplemented with 2,4-D alone generally outperformed those cultured on media supplemented with both 2,4-D and kinetin for embryogenic callus induction. In contrast, the results of Arulselvi and Krishnaveni (2009) revealed that increasing the level of kinetin to a concentration of 0.5 mgL^{-1} in the I6 medium than in MS medium increased the frequency of embryogenic calli. This controversy could be attributed to the modification made in the I6 medium. Similarly, Pola et al. (2008) reported that the addition of kinetin to MS medium supported and improved frequency of embryogenesis.

The results (Table 3) also reveal that the mean number of embryogenic calli produced from different sorghum genotypes on media CI1, CI2 and CI3 were not significantly different. These three media, although contain the same concentration of 2,4-D (1.5 mgL^{-1}) and L-proline, they have different constituents. These results point out the importance of the auxin, 2,4-D as a main component of the embryogenic callus induction media. In accordance with our results, Rueb et al. (1994), Vikrant and Rashid (2003), Jogeswar et al. (2007) and Zhao et al. (2010) reported that the auxin 2,4-D is critical in the induction of primary calli and embryogenic calli in monocotyledon plants. In this respect, Muhumuza and Okori (2013) pointed out that the combined effects of kinetin and 2,4-D in callus induction medium, highly

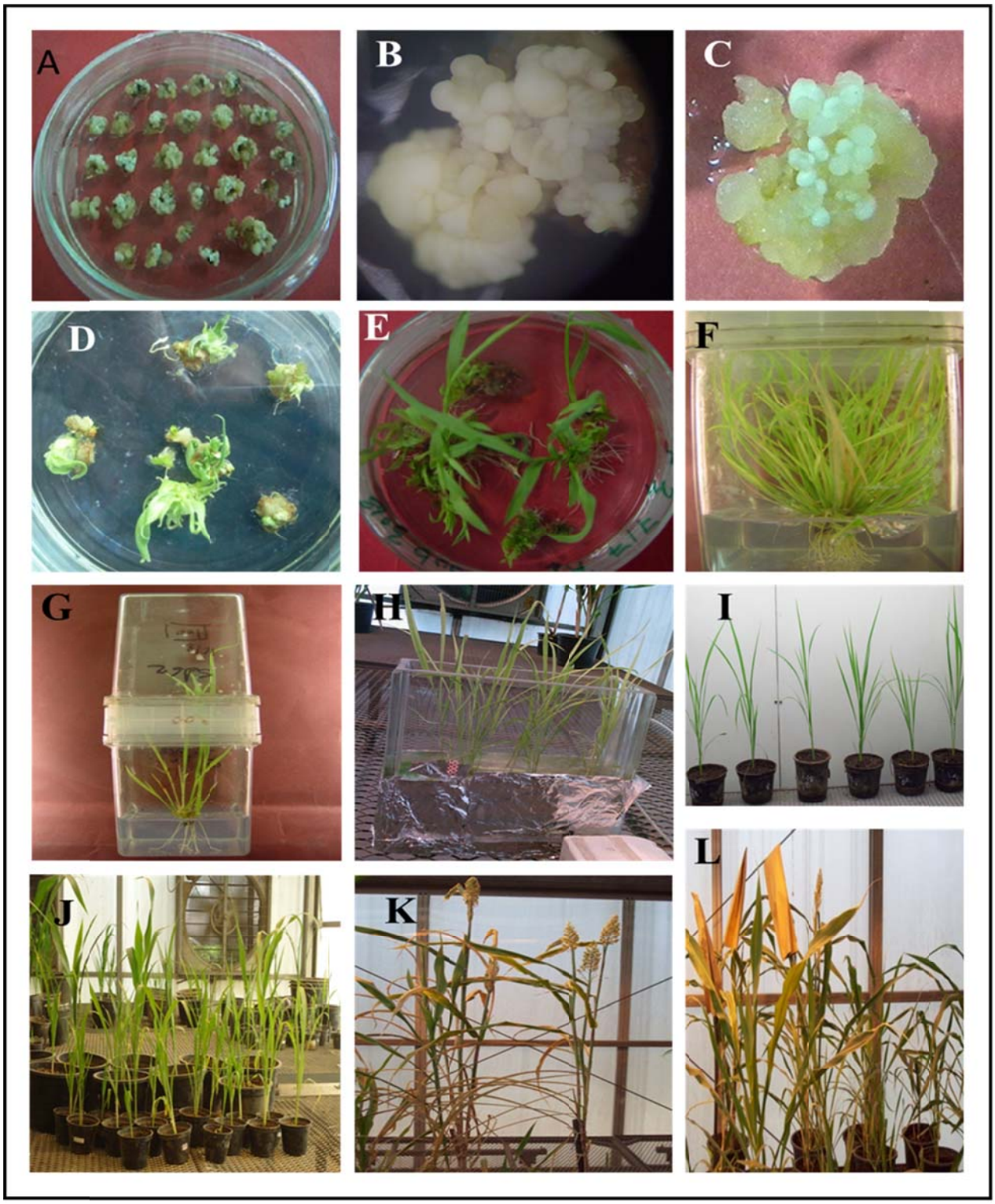

Figure 1. Steps for callus induction and regeneration of sorghum. **(A)** A plate of embryogenic calli. **(B)** White compact embryogenic callus. **(C)** Yellowish white embryogenic callus. **(D)** Shoot induction from embryogenic calli on regeneration media. **(E)** Shoot and root formation on shooting media. **(F)** Rooting with multiple shoots per callus. **(G)** Rooting with few shoots per callus. **(H)** Regenerated sorghum plantlets acclimatized in Hoagland solution in aquarium. **(I)** Regenerated sorghum plantlets acclimatized in growth chambers. **(J)** Regenerated plants in the greenhouse. **(K and L)** Mature regenerated plants in greenhouse.

Table 3. Influence of media composition on embryogenic callus induction in primary cultures of immature embryos of different sorghum lines.

Genotype	Type[b]	Percentage of embryogenic callus[a]					
		CI1	C12	C13	C15	C16	Mean
LG3	L	73.2 ± 7.2^{aA}	63.6 ± 6.9^{aAB}	54.3 ± 4.7^{bB}	17.0 ± 2.7^{abcdC}	29.6 ± 4.8^{aC}	47.5 ± 6.0^{A}
LG4	L	59.6 ± 11.5^{abA}	43.2 ± 7.4^{bcdeA}	51.6 ± 6.5^{bcA}	22.0 ± 3.0^{abcB}	19.1 ± 7.6^{abcB}	39.1 ± 5.2^{B}
LG8	L	50.9 ± 4.0^{bA}	36.8 ± 5.0^{cdefAB}	21.9 ± 6.3^{deBC}	13.6 ± 1.2^{abcdC}	17.2 ± 0.4^{abcC}	28.1 ± 4.0^{B}
Dorado	C	54.2 ± 7.5^{bA}	53.5 ± 4.0^{abA}	50.0 ± 12.3^{bcA}	13.0 ± 5.0^{bcdB}	7.0 ± 0.8^{bcdB}	35.5 ± 6.2^{BC}
Keyman	E	30.2 ± 5.1^{cB}	34.5 ± 5.8^{defB}	83.4 ± 5.1^{aA}	25.6 ± 3.9^{abB}	19.5 ± 0.9^{abcB}	38.6 ± 6.3^{B}
SPP462	E	21.5 ± 5.9^{cAB}	30.2 ± 5.1^{efA}	12.1 ± 1.7^{eBC}	3.3 ± 1.9^{dC}	0.0 ± 0.0^{dC}	13.4 ± 3.3^{G}
TX2794	E	15.1 ± 4.3^{cAB}	26.4 ± 0.9^{fA}	22.7 ± 1.3^{deAB}	7.3 ± 2.1^{cdB}	8.8 ± 0.6^{bcdB}	16.1 ± 2.1^{FG}
SPGM94021	E	50.3 ± 5.1^{bA}	52.6 ± 10.7^{abcA}	60.7 ± 2.6^{bA}	17.0 ± 6.9^{abcdB}	4.1 ± 3.5^{cdB}	36.9 ± 6.4^{BC}
SPMD94001	E	19.5 ± 3.1^{cA}	23.0 ± 3.6^{fA}	15.0 ± 4.6^{eA}	29.6 ± 3.0^{aA}	23.4 ± 3.5^{abA}	22.1 ± 1.8^{EF}
TX631	E	50.1 ± 12.0^{bA}	49.6 ± 10.7^{abcdA}	36.3 ± 5.4^{cdA}	19.3 ± 5.8^{abcdB}	0.0 ± 0.0^{dC}	31.0 ± 5.9^{CD}
Mean		42.4 ± 3.9^{A}	41.3 ± 2.9^{A}	40.8 ± 4.3^{A}	16.8 ± 1.7^{B}	12.9 ± 2.0^{B}	30.8 ± 1.7

[a]Each value is the mean percentage of three individual experiments. Numbers (mean ± S.E.) with different letters within the same line (uppercase) and column (lowercase) differ significantly at α=0.05. [b]L, local germplasm; E, elite line; C, cultivar.

significantly affected callus growth. The addition of kinetin hormone in the callus induction medium at low levels of 2,4-D significantly reduced the callus induction frequency of sorghum. Seemingly, higher 2,4- D levels exhibited inhibitory effects. Moreover, the addition of L-proline to the medium has been reported to enhance the frequency of embryogenic callus formation in cereals (Armstrong and Green, 1985; Perez et al., 1993; Rao et al., 1995; El-Itriby et al., 2003). Furthermore, the present results reveal that the N6-based medium (CI3) did not exert significant effect on callus induction from nine out of the 10 assayed genotypes, as compared to the MS -based media (CI1 and CI2) (Table 3).

Effect of the sorghum genotype on callus induction

For each genotype, an average of 150 immature embryos in three replicates was used as explants for the experiments of callus induction. As shown in the results (Table 3), callus induction was found to be highly variable and genotype dependent. The line LG3 revealed the highest average number of calli (47.5 ± 6.0%) across the media tested. This average number was significantly higher than its corresponding value for all the other genotypes. The mean number of calli induced by the different sorghum genotypes differed significantly with the lowest frequency of induced calli (13.4 ± 3.3%) produced by SPP462. Previous investigations on sorghum tissue culture also revealed that callus induction and regeneration are highly genotype dependent (Thomas et al., 1977; Cai and Butler, 1990; Devi and Sticklen, 2001; Arulselvi and Krishnaveni, 2009; Muhumuza and Okori, 2013).

Interaction between callus induction media and sorghum genotypes

Results presented in Table 3 show that the influence of the media composition on the induction of embryogenic callus varied significantly among tested sorghum genotypes.

The genotypes LG3, LG4, LG8, Dorado and TX631 showed the highest average of callus induction on medium CI1 which was the only MS-based medium supplemented with exogenous vitamins. In CI1 medium, the concentrations of the nicotinic acid (0.5 mgL^{-1}), pyridoxine (0.5 mgL^{-1}) and myoinositol (0.1 mgL^{-1}) were equal to their respective concentrations in the other tested medium supplemented with the MS vitamins. However, the concentration of thiamine was higher in medium CI1 (10 mgL^{-1}) as compared to 0.1 mgL^{-1} in MS with vitamins. This reflects the positive effect of increasing the thiamine concentration in the medium of certain genotypes. Thiamine has been reported to exert diverse physiological functions in plants and serves as cofactor in enzymatic reactions, but plant cell requirements for vitamin concentration vary according to the plant species and type of culture (Goyer, 2010; Abrahamian and Kantharajah, 2011). Moreover, medium CI1 had an additional 10 mgL^{-1} ascorbic acid. Ascorbic acid acts in plants as an antioxidant in addition to its enzymatic cofactor activity, thus reducing phenolics secretion and improving regenerability. This finding is consistent with those of Abdelwahd et al. (2008) on *Vicia faba*.

The two genotypes SPP462 and TX2794 responded better on callus induction medium C12 containing MS with vitamins. Thus, revealing that excess thiamine and

Table 4. ANOVA for callus induction in sorghum genotypes.

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F* value
Genotypes (G)	9	16094.87260	1788.31918	17.1955**
Media (M)	4	25969.57493	6492.39373	62.4273**
Interaction (GXM)	36	17930.25973	498.06277	4.7891**
Treatment	49	59994.70727	1224.38178	11.7730**
Error	100	10399.92667	103.99927	

**Significant at a level of 1% of probability ($p < 0.01$).

ascorbic acid did not increase the capability of these genotypes to induce embryogenic calli.

The frequency of embryogenic calli of only genotype "Keyman" was significantly higher on medium CI3 (N6-based medium) than on the MS-based media. This result is in partial accordance with Sato et al. (2004). They did not observe any embryogenic callus on N6 medium with four sorghum tested lines. The variable responses of the different sorghum genotypes during callus induction and embryogenesis on MS and N6 media can be attributed to the different nitrogenous compounds in these two media. This is in consistence with the suggestion of Armstrong and Green (1985) with maize. Moreover, Hodges et al. (1986) pointed out that the form of nitrogen, which is different in N6 and MS media, may have a fundamental role in the expression of genes that control the embryogenic process.

The frequency of embryogenic calli of the genotype SPMD94001 was generally low on all the media tested. However, the highest frequency of calli in this genotype was induced by medium CI5 (29.6%) which is an MS-based medium supplemented by 0.1 mgL^{-1} kinetin. Conversely, the lowest frequency of calli was induced by the N6-based medium CI3. These result reveals the slightly positive effect of low level of kinetin on the callus induction ability of this genotype, which is in disagreement with Mastellar and Holden (1970), Wernicke and Brettell (1982), Zao et al. (2010) and Muhumuza and Okori (2013).

The analysis of variance (ANOVA) (Table 4) indicated that the response to callus initiation was influenced by genotype and callus induction medium independently and thus their interaction had a greater effect. In addition, media effect was higher than genotype effect (6462.39373, for media effect vs. 1788.31918, for genotype effect).

Secretion of phenolic compounds by callus tissues had a great effect on the embryogenic callus induction and regeneration frequency. Although 1% of PVP was added to all callus induction media, our observations indicate that some genotypes produced phenolic compounds in presence of PVP, which had negative effect on the formation of embryogenic callus as well as regeneration ability. To overcome this problem, frequent subcultures have been carried out in less than two weeks intervals. Dorado and LG3 showed no secretion of phenolics. In contrast, all the other tested lines showed different degrees of phenolics, at least in one experiment or on a certain type of media. The genotypes LG8 and TX631 rarely secreted phenolic compounds. This was followed by SPGM94021 and SPMD94001 which showed weak phenolics secretion. The line LG4 revealed medium secretion, while the lines Keyman, SPP462 and TX2794 exhibited high degree of phenolic secretion.

Embryogenic callus cultures in cereal species were previously described and classified into two morphotypes, differing by morphology, growth rate and extent of embryo differentiation. These two types are: compact white embryogenic (type I) and friable embryogenic (type II) (Elkonin and Pakhomova, 2000). In maize and other cereals, type II embryogenic cultures have higher growth rate and higher regeneration frequency than the compact type I callus cultures (Armstrong and Green, 1985; El-Itriby et al., 2003). With different genotypes of sorghum, we observed the two types of embryogenic callus cultures. Unlike maize, both types of embryogenic callus were regenerable and in some cases, the white compact type I had higher frequencies of regeneration than type II embryogenic callus. In consistence with the present results, Grootboom et al. (2008) reported the formation of highly embryogenic totipotent type I callus in sorghum.

Regeneration of the sorghum genotypes

The different sorghum genotypes exhibited different regeneration ability. Out of the 10 tested sorghum genotypes, only seven were successfully regenerated on at least two of the three examined regeneration media. The number of shoot per callus ranged from 2 to 17 shoots (Figure 1F and G). The calli of the genotypes Keyman, SPP462 and TX2794 failed to regenerate on any of the three regeneration media. Although these lines produced a high frequency of embryogenic calli with mean values of 38.7, 13.4 and 16.0%, respectively, across the seven tested media, these calli failed to develop any shoots. This could be attributed to the high level of phenolic compounds produced by these genotypes. Phenolic compounds secretion has been previously revealed by many authors in sorghum among

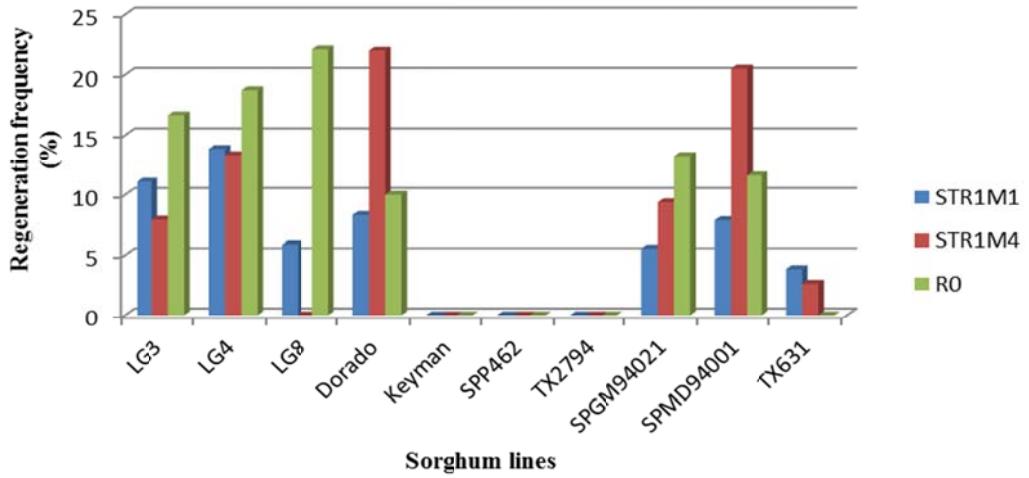

Figure 2. Effect of media composition on shoot formation of different sorghum lines.

other plant species to exert an inhibitory effect on callus regeneration (Oberthur et al., 1983; Dicko et al., 2006; Zhao et al., 2010).

Interaction between regeneration media and sorghum genotypes

The effect of the three investigated regeneration media on the capability of the 10 sorghum genotypes to develop shoots is represented as a histogram (Figure 2). The regeneration percentage ranged from 0 to 22.1%. The genotypes LG3, LG4, Dorado, SPGM94021 and SPMD94001 succeeded to form shoots on the three tested media. Nevertheless, the genotypes LG8 and TX2794 produced shoots on only two out of the three media.

The local lines LG3, LG4 and LG8, in addition to the genotype SPGM94021 revealed the highest percentage of regenerated shoots on medium R0 (16.66, 18.75, 22.1 and 13.25%, respectively). The highest number of shoots formed by the genotypes Dorado and SPMD94001 was on medium STR1M4. Meanwhile, the genotype TX631 formed shoots on only two regeneration media (STR1M1 and STR1M4) with low frequency, although it was slightly higher on STR1M1 than on MSTRTM4.

The regeneration medium R0 in contrast to the other two regeneration media was supplemented with different growth regulators, IAA, zeatin, TDZ and abscisic acid (ABA). This might reflect the demand of the genotypes LG3, LG4, LG8 and SPGM94021 to these growth regulators for enhancing the process of shoot formation and development; while, shoot formation in Dorado and SPMD94001 is more influenced by the addition of BA and IAA (2 and 500 mgL^{-1}, respectively). In this respect, different authors reported the use of different growth regulators to improve the regeneration efficiency. Visarada et al. (2003) obtained efficient regeneration from immature embryo derived calli using 0.5 mgL^{-1} BA + IBA. Rathus et al. (2004) revealed that a combination of 1 mgL^{-1} IAA and 1 mgL^{-1} zeatin gave the best results for shoot regeneration in sorghum. Correspondingly, Zhao et al. (2010) observed the best shoot induction when the explants were cultured on MS medium supplemented with 1 mgL^{-1} IAA and 3 mgL^{-1} BA and on MS medium supplemented with 2 mgL^{-1} BA and 0.5 mgL^{-1} kinetin.

The regenerated shoots were transferred to rooting media. Two rooting media that is "rooting 0" containing 0.5 mgL^{-1} of each IBA and α-naphthaleneacetic acid (NAA) and "rooting 1" free of growth regulators were examined. In all the tested genotypes, short, thick and poor rooting were observed on Rooting 0, while, long, strong and well develop roots were observed on rooting 1. Conversely, Rao and Kavikishore (1989) and Pola and Mani (2006) obtained good rooting on MS medium supplemented with NAA. Seemingly, Zhao et al. (2010) transferred the regenerated shoots onto MS medium supplemented with 3 mgL^{-1} BA for rooting.

Some of the plantlets died when rooted plantlets were transferred directly to pots in greenhouse for acclimatization. This loss in the regenerated plantlets was significantly reduced when plantlets were transferred to an aquarium containing Hoagland solution (Figure 1H) before transferring to the pots and all of the survived plants reached maturity and found to be fertile (Figure 1K and L). Hydroponic culture were found to aid in plantlets acclimatization by providing a humid atmosphere, allowing the formation and ramification of new roots. In this respect, Pospíšilová et al. (1999) pointed out that the roots of the regenerated plantlets are fragile and susceptible to mechanical damage. For that reason, the plantlets may die shortly after transplanting into pots. Similarly,

Zapata et al. (2003) reported a significant increase in the number of leaves and roots in hydroponic system compared to control.

The results of the present investigation revealed different responses to *in vitro* culture and clear interactions of the sorghum genotypes with the different nutrient media. Thus, determination of the best genotype–medium combination is essential to obtain satisfactory regeneration frequency. This finding has been also reported by Assem (2001), Grootboom et al. (2008) and Arulselvi and Krishnaven (2009). Moreover, these results confirm that in sorghum, as in other cereals, *in vitro* response, that is the efficiency of callus induction and plant regeneration frequency are highly genotype dependent. This has been previously reported by different authors: in wheat (Yadava and Chawla, 2001), in maize (El-Itriby et al., 2003), in barley (Hussein et al., 2010) and in sorghum (Jogeswar et al., 2007; Grootboom et al., 2008).

In conclusion, most of the previous studies on *in vitro* culture of sorghum were on highly regenerable and tissue culture amenable genotypes, such as, P898012, BTX 430 and BTX 623 (Kishore et al., 2006; Girijashankar et al., 2007; Grootboom et al., 2008). Nevertheless, the present work investigated different Egyptian adapted sorghum genotypes of different origins and agronomical importance. These genotypes include tall, short, drought tolerant, drought susceptible, cultivated and tropical sorghum.

The genotypes tested showed a variation of genotypic responses to *in vitro* culture. From the obtained *in vitro* culture results, the genotypes of choice for transformation experiments would be in that order of priority, LG3 with media CI1 and R0, SPMD94021 with media CI3 and R0, LG4 with media CI1and R0, Dorado with media CI1 and STR1M4 and LG8 with medium CI1 or CI2 and R0. These combinations display the best regeneration potential and should then increase the probability of producing transgenic plants using these lines.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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REFERENCES

Abdelwahd R, Hakam N, Labhilili M, Udupa SM (2008). Use of an adsorbent and antioxidants to reduce the effects of leached phenolics in *in vitro* plantlet regeneration of faba bean. Afr. J. Biotechnol. 7: 997-1002.

Abrahamian P, Kantharajah A (2011). Effect of vitamins on *in vitro* organogenesis of plant. Amer. J. Plant Sci. 2:669-674.

Armstrong CL, Green CE (1985). Establishment and maintenance of friable, embryogenic maize callus and the involvement of L-proline. Planta 164:207-214.

Arulselvi IP, Krishnaveni S (2009). Effect of hormones, explants and genotypes in *in vitro* culturing of sorghum. J. Biochem. Technol. 1(4):96-103.

Assem SK (2001). Callus production and plant regeneration in Egyptian maize genotypes. Arab J. Biotechnol. 4(2):247-256.

Bhaskaran S, Neumann AJ, Smith RH (1988). Origin of somatic embryos from cultured shoot tips of *Sorghum bicolor* (L.) Moench. *In vitro* Cell Dev. Biol. 24:947-950.

Bhaskaran S, Smith RH (1988). Enhanced somatic embryogenesis in *Sorghum bicolor* from shoot tip culture. *In Vitro* Cell. Dev. Biol. 24:65-70.

Bhaskaran S, Smith RH (1989). Control of morphogenesis in sorghum by 2,4-dichlorophenoxy acetic acid and cytokinins. Ann. Bot. 64:217-224.

Boyes CJ, Vasil IK (1984). Plant regeneration by somatic embryogenesis from cultured young inflorescences of *Sorghum arundinaceum* (Desv.) Stapf var. *sudanese* (Sudan grass). Plant Sci. Lett. 35: 153-157.

Brar DS, Rambold S, Gamborg O, Constable F (1979). Tissue culture of corn and sorghum. Z. Pflanzenphysiol. 95: 377-388.

Cai T, Butler L (1990). Plant regeneration from embryogenic callus initiated from immature inflorescence of several high tannins sorghum. Plant Cell Tissue Org. 20: 101-110.

Chandrakanth E, Sunil KG, Rathore KS (2002). Transgene silencing and reactivation in sorghum. Plant Sci. 162: 181-192.

Chu CC, Wang CC, Sun CS, Hsu C, Yin KG, Chu CY, Bi FY (1975). Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci. Sinica 5: 659-668.

Devi PB, Sticklen MB (2001). Culturing shoot tip clumps of *Sorghum bicolor* and optimal microprojectile bombardment parameters for transient expression. J. Cytol. Genet. 2: 89-96.

Dicko MH, Gruppen H, Traore AS, Voragen AG, Vanberkel WJH (2006). Phenolic compounds and related enzymes as determinants of sorghum for food use. Biotechnol. Mol. Biol. Rev. 1: 21-38.

Eapen S, George L (1990). Somatic embryogenesis and plant regeneration in inflorescence segments of *Sorghum versicolor*. Maydica 35: 55-58.

El-Itriby HA, Assem SK, Hussein, EHA, Abdel-Galil FM, Madkour MA (2003). Regeneration and transformation of Egyptian maize inbred lines *via* immature embryo culture and biolistic particle delivery system. *In Vitro* Cell. Dev. Biol. 39(5): 524-531.

Elkonin LA, Pakhomova NV (2000). Influence of nitrogen and phosphorus on induction embryogenic callus of Sorghum. Plant Cell Tissue Org. 61: 115-123.

FAO, STAT (2010). www.Fao.org.

Girijashankar V, Sharma KK, Balakrishna P, Seetharama N (2007). Direct somatic embryogenesis and organogenesis pathway of plant regeneration can seldom occur simultaneously within the same explant of sorghum. J. SAT Agric. Res. 3(1): 3 pp.

Goyer A (2010). Thiamine in Plants: Aspects of Its Metabolism and Functions. Phytochemistry 71(14-15): 1615-1624.

Grootboom AW, O'Kennedy MM, Mkhonza NL, Kunert K, Chakauya E, Chikwamba RK (2008). *In vitro* culture and plant regeneration of sorghum genotypes using immature zygotic embryos as explant source. Int. J. Bot. 4(4): 450-455.

Gupta S, Khanna VK, Rameshwar S, Garg GK (2006). Strategies for overcoming genotypic limitations of *in vitro* regeneration and determination of genetic components of variability of plant regeneration traits in Sorghum. Plant Cell Tissue Organ Cult. 86: 379-388.

Hagio T (2002). Adventitious shoot regeneration from immature embryo of sorghum. Plant Cell Tissue Org. 68: 65-72.

Harshavardhan D, Rani TS, Ulaganathan S, Seetharama N (2002). An improved protocol for regeneration of *Sorghum bicolor* from isolated shoot apices. Plant Biotechnol. 19(3): 163-171.

Hodges TK, Kamo KK, Imbrie CW, Beswar MR, Schroll S (1986). Genotype specificity of somatic embryogenesis and regeneration in maize. Nat. Biotechnol. 4: 219-223.

Hussein EHA, Sakran MA, Hazman MY, Haider ASE, El–Wassief AM (2010). Development of an efficient regeneration system for Egyptian cultivar(s) of barley *(Hordeum vulgare* L.). Egypt J. Genet. Cytol. 39:259-270.

Ignacimuthu S, Premkumar A (2014). Development of transgenic *Sorghum bicolor* (L.) Moenchresistant to the *Chilo partellus* (Swinhoe) through *Agrobacterium*-mediated transformation. Mol. Biol. Genet. Eng. 2:1.

Jeoung JM, Krishnaveni S, Jayaraj J, Haying Y, Trick HN, Muthukrishna S, Liang GH (2002). *Agrobacterium* mediated transformation of grain Sorghum. In: "Sorghum Tissue Culture and Transformation" (eds. Seetharama N, Godwin I). Oxford IBH Pub. 57-64.

Jogeswar G, Ranadheer D, Anjaiah V, KaviKishor PB (2007). High frequency somatic embryogenesis and regeneration in different genotypes of *Sorghum bicolor* (L.) moench from immature inflorescence explants. *In vitro* Cell. Dev. Biol. Plant 43:159-166.

Johnson CM, Stout PR, Broyer RC, Carlton AB (1957). Comparative chlorine requirements of different plant species. Plant Soil 8:337-353.

Kingsley AP, Ignacimuthu S (2014). Enhanced plant regeneration involving somatic embryogenesis from shoot tip explants of *Sorghum bicolor* (L. Moench). Asian J. Plant Sci. Res. 4: 26-34.

Kishore SN, Visarada KBRS, Aravinda LY, Pashupatinath E, Rao SV, Seetharama N (2006). *In vitro* culture methods in sorghum with shoot tip as the explant material. Plant Cell Rep. 25: 174-182.

Lazar M, Collins G, Vian W (1983). Genetic and environmental effects on the growth and differentiation of wheat somatic cell cultures. J. Hered. 74:353-358.

Li Y, Gao J, Fei S (2009). High frequency *in vitro* embryogenic callus induction and plant regeneration from indiangrass mature caryopsis. Sci. Hortic. 119(3):306-309.

Maheswari M, Jyothilakshmi N, Yadav SK, Varalaxmi Y, Vijaya LA, Vanaja M, Venkateswarlu B (2006). Efficient plant regeneration from shoot apices of sorghum. Biol. Plantarum 50(4): 741-744.

Manjula SM, Kuruvinashetti MS, Harti CC (2000). Regeneration establishment and evaluation of somaclones in *Sorghum bicolor* (L.) Moench. Euphytica 115(3): 173-180.

Mastellar VJ, Holden DJ (1970). The growth of and organ formation from callus tissue of sorghum. Plant Physiol. 45: 362-364.

Mishra A, Khurana P (2003). Genotype dependent somatic embryogenesis and regeneration from leaf base cultures of *Sorghum bicolor*. J. Plant Biochem. Biotechnol. 12:53-56.

Muhumuza JB, Okori P (2013). Development of a highly efficient *in vitro* culture system for Ugandan adapted sorghum genotypes. Int. Res. J. Plant Crop Sci. 1(1):1-11.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473-497.

Nahdi S, de Wet JMJ (1995). *In vitro* regeneration of *Sorghum bicolor* lines from shoot apices. ISMN 36:89-90.

Nguyen T, Thu TT, Claeys M, Angenon G (2007). *Agrobacterium*-mediated transformation of sorghum (*Sorghum bicolor* (L.) Moench) using an improved *in vitro* regeneration system. Plant Cell Tissue Organ 91:155-164.

Oberthur E, Nicholson RL, Butler LG (1983). Presence of polyphenolic materials, including condensed tannins, in sorghum callus. J. Agric. Food Chem. 31: 660-662.

Perez EA, Patena LF, Logrono ML (1993). Embryogenic and organogenic callus induction, maintenance, and plantlet regeneration in maize (*Zea mays* L,) cv IPB Var 4 and inbred line Pi23. Philip. J. Crop Sci. 18(1): 31-35.

Pola S, Mani NS, Rammana T (2008). Plant tissue culture studies in *Sorghum bicolor*: immature embryo explants as the source material. J. Plant Prod. 2(1):1-14.

Pola SR, Mani NS (2006). Somatic embryogenesis and plantlet regeneration in *Sorghum bicolor* (L.) Moench, from leaf segments. J. Cell. Mol. Biol. 5:99-107.

Pospíšilová J, Synková H, Haisel D, Čatský J, Wilhelmová N, Šrámek F (1999). Effect of elevated CO_2 concentration on acclimation of tobacco plantlets to *ex vitro* conditions. J. Exp. Bot. 50: 119-126.

Raghuwamshi A, Birch RG (2010). Genetic transformation of sweet sorghum. Plant Cell Rep. 29(9):997-1005.

Rao AM, Kavikishore KPB (1989). *In vitro* plant regeneration potential from callus cultures of grain sorghum. Curr. Sci. 58:693-696.

Rao AM, Padma SK, Kavi KPB (1995). Enhanced plant regeneration in grain and sweet sorghum by asparagine, proline and cefotaxime. Plant Cell Rep. 15(1-2):72-75.

Rashid U, Ali S, Ali GM, Ayub N, Masood MS (2009). Establishment of an efficient callus induction and plant regeneration system in Pakistani wheat (*Triticum aestivum*) cultivars. eJ. Biotechnol. 12 (3):1-12.

Rathus C, Nguyen T, Able JA, Gray SJ, Godwin D (2004). Optimizing sorghum transformation technology *via* somatic embryogenesis. In: "Sorghum Tissue Culture and Transformation" (eds. Seetharama N, Godwin I). Sci. Pub. pp. 25-34.

Rueb S, Leneman M, Schilperoor RA, Hensgens LAM (1994). Plant regeneration through somatic embryogenesis from callus induced on mature rice embryos (*Oryza sativa* L.). Plant Cell Tissue Org. 36(2): 259-264.

Sairam RV, Devi PS, Murty UR, Seetharama N, Potrikus I (1999). Plant regeneration from mesophyl protoplasts in sorghum (*Sorghum bicolor* (L.) Moench). Plant Cell Rep. 18: 972-977.

Sairam RV, Seetharama N, Shyamala T (2000). Plant regeneration from scutella of immature embryos of diverse sorghum genotypes. Cereal Res. Commun. 28: 279-285.

Sato S, Clemente T, Dweikat I (2004). Identification of an elite sorghum genotype with high *in vitro* performance capacity. *In vitro* Cell. Dev. Biol. Plant 40: 57-60.

Seetharama N, Sairam RV (1997). Regeneration of plants from and transient gene expression in mesophyll protoplasts of sorghum. In: "Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl millet". Lubbock, Texas, U. S. A. 22- 27 Sep. 1996, Collaborative Research Support Program on Sorghum and Pearl Millet, Lincoln, Nebraska, U.S. A. pp. 674-675.

Seetharama N, Sairam RV, Rani TS (2000). Regeneration of *Sorghum bicolor* (L.) Moench from shoot tip cultures. Plant Cell Tissue Org. 61: 169-173.

Silva FASE, Azevedo CAV (2009). Principal components analysis in the software Assistat-Statistical Attendance. In: "World Congress on Computers in Agriculture, 7" American Society of Agricultural and Biological Engineers, Reno-NV-USA.

Thomas E, King PJ, Potrykus I (1977). Shoot and embryo-like structure formation from cultured tissues of *Sorghum bicolor*. Naturewissenschaften 64: 587-595.

Vikrant, Rashid A (2003). Somatic embryogenesis or shoot formation following high 2,4-D pulse-treatment of mature embryos of *Paspalum scrobiculatum*. Biol. Plant 46:297-300.

Visarada KBRS, SaiKishore N, Balakrishna D, Rao SV (2003). Transient *gus* expression studies in Sorghum to develop a simple protocol for *Agrobacterium* mediated genetic transformation. J. Genet. Breed. 57:147-154.

Wernicke W, Brettell RIS (1982). Morphogenesis from cultured leaf tissue of *Sorghum bicolor* (L.) culture initiation. Protoplasma 111:19-27.

Yadava R, Chawla HS (2001). Interaction of genotype, growth regulators and amino acids on plant regeneration from different developmental states of influorescence in wheat. J. Genet. Breed. 55: 261-267.

Zapata EV, Morales GS, Lauzardo ANH, Bonfil BM, Tapia GT, Sanchez ADJ, Valle MVD, Aparicio AJ (2003). *In vitro* regeneration and acclimatization of plants of turmeric (*Curcuma longa* L.) in a hydroponic system. Biotecnol. Appl. 20:25-31.

Zarif M, Sadia B, Kainth RA, Khan IA (2013). Genotypes, explants and growth hormones influence the morphogenesis in Pakistani sorghum (*Sorghum bicolour*): preliminary field evaluation of sorghum somaclones. Int. J. Agric. Biol. 15: 1157-1162.

Zhao L, Liu S, Song S (2010). Optimization of callus induction and plant regeneration from germinating seeds of sweet sorghum (*Sorghum bicolor* Moench). Afr. J. Biotechnol. 9(16): 2367-2374.

Zhao ZY Cai T, Tagliani L, Miller M, Wang N, Pang H, Rudert M, Schroeder S, Hondred D, Seltzer J, Pierce D (2000). *Agrobacterium*-mediated sorghum transformation. Plant Mol. Biol. 44: 789-798.