African Journal of Biotechnology Vol. 7 (11), pp. 1759-1764, 3 June, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Regeneration of Sudanese maize inbred lines and open pollinated varieties

Rasha Adam Omer[1, 2], Abdelbagi M. Ali [2], Jonathan M. Matheka[1] and Jesse Machuka[1]*

[1]Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844, Nairobi, Kenya.
[2]Agricultural Research Corporation, P.O. Box 126, Wad Medani, Sudan.

Accepted 10 January, 2008

Eight maize inbred lines and three open pollinated varieties from Sudan were evaluated for their response to tissue culture. Immature embryos obtained 16 days after pollination were used as explants for callus induction. Calli were induced on LS medium supplemented with 2 mg/L 2,4-dichlorophenoxyacetic acid. Callus induction capacity was highest in inbred lines IL3, IL15 and IL1. The Varieties Hudiba-2 and Hudiba-1 were not statistically different (p >0.05) in callus induction. The capacity for embryogenic callus formation was highest in inbred line IL3 followed by IL1 and IL38 and in varieties Hudiba-2 and Hudiba-1. Inbred lines IL16, IL42, IL43 and IL28 had the lowest embryogenic callus formation capacity. Plant regenerating genotypes were IL3, IL38, IL15, IL1, Hudiba-2 and Mojtamaa-45. Inbred line IL3 was the most regenerable genotype with a shoot formation frequency of 76% averaging 6 shoots per callus. The highest regenerating variety was Mojtamaa-45, which averaged 5 shoots per callus.

Keywords: Sudanese maize genotypes, embryogenic callus, regeneration, and tissue culture.

INTRODUCTION

Maize is an important food and forage crop in the Northern and Southern regions of Sudan (Abdelrahman et al., 2001; FAO, 2000). Identification of Sudanese genotypes that respond well to embryogenic callus induction and plant regeneration is a necessary initial step for their successful genetic transformation. Regeneration of tropical (Kenyan) maize was reported by (Oduor et al., 2006). However there is no report on tissue culture response of Sudanese maize. This study therefore sought to identify Sudanese inbreds and open pollinated varieties (OPV) that are good responders to embryogenic callus formation and plant regeneration.

Maize tissue cultures have been initiated from virtually all plant parts but these have been found to be largely non regenerable. Immature embryos have been the most widely used explants for initiation of regenerable tissue cultures (Armstrong and Green, 1985; Phillips et al., 1988). Immature embryos can initiate two types of callus cultures from their scutella surfaces: Type I and type II callus. Type I is compact and organogenic and easily obtained from immature embryos. On the other hand, type II is friable and embryogenic and is initiated at a lower frequency than type I (Carvalho et al., 1997). Only a few tropical genotypes have been shown to be capable of initiating type II callus (Oduor et al., 2006; Carvalho et al., 1997). Type II callus has been found to be more regenerable than type I (Armstrong and Green, 1985).

Maize plant regeneration can take place through two avenues, that is, organogenesis or somatic embryogenesis. Organogenesis involves the formation of organs (shoots and roots) directly from the explant, or indirectly via callus formation first. On the other hand somatic embryogenesis involves the formation of scutella-like structures from the explant or callus (Slater et al., 2004). Somatic embryogenesis is the most common avenue of plant regeneration (Odour et al., 2006; Phillips et al., 1988).

This study reports, for the first time, the embryogenic callus induction and plant regeneration response of Sudanese maize inbred lines and open pollinated varieties.

*Corresponding author. E-mail: machuka@ku.ac.ke. Tel: +254-020-810245. Fax: +254-020-811575.

Abbreviations: IM, induction medium; PM, proliferation medium; MM, maturation medium; R1, regeneration medium.

Table 1. Genetic background of selected Sudanese maize inbred line studied.

Code	Pedigree
IL1	(Local x Ring-1)-A-S1-1
1L3	(Local x Ring-1)-B-S1-1
IL15	(Ring-7 x L.Abiad)-B-S1-3
IL16	(Ring-7 x L.Abiad)-C -S1-1
IL28	(Ring-8)-S1-1
IL38	(Ring-8)-A-S1-7
IL42	(Ring-8)-B-S1-4
IL43	(PR-89B-5655)-S1-1

The Sudanese maize genotypes were obtained from the Agricultural Research Cooperation (ARC) in Sudan.

MATERIALS AND METHODS

Plant material

Eight inbred lines (Table 1) and three OPVs (Hudiba 1, Hudiba 2 and Mojtamaa-45) were obtained from the Agricultural Research Cooperation (ARC) in Sudan and evaluated in tissue culture to identify genotypes capable of high embryogenic callus induction and plant regeneration. Seeds were planted in the screen house of Plant Transformation Laboratory (PTL) at Kenyatta University.

Callus formation

Immature embryos were extracted from plants, 16 days after pollination, under sterile conditions as reported by Oduor et al. (2006). To induce the formation of embryogenic callus, the immature embryos were cultured on the basal medium reported previously (Oduor et al., 2006) consisting of LS salts and vitamins (Linsmaier and Skoog, 1965), supplemented with 30 g l^{-1} sucrose, 2.9 g l^{-1} proline, 0.1 g l^{-1} casein hydrolysate and 2 mg l^{-1} 2,4-D (2,4-dichloroacetic acid). This media formulation was referred to as IM. Callus induction was achieved using thirty embryos per Petri dish (15 x 100 mm) with four Petri dishes per genotype. Callus cultures were maintained on proliferation media (PM) (Odour et al., 2006) and the PM refreshed after every 14 days. The percentage of type I and type II callus was determined after 21 days of culture on PM.

Plant regeneration

After six weeks of callus induction, all calli except the necrotic and dying ones, were transferred to LS basal medium (Linsmaier and Skoog, 1965) containing 60 g l^{-1} sucrose and lacking 2,4-D (referred to as maturation or RI medium) and cultured for 14 days to initiate shoots. For induction of a strong root system, shoot-inducing calli were then transferred to rooting medium (RII) comprising MS basal salts and vitamins (Murashige and Skoog, 1962), supplemented with 30 g l^{-1} sucrose and devoid of hormones, casein hydrolysate or proline. The number of regenerated plantlets per calli was evaluated 24 days after regeneration.

Data analysis

Differences in embryogenic callus induction and regeneration frequency among the genotypes were analysed using the Genstat for windows (Discovery edition) statistical software. Data were square root transformed before analysis.

RESULTS AND DISCUSSION

Callus formation

Zygotic embryos responded to culture on IM by swelling to almost twice the initial size after 3-5 days. The colour of the embryos was unchanged during culture on IM. Callus formation was induced for all the genotypes. After three to seven days of embryo culture on IM, callus formation started with the swelling of the embryo on the middle portion of scutellum as well as the basal side.

The inbred lines IL15, IL28, IL16 and the OPV Hudiba-2 formed hair-like structures on the scutella surface of their embryos on IM medium (Figure1C). Such a response has also been reported by Bronsema et al (1997) who observed hair formation on embryo scutella surface of the temperate tropical inbred line A632 on callus induction media. This response is genotype specific since the other inbred lines and OPVs did not respond similarly.

Callus induction varied from 85.0% for IL3 to 23.33% for IL16 (Table 2). Inbred lines IL3 and IL15 were observed to induce calli easily and rapidly. These genotypes had significantly higher ($p < 0.05$) callus induction capacity than the other inbred lines (Table 2). Inbred line IL16 produced calli with difficulty and embryos were observed to swell marginally before becoming necrotic and dying. Among the OPVs, Hudiba-2 had the highest capacity to form callus (Table 2).

The type of calli induced by the different genotypes became apparent on PM. Some genotypes produced callus comprising somatic embryos while others formed nonembryogenic calli. These two different callus types have been designated type I and type II (Armstrong and Green, 1985). Type I callus is dirty white, compact and organogenic while type II is white friable and embryogenic. The genotypes IL3, Hudiba-2, Mojtamaa-45, IL15, IL1 and IL38 formed Type II calli (Figure1A). Among the inbreds IL3 showed the highest capacity for type II callus formation, while IL15 showed the lowest (Table 2). All OPVs formed embryogenic calli. However the OPV Hudiba-2 was observed to have the highest capacity for type II callus induction than Hudiba-1. Type I callus was formed by IL28, IL16, IL43 and IL42 (Figure 1B).

The type of callus induced has been shown to be dependent on the genotype (Carvalho et al., 1997). Two genes have been implicated in the inheritance of callus induction and plant regeneration. These genes find expression in the middle and basal portion of the scutellum of immature embryo. In case of responsive genotypes, these regions proliferate to form embryogenic callus while in non-embryogenic genotypes, they do not (Bronsema et al., 1997).

The two types of callus were also observed on the same embryo for some genotypes such as IL15. This observation is similar to that of Bronsema et al. (1997)

Table 2. Effect of genotype on embryogenic callus and callus induction among Sudanese maize.

Genotype	Number of experiments	Embryogenic callus induction (%)	Callus induction (%)
Mojtamaa-45	4	15.000±0.950	68.332±0.213
Hudiba 1	4	19.168±0.2 73	74.168±0.175
Hudiba 2	4	30.833±0.842	83.332±0.195
IL3	4	54.168±0.973	85.000±0.188
IL16	4	0.000±0.000	23.333±0.219
IL38	4	18.333±0.752	63.332±0.045
IL42	4	0.000±0.000	56.665±0.165
IL28	4	0.000±0.000	66.668±0.079
IL15	4	14.165±0.321	76.668±0.192
IL43	4	0.000±0.000	46.668±0.103
IL1	4	20.000±0.142	70.835±0.128
P value		<0.0001	<0.0001
LSD		6.599	9.425

Values are means of four replicates ± S.E.

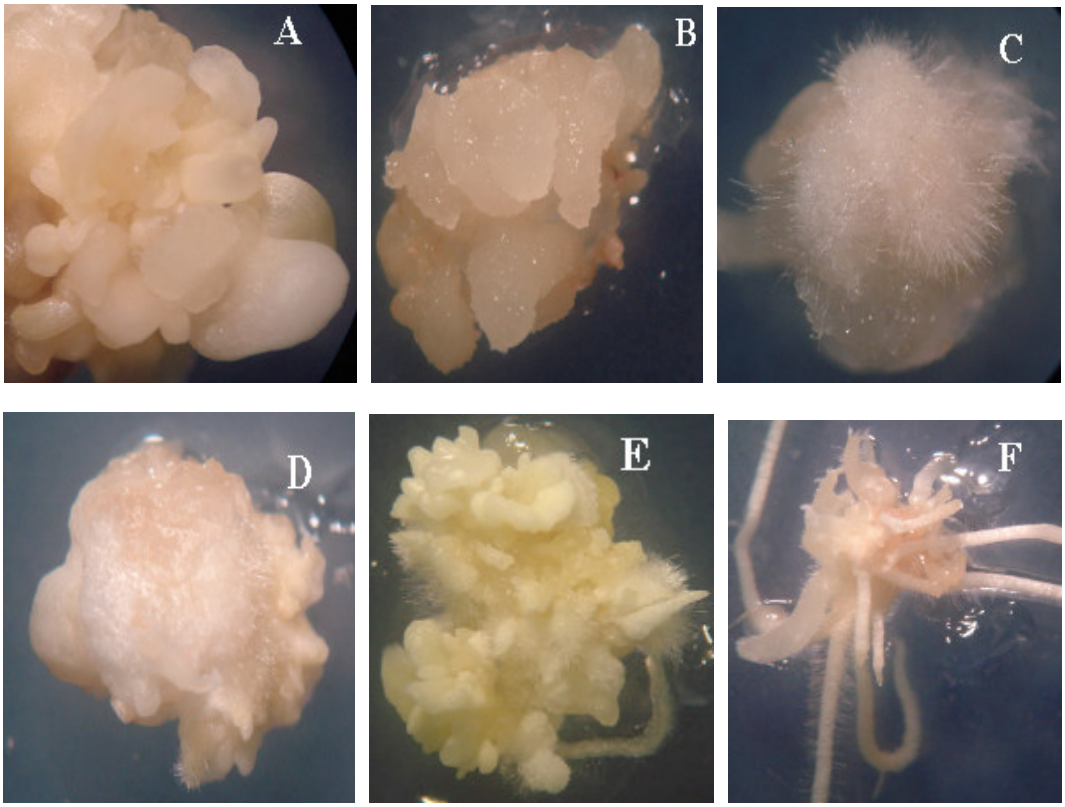

Figure 1. Callus formation and plant regeneration of Sudanese maize. (A) IL3 callus showing somatic embryos. (B) Calli of IL43 that is watery and lacks somatic embryos. (C) Calli of inbreds IL15 showing hair-like structures on the scutella surfaces of the embryos. (D) Poor regenerable callus of IL16 showing calli with hard tissue. (E) Maturing Hudiba-2 embryogenic callus showing formation of shoot- and roots-like structures on regeneration I medium (F) Rhizogenic callus of Hudeiba-1 producing roots on regeneration I medium.

Figure 2. Regeneration of plantlets and screen house growth of Sudanese maize. (A) Plantlets Regenerating from IL3 callus cultured on regeneration II medium. Bar = 2 cm (B) Hardening of regenerated plantlets at the screen house. Bar = 25 cm. (C) Regenerated plants maturing in the Screen house. Plants growing in 20-liter pots containing loam soil. Bar = 30 cm. (D) Seeds obtained from mature regenerants. Bar = 0.5 cm.

who reported formation of compact embryogenic callus at the middle part of the scutellum and friable embryogenic callus at the basal side of the scutellum.

Plant regeneration

Calli from all the genotypes were placed on maturation (Regeneration 1) media and cultured for 14 days in the dark (Figure 1). During this period, somatic embryos of IL3, IL15, IL1, IL38, Mojtamaa-45 and Hudeiba-2 calli matured, that is, they formed a scutellum (that later turned white), a coleoptile-like structure (that turned green) and a rootlet (Figure 1E). Calli of the other genotypes had no observable response to maturation.

Mature calli transferred to shoot induction media (Regeneration II) started greening 2 - 5 days after transfer to light. From majority of the calli, shoots and roots regenerated simultaneously while on R1 (Figure 2A) and transfer to rooting media was therefore unnecessary. The calli of Hudeiba-1 and IL28 cultured on regeneration I media responded by forming roots, which were mostly pigmented red. Calli of Mojtamaa-45, IL1, IL43 and IL28 had no other observable response to regeneration except slight greening.

Although regeneration was attempted for all the genotypes, however, not all genotypes could regenerate plants. Regeneration was achieved only in IL3, IL15, IL1, IL38, Hudeiba- 2 and Mojtamaa-45 (Table 3). The highest regeneration frequencies of 75.83 and 61.6% and was observed for IL3 and Hudeiba-2, respectively. The lowest regeneration frequency (40%) was observed for the inbred line IL38 (Table 3).

For some genotypes such as IL28 initiated Type II callus, which failed to regenerate into plants but rather continued forming roots on maturation media (Figure 1F). Carvalho et al. (1997) reported that not all tropical genotypes that initiated embryogenic calli could regenerate plants and some genotypes classified as none embryogenic produced plants. They concluded that such a classification does not accurately predict the regenerative ability of a calli from a given genotype (Carvalho et al.,

Table 3. Regeneration frequency and shoot induction among Sudanese maize genotypes.

Genotype	Number of experiments	Number of shoots	Regeneration frequency (%)
Mojtamaa-45	4	5.250±0.629	44.168
Hudeiba 2	4	3.000±0.408	61.665
IL3	4	6.000±0.707	75.833
IL38	4	4.250±0.854	40.000
IL15	4	1.500±0.289	47.500
IL1	4	3.250±0.479	49.165
P value		<0.0005	<0.0010
LSD		1.760	15.219

Values are means of four replicates ± S.E.

1997).This implies that plant regeneration is achievable for both embryogenic and non-embryogenic genotypes under appropriate tissue culture conditions.

Hudiba-1, IL28 and IL15 regenerated roots on PM and regeneration medium despite forming compact embryogenic callus. This type of callus (rhizogenic callus) has been observed in both embryogenic and non-embryogenic maize genotypes (Green and Phillips, 1975; Tomes, 1985). Rao et al. (2006), observing a similar response, reported that such a response is dependent on the genotype used. It has been shown for some maize genotypes that the transition from embryogenesis to rhizogenesis can be prevented by addition of sugars such as mannitol in the callus proliferation media (Emons and Kieft, 1995). Plant regeneration from rhizogenic calli could not be achieved under these culture conditions. It is possible that differences in responses to regeneration of a rhizogenic callus depends on the presence of other factors in the culture media (Duncan et al., 1985).

Acclimatization and growth of regenerants in screen house

After about 2 - 3 weeks of culture in shooting or rooting media, plantlets with a well-developed root system were first hardened (Figure 2B) according to Oduor et al. (2006) before transfer to the screen house. Most of the plantlets survived hardening and were transferred into the soil in the screen house and maintained to maturity (Figure 2C).

Although regenerated plants appeared fertile, some abnormal phenotypes were observed in regenerated plants. The most commonly observed abnormalities in R_0 regenerants were tussel ear formation and dwarfism. These abnormalities are typical of tissue-cultured cells, plants derived from such cells, and progenies of such plants. They have been termed as somaclonal variations and have been described either as epigenetic or genetic in occurrence (Larkin and Scowcroft, 1981). Epigenetic changes are physiological and so cannot be passed on from one generation to another. On the other hand, genetic changes are heritable and arise as a result of changes in the chromosome structure and number. Despite the presence of somaclonal variations, plants formed seeds, some of which were established from the tassels of some mature plants (Figure 2D).

In conclusion, this study identified Sudanese genotypes responding well to embryogenic callus induction and plant regeneration. Future biotechnological applications in maize such as tissue culture and genetic transformation should make use of these good responders.

ACKNOWLEDGEMENTS

The work was done at the PTL in Kenyatta University and was accomplished through fundings provided by the ASARECA. The technical help provided at the PTL is highly appreciated. The ARC in Sudan is also thanked for providing the maize genotypes used in this study.

REFERENCES

Abdelrahman AMA, Magboul EL, Nour A (2001). Effects of sowing date and cultuvar on the yield and yield components of maize in nothern Sudan. Seventh Eastern and Southern Africa regional maize conference 11th-15th February, pp. 295-298.

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

Bronsema FBF, Oostveen WJF, Lammeren AAM (1997). Comparative analysis of callus formation and regeneration on cultured immature maize embryos of the inbred lines A188 and A632. Plant Cell, Tissue Organ Cult. 50: 57-65.

Carvalho CHS, Bohorova N, Bordallo PN, Abreu LL, Valicente FH, Bressan W, Paiva E (1997). Type II callus production and plant regeneration in tropical maize genotypes. Plant Cell Rep., 17: 73-76.

Duncan DR, Williams ME, Zehr BE, Widholm JE (1985). The production of callus capable of plant regeneration from immature embryos of numerous Zea mays genotypes. Planta. 165: 322–333.

Emons AMC, Kieft H (1995). Somatic embryogenesis in maize (*Zea mays* L.). In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry: Somatic Embryogenesis and Synthetic Seed, Springer-Verlag, Berlin, pp. 24-39.

FAO (2000). FAO/WFP crop and food supply assessment mission to Sudan. FAO global information and early warning system on food and agriculture world food programme. http://www.fao.org/giews/ assessed on 12/05/2007.

Green CE, Phillips R (1975). Plant regeneration from tissue cultures of maize. Crop Sci. 15: 417-421.
Larkin PJ, Scowcroft WR (1981). Somaclonal variation: a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197-214.
Linsmaier EM, Skoog F (1965). Organic growth factor requirements of tabacco tissue cultures. Physiol. Plant. 18: 100–127.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15: 473-497.
Oduor RO, Ndung'u S, Njagi EN, Machuka J (2006). *In Vitro* regeneration of dryland Kenyan maize genotypes through somatic embryogenesis. Int. J. Bot. 2: 146-151.
Phillips RL, Somers DA, Hibberd KA (1988). Cell/tissue culture and *in vitro* manipulation. In: Sprague GF, Dudley JW (eds). Corn and corn improvement. 3rd edn. Madison: ASA/CSSA/SSSA, pp. 345-387.
Rao AQ, Hussain SS, Shahzad MS, Bokhari SYA, Raza MH, Rakha AMA, Shahid AA, Saleem Z, Husnain TR (2006). Somatic embryogenesis in wild relatives of cotton (*Gossypium Spp.*). J. Sci 7: 291-298.
Slater A, Scott NW, Fowler MR (2004). Plant biotechnology. The genetic manipulation of plants. Oxford university press Inc. N.Y., USA: 35-52.
Tomes DT (1985). Cell culture, somatic embryogenesis and plant regeneration in maize, rice, sorghum and millets. In: Bright SWJ, Jones MGK (eds.) Cereal Tissue and Cell Culture. Martinus Nijhoff/Dr.W.Junk Publishers, Dordrecht, pp 175–205.