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SOMATIC EMBRYOGENESIS ABILITY IN SELECT GEORGIA AND PEE DEE COTTON LINES

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Abstract

Fifteen Georgia and Pee Dee cotton germplasm lines along with Coker 312 (used as a positive control) were screened for their regenerability potential using nine embryo initiation/development media. In addition to Coker 312, somatic embryos and plants were obtained in PD 97019, PD 97021 and PD 98033 lines. Close to 95% of the Coker 312 seeds tested produced somatic embryos. However, extensive seed to seed variation was found in the regenerable Pee Dee lines. Efforts are under way to evaluate the effects of the various media used and develop pure regenerable lines to improve the regeneration efficiency of these newly found responsive Pee Dee genotypes.

Introduction

In vitro plant regeneration, a key step in cotton transformation, can be achieved through either shoot differentiation (Agrawal et al., 1997; Hemphill et al., 1998) or somatic embryogenesis (Shoemaker et al., 1986; Trolinder and Goodin, 1987). The ideal cotton transformation method, however, remains the one using somatic embryogenesis because somatic embryos are believed to originate from single cells (Merkle et al., 1995; Rajasekekaran et al., 2001), but the genetic specificity of cotton somatic embryogenesis has been well documented (Trolinder and Xhixian, 1989). This genetic specificity has led to a heavy reliance on Coker 312, an obsolete cotton but dependable regenerable line, for cotton transformation. Research in overcoming this problem is lacking and further experimentation is necessary to increase the regeneration efficiency of commercial cotton varieties (Rajasekaran et al., 2001). Therefore, the focus of this study was to screen Pee Dee and the University of Georgia germplasm for their regeneration potential using a modified version of a tissue culture protocol that we had developed and used to successfully regenerate embryos and plants in three different cotton species (Sakhanokho et al, 2000; 2001). We also tested the effect of a high nitrogen compound, namely putrescine, on cotton embryogenesis. This hormone-like substance has been shown to induce or enhance somatic embryogenesis and plant production in carrot (Anderson et al., 1998) and oat (Kelley et al., 2002) plants, but its effect on cotton has not been investigated.

Material and Methods

Plant Material and Culture Media

The genotypes studied included Georgia germplasm lines developed by Dr. Shelby Baker, former University of Georgia cotton breeder, and the Pee Dee material from the USDA/ARS, Florence, South Carolina, which was integrated with the Georgia breeding material by Dr. O. Lloyd May, research cotton breeder and geneticist at the University of Georgia, Tifton, Georgia. These genotypes were GA 161, GA 94894, GA 96199, GA 96211, GA 9654, PD 97006, PD 97019, PD 97021, PD 97047, PD 97072, PD 97100, PD 97101, PD 98015, PD 98033, and PD 98084. Seeds of Coker 312, the most regenerable cotton variety, were obtained from Dr. K. Rajasekaran, USDA/ARS, New Orleans, Louisiana, and included in the study as positive control. The various culture media used in this experiment are shown in Table 1and were described earlier (Sakhanokho et al., 2000; 2001).

Seed Sterilization and Germination

Seeds were placed in 250-mL flasks and surface sterilized in consecutive washings of 100% ethanol (30 to 60 s) and 23% commercial bleach [5.25% (v/v) NaOCl] with one drop of Tween 20 (polyoxyethylene-sorbitan monolaurate, Sigma, St. Louis, MO). The seeds were then shaken for 20 min at 110 rpm, rinsed three to four times with and then stored overnight in sterile distilled water. The next day, the seeds were rinsed again with sterile distilled water to remove any soap and/or bleach residues. Then the seed coats were removed for better germination efficiency and synchronization before placing the seeds on MS0 medium containing MS salts, 30 g L^{-1} glucose, 2 g L^{-1} Gelrite (Merck & Co., Inc., Rahway, NJ) and 0.75 mg L^{-1} $MgCl_2$ (pH 6.8). Seed germination took place under a 16 h light, 8 h dark light condition at 28 ± 2°C.

Callus Initiation and Proliferation

Explants used for callus initiation consisted of 4- to 10-d-old hypocotyls and cotyledons. Hypocotyl segments (5-mm sections) were longitudinally split and each cotyledon was cut into 7 to 8 pieces. These hypocotyl and cotyledon sections were aseptically transferred to Petri dishes containing callus induction medium (CIM), which was made up of MS salts supplemented with 0.4 mg L^{-1} thiamine, 2.0 mg L^{-1} NAA, 1.0 mg L^{-1} kinetin, 100 mg L^{-1} myo-inositol, 30 g L^{-1} glucose, 2 g L^{-1} Gelrite, and 0.75 g L^{-1} $MgCl_2$ (pH 5.8). Most explants, especially the hypocotyl explants, formed callus after about 4 wk. Preembryogenic callus was selected based on the morphology and characteristics. The prolifically growing and loose

preembryogenic callus with smaller cells and very dense cytoplasm was selected and then transferred onto fresh CIM medium until an adequate and desired amount of callus (1-3 g) was obtained and transferred into EIML liquid medium.

Liquid Culture Step

Potential embryogenic calli, identified as sections of small, less vacuolate and densely cytoplasmic cells, selected and proliferated on CIM medium, were then transferred into liquid somatic embryo initiation medium (EIML). EIML medium consisted of MS salts in which NH_4NO_3 was omitted and the amount of KNO_3 was doubled and supplemented with 10 mg L^{-1} thiamine, 100 mg L^{-1} myo-inositol, 1 mg L^{-1} nicotinic acid, 1 mg L^{-1} pyridoxine, 1 g L^{-1} glutamine, 0.5 g L^{-1} asparagine, and 30 g L^{-1} glucose (pH 5.8). One to 3 g of selected friable embryogenic callus were transferred into 250-mL, wide-mouth flasks containing 100 mL of EIML. These flasks were shaken at 130 rpm under a 16 h/8 h light/dark cycle at 28°C. The cultures were checked on a regular basis to monitor development of somatic embryos.

Embryo Development/Maturation Media

After four, six or even eight weeks, cultures showing embryos or embryo-like structures were strained through a tea strainer and 40-mesh screens to enrich for embryogenic cells that were then placed on various development/maturation media (Table 1).

Results and Discussion

In addition to Coker 312, somatic embryos and plants (Figures 1 and 2) were obtained in PD 97019, PD 97021 and PD 98033 lines. Close to 95% of the Coker 312 seeds tested produced somatic embryos. However, extensive seed to seed variation was found in the regenerable Pee Dee lines, which may be due to breeding by selection and bulking and not by pure line breeding (Trolinder and Xhixian, 1989). Efforts are under way to evaluate the effects of the various media used and develop pure regenerable lines to improve the regeneration efficiency of these newly found responsive Pee Dee genotypes.

Acknowledgements

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References

Agrawal, D.C., A.K. Banerjee, R.R. Kolala, A.B. Dhage, A.V. Kulkanmi, S.M. Nalawade, S. Hazra, and K.V. Krishnmurthy. 1997. *In vitro* induction of multiple shoots and plant regeneration in cotton *(Gossypium hirsutum* L.). Plant Cell Rep. 16:647-652.

Andersen, S.E., D.R. Bastola, and S.C. Minocha. 1998. Metabolism of polyamines in transgenic cells of carrot expressing a mouse ornithine decarboxylase cDNA. Plant Physiol. 116:299-307.

Hemphill, J.K., C.G.A. Maier, and K.D. Chapman. 1998. Rapid *in-vitro* plant regeneration of cotton (*Gossypium hirsutum* L.). Plant Cell Rep. 17:273-278.

Kelley, R.Y., A. Zipf, D. Wesenburg, G. Sharma. 2002. Putrescine-enhanced somatic embryos and plant numbers from elite oat (Avena spp. L.) and reciprocal crosses. *In Vitro* Cellul. Dev. Biol. Plant 38:508-512.

Merkle, S.A., W.A. Parrot, B.S. Flinn. 1995. Morphogenic aspects of somatic embryogenesis. *In In Vitro* Embryogenesis in Plants. T.A. Thorpe (ed.). Kluwer Academic Publishers, Dordrecht. 155-203.

Rajasekaran, K., C. A. Chlan, and T.E. Cleveland. 2001. Tissue culture and genetic transformation of cotton. pp. 270-290. *In* Genetic Improvement of Cotton: Emerging Technologies, J.N. Jenkins and S. Saha (eds). Science Publishers, Inc. Enfield, NH, U.S.A.

Sakhanokho, H. F., A. Zipf, K. Rajasekaran, S. Saha, and G. C. Sharma. 2000. Embryogenic regeneration in three cotton species. *In* Proc. 3rd International Crop Science Congress 2000, Hamburg, Germany. 17-22 Aug., 2000.

Sakhanokho, H. F., A. Zipf, K. Rajasekaran, S. Saha, and G. C. Sharma. 2001. Induction of highly embryogenic calli and plant regeneration in Upland and Pima cottons. Crop Sci. 41:12351240.

Shoemaker, R.C., L. J. Couche, and D. W. Galbraith. 1986. Characterization of somatic embryogenesis and plant regeneration in cotton (*Gossypium hirsutum*). Plant Cell Rep. 3:178-181.

Trolinder, N. L. and J. R. Goodin. 1987. Somatic embryogenesis and plant regeneration in cotton (Gossypium L.). I. Effects of source of explant and hormone regime. Plant Cell Rep. 6:231-234.

Trolinder, N. L. and C. Xhixian. 1989. Genotype specificity of the somatic embryogenesis response in cotton. Plant Cell Rep. 8:133-136.

Table 1. Media used to screen for the potential of several cotton varieties to be cultured into plants.

	Hormone/growth regulator (mg/L)				Carbon source (g/L)		
Medium	**NAA**	**2, 4-D**	**Kinetin**	**Putrescine**	**Glucose**	**Sucrose**	**Purpose**
MS0					30		Seed germination
CIM	2		1		30		Callus initiation
EIML					30		Embryo initiation
$EMMS_2$	0.5		0.05		30		Embryo initiation/development
$EMMS_4$		0.1	0.5		30		Embryo initiation/development
S15g	0.05					15	Embryo initiation/development
$EMMS_2$.25Put	0.5		0.05	0.25	30		Embryo initiation/development
$EMMS_2$.5Put	0.5		0.05	0.5	30		Embryo initiation/development
$EMMS_4$.25Put		0.1	0.5	0.25	30		Embryo initiation/development
$EMMS_4$.5Put		0.1	0.5	0.5	30		Embryo initiation/development
S15g.25Put	0.05			0.25		15	Embryo initiation/development
S15g.5Put	0.05			0.5		15	Embryo initiation/Development

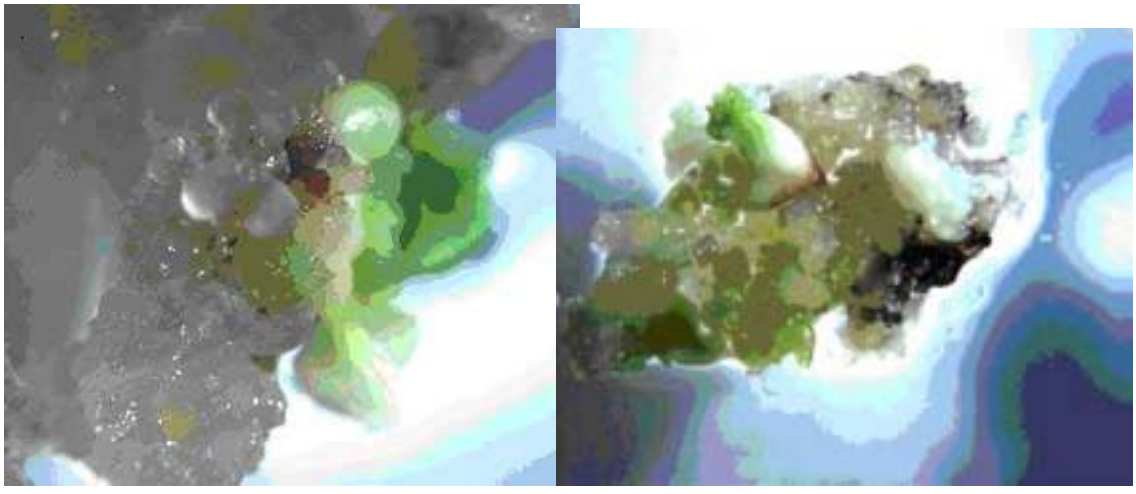

Figure 1. Somatic embryogenesis (from left to right) in PD97021 PD98033 cotton genotypes

Figure 2. Regenerated plants through embryogenesis from Coker 312, PD 98033, PD 97021, and PD 97019.