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Effect of genotype on somatic embryogenesis from axes of mature peanut embryos

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Abstract

Genotypes representing the three botanical varieties of peanut (*Arachis hypogaea* L.) were assessed for somatic embryogenesis and subsequent plant conversion from mature zygotic embryo axes. Explants were initially cultured on Murashige and Skoog medium supplemented with 12.42 μM 4-amino-3,5,6-trichloropicolinic acid. Individual somatic embryos were isolated from explant tissue and used to initiate repetitive liquid cultures. There were significant differences among genotypes and varieties for somatic embryo formation and plant regeneration using a single media sequence. Botanical variety *fastigiata* had a lower embryogenic frequency and produced significantly fewer embryos than either *hypogaea* or *vulgaris*, which were similar in response.

Abbreviations: EA – zygotic embryo axes, MS – Murashige and Skoog (1962) medium, picloram – 4-amino-3, 5, 6-trichloropicolinic acid

Introduction

Peanut improvement through biotechnology will largely depend on efficient regeneration systems. Plant regeneration via somatic embryogenesis has been obtained from mature zygotic embryo axes (McKently 1991), immature zygotic embryos (Ozias-Akins *et al.* 1992; Sellars *et al.* 1990; Hazra *et al.* 1989), and immature leaflets (Baker & Wetzstein 1992). The various genotypes tested in these studies all formed embryos, but the frequency of response was varied. Sellars *et al.* (1990) and Ozias-Akins *et al.* (1992) studied the effect of genotype on somatic embryo formation from various immature embryo explants, and found significant differences among genotypes. Sellars *et al.* (1990) regenerated somatic embryos from the cotyledons and embryo axes of intact explants at varying rates from three cultivars. Ozias-Akins *et al.* (1992) observed significant differences among seven genotypes for somatic embryo formation and subsequent plant regeneration.

Although routine, the use of immature embryo explants is limited by the need to maintain sufficient plant material at specific maturity and to categorize the stages of embryo development. Recovering somatic embryos from the mature axes of a wide variety of genotypes provides a significant advantage for establishing embryogenic cultures of peanut. In this report, the effect of genotype on the frequency of somatic embryogenesis from mature embryo tissue was evaluated. The objectives of this study were to evaluate the embryo-forming capacity of mature zygotic embryo axes from 14 peanut genotypes representing the three botanical varieties of commercial peanut, *A. hypogaea* ssp. *hypogaea* var. *hypogaea*, and *A. hypogaea* ssp. *fastigiata* vars. *vulgaris* and *fastigiata*. Primary embryogenesis, long-term repetitive culture, and plant regeneration were evaluated.

Materials and methods

Seed from 14 peanut genotypes (Table 1) were harvested from greenhouse-grown plants at the R8 stage (Boote 1982), in which inner pericarp and testa are coloured. Mature embryo axes (EA) were removed

from the seed and cultured according to the method of McKently (1991). Briefly, EA 8 to 10 mm long were surface disinfested in 1.6% NaOCl for 3 min, 70% ethanol for 1 min, and rinsed 3 times with sterile deionized water. Sixty explants of each genotype were placed in 100 × 15-mm petri dishes (three EA per dish) containing 25 ml of medium. The medium consisted of MS salts and vitamins, 12.42 μM picloram, 25 g l^{-1} sucrose, and 8 g l^{-1} Difco Bactoagar (pH 5.8). Cultures were maintained at 25 °C under a 16-h photoperiod (cool-white fluorescent lamps, 90–98 μmol m^{-2} s^{-1}). The EA were transferred to fresh medium every 28 days, and evaluated for somatic embryo formation 65 days after culture initiation.

Resulting somatic embryos were removed from explant tissue and transferred to liquid MS medium containing 4.14 μM picloram to initiate long-term repetitive cultures. Ten somatic embryos were placed in 250-ml Erlenmeyer flasks, each containing 50 ml medium. The cultures were routinely subcultured every 2 weeks for 10 months. The most proliferative embryo clusters were separated to approximately 8 mm in diameter and used for the subcultures. Thirty clusters of each genotype were routinely selected to inoculate the fresh cultures. At 10 months, individual somatic embryos were isolated from embryogenic masses and transferred to solid MS basal medium for plant development. The cultures were transferred to fresh medium every 28 days, and evaluated for shoot formation after 21 weeks.

Data from the primary culture period were used to calculate the percentage of cultured EA that gave rise to somatic embryos and the number of somatic embryos that formed per responding EA. Cultures were scored for plant regeneration when a tetrafoliolate leaf was present. The numerical variables described above were analyzed using the SAS procedures GLM and FREQ. To assess the potential differences among the genotypes, the chi-square goodness of fit test was used. Following one-way ANOVA analyses, mean separation was performed using Duncan's multiple range test (DMRT).

Results and discussion

Direct somatic embryogenesis was observed within 30 days of culture initiation from all of the genotypes examined. The embryos arose from a 2 mm band of hypocotyledonary tissue surrounding explant epicotyls (Fig. 1a). At the end of the 65-day culture period, the number of responding EA and the mean number of somatic embryos formed per responding EA were determined for each genotype. Only clearly identifiable somatic embryos with single axes and two cotyledons were counted. Conglomerates of two or more fused axes and structures lacking apical development were not scored. Significant differences in the frequency of response were observed among the 14 genotypes ($\chi^2 = 57.25$, 13 df, $p < 0.001$). Response ranged from 10% for genotype 803 to 58% for Sunrunner (Table 1). The number of somatic embryos that formed per embryogenic EA was not significantly different among the genotypes ($p = 0.17$). A highly variable number of somatic embryos formed from the individual responding explants within each genotype. There was, however, a strong correlation ($r = 0.96$, $p = 0.0001$) between the percentage of responding EA and the number of somatic embryos per responding unit, which indicated that a genotype showing a higher percentage of

Table 1. Somatic embryogenesis from the embryo axes of mature seed of 14 peanut genotypes.

Genotype	Responsive explants[1] (% ± SE)	No. embryos per responding explant (Mean ± SE)	Frequency of shoot development (% ± SE)
(*hypogaea*)[2]			
Sunrunner	58 ± 6	4.7 ± 0.8	33 ± 7
Okrun	42 ± 11	3.8 ± 1.1	40 ± 7
Florunner	38 ± 6	3.2 ± 0.4	30 ± 7
487B	50 ± 6	4.4 ± 0.6	45 ± 8
NC-7	42 ± 8	3.6 ± 0.5	40 ± 7
393-7(BL)[3]	38 ± 7	3.3 ± 0.6	13 ± 5
(*vulgaris*)			
558A(BL)	41 ± 5	3.6 ± 0.4	20 ± 8
Spanco	38 ± 7	3.3 ± 0.3	7 ± 2
Tamnut	50 ± 7	4.4 ± 0.7	13 ± 5
435	26 ± 6	2.6 ± 0.2	8 ± 3
Chico	12 ± 5	2.6 ± 0.8	7 ± 2
(*fastigiata*)			
623B(BL)	20 ± 4	2.3 ± 0.2	43 ± 8
640A(BL)	24 ± 4	2.6 ± 0.4	33 ± 7
803(BL)	10 ± 4	1.8 ± 0.3	3 ± 1

[1] n = 60.
[2] Botanical varieties; *Arachis hypogaea* ssp. *hypogaea* var. *hypogaea*, *A. hypogaea* ssp. *fastigiata* vars. *vulgaris* and *fastigiata*.
[3] BL = Breeding line, University of Florida peanut program.

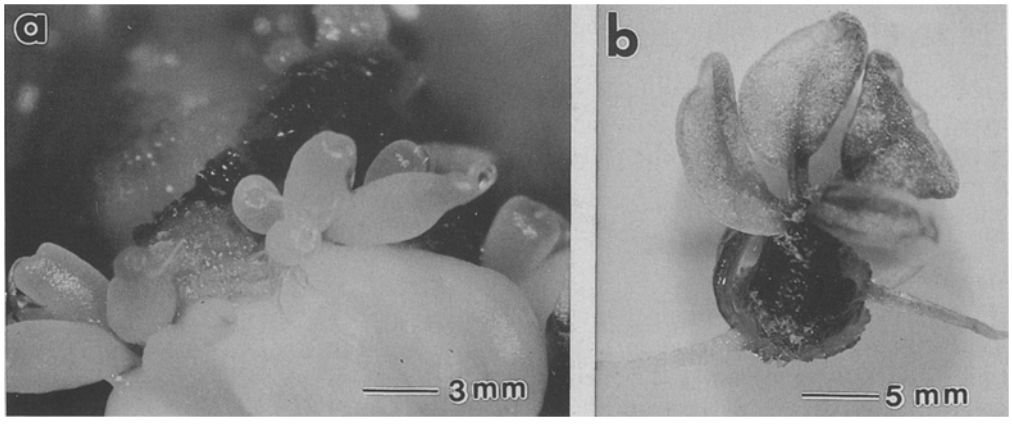

Fig. 1. (*a*) Somatic embryos originating from a mature zygotic embryo axis of Florunner peanut cultured on 12.42 μM picloram. (*b*) Germination of Florunner peanut somatic embryo after 21 weeks culture on MS basal medium.

response also had a higher capacity to form somatic embryos per responding unit. This was consistent with the observation by Ozias-Akins *et al.* (1992) in their study of somatic embryogenesis from immature cotyledons of peanut. This same relationship between response frequency and number of somatic embryos formed has also been observed with soybean cotyledons (Parrott *et al.* 1989; Tian *et al.* 1994).

Given the classification of the 14 genotypes into the three botanical varieties, an analysis was performed to detect differences at this level. Such an aggregation gave larger sample sizes that enhanced the power of detecting differences obscured at the individual genotype level. Explant response rates were previously shown to be significantly different among the genotypes. Upon aggregation, this conclusion is retained, as significant differences were observed in the frequency of response among the three varieties (χ^2 = 31.2, 2 df, $p < 0.001$). Grouping for the mean number of somatic embryos formed per embryogenic explant revealed significant differences among varieties ($p < 0.01$, DMRT). These had been obscured at the individual genotype level. Mean separation suggested that variety *fastigiata* had a lower embryogenic frequency and produced significantly fewer somatic embryos than either *hypogaea* or *vulgaris*, which were similar in response.

Twenty somatic embryos of each genotype were used to initiate the liquid secondary embryogenic cultures. When placed in liquid medium, approximately 75% of the embryos enlarged and produced secondary embryos. These secondary embryos were arrested at the torpedo development stage and gave rise to additional embryos and the subsequent formation of embryo masses. This observation concurs with that of Durham & Parrott (1992) who initiated embryogenic suspension cultures of peanut using somatic embryos induced from immature cotyledons. At each subculture, the masses were divided into clusters approximately 8 mm in diameter consisting of approximately 5–10 embryos. Thirty clusters of each genotype were selected to inoculate the fresh cultures. After 10 months, 50 embryos of each genotype were individually transferred to solid MS basal medium for germination and plant development.

The presence of tetrafoliolate leaves and developed roots were the criteria for evaluating somatic embryo conversion to plantlets (Fig. 1b). Significant differences in the frequency of conversion were observed among the 14 genotypes, as determined by chi-square analysis ($\chi^2 = 56.2$, 13 df, $p < 0.0001$). The percentage of somatic embryos forming shoots ranged from 3% for genotype 803 to 45% for 487B (Table 1). An analysis was performed to detect conversion differences at the variety level, and significant differences were detected ($\chi^2 = 28.9$, 2 df, $p < 0.001$).

Conclusions

The *in vitro* responses reported here indicate that a wide range of peanut genotypes are competent for plant regeneration via somatic embryogenesis from mature embryo axis explants. Highly significant differences were, however, observed among the genotypes for the characters examined. Genotypes from vari-

eties *hypogaea* and *vulgaris* had a higher embryogenic frequency and produced significantly more somatic embryos than those from variety *fastigiata*. All genotypes showed good proliferation during the liquid subculture period. Subsequent studies to optimize media and culture requirements may improve the *in vitro* response of individual genotypes.

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