Evaluation of Runner and Virginia Market Types for Tissue Culture Responses

K. Chengalrayan and M. Gallo-Meagher[1]*

ABSTRACT

Studies on somatic embryogenesis and organogenesis of peanut (*Arachis hypogaea* L.) derived from mature zygotic embryo-derived leaflets of 10 cultivars and two breeding lines representing both runner and virginia market types were conducted using tissue culture procedures originally optimized for the spanish market type, J.L. 24. The somatic embryogenesis protocol used was a multi-step process which included embryogenic mass induction, embryo development, germination, and conversion. The percentage of embryogenic mass induction, embryo development, and conversion was genotype-dependent. However, the number of embryos produced per explant and germination to plantlets was genotype-independent. SunOleic® 95R consistently ranked highest for its response during all stages of embryo development, and runner market types were generally more responsive than virginia market types. The percentage of shoot induction and the number of shoot buds per explant during organogenesis were genotype-dependent, and runner market types were more responsive for organogenesis than virginia market types. Genotypes with a high embryogenic response also were highly responsive for organogenesis.

Key Words: 6-benzylamino purine (BAP), 2,4-dichlorophenoxyacetic acid (2,4-D), mature zygotic embryo-derived leaflets, organogenesis, somatic embryogenesis, thidiazuron.

A tissue culture system with a high capacity for plant regeneration is usually necessary for successful biotechnology applications. In peanut (*Arachis hypogaea* L.), plant regeneration has been obtained via embryogenesis (Hazra *et al.*, 1989; Ozias-Akins, 1989; Chengalrayan *et al.*, 1994, 1997, 2001; Akasaka *et al.*, 2000; Lakshmanan and Taji, 2000; Little *et al.*, 2000) and organogenesis (Chengalrayan *et al.*, 1995, 2001; Gill and Ozias-Akins, 1999; Pestana *et al.*, 1999). For many plant species, including peanut, both the embryogenic and organogenic responses remain limiting factors due to strong genotype dependence. Before embarking upon a program of peanut improvement using *in vitro* techniques, it is of utmost importance to evaluate the available cultivars for their morphogenetic potential. Thus, the effects of various peanut genotypes on embryogenesis (Ozias-Akins *et al.*, 1992; Reddy and Reddy, 1993; McKently, 1995; Chengalrayan *et al.*, 1998) and organogenesis (Mroginski *et al.*, 1981; McKently *et al.*, 1991; Cheng *et al.*, 1992) have been studied. The protocols described for embryogenesis involve multiple steps, starting from embryo induction to plant formation. Significant differences among genotypes for somatic embryo formation, subculture capacity, and plant regeneration were demonstrated in peanut using the same media sequence (Ozias-Akins *et al.*, 1992; McKently, 1995). Chengalrayan *et al.* (1998) observed that, with the exception of root meristem differentiation and subsequent radicle emergence, each developmental stage of embryogenesis was genotype-dependent. Cheng *et al.* (1992) reported that plant regeneration via organogenesis was observed only in valencia market-type cultivars or hybrid derivatives with a valencia background. However, there are no reports comparing the ability of various peanut genotypes to undergo both somatic embryogenesis and organogenesis.

Protocols previously optimized for embryogenesis and organogenesis of mature zygotic embryo-derived leaflets (MZELs) of J.L. 24, a spanish market type, were extended to 10 U.S. peanut cultivars and two breeding lines. These protocols were selected due to their previous ability to produce a high frequency of regeneration independent of genotype and because they are the only protocols available to study both embryogenesis and organogenesis from the same explant source. The objective of this study was to evaluate 12 U.S. genotypes, representing runner and virginia market types, for their morphogenetic ability (embryogenesis and organogenesis) *in vitro*.

Although the first report on plant *in vitro* regeneration was in the late 1930s, gene regulation of embryogenesis and organogenesis remains largely unknown. A system with a high frequency of regeneration via both somatic embryogenesis and organogenesis from the same explant source should facilitate gene identification for these two different *in vitro* developmental pathways. Therefore, the outcome of this research may provide a model *in vitro* peanut system to identify genes regulating plant development.

Materials and Methods

Explants. Ten peanut cultivars and two breeding lines, belonging to runner and virginia market types (*Arachis hypogaea* spp. *hypogaea* var. *hypogaea*), were selected for this study due to their commercial value, and their relevant agronomic characteristics are described in Table 1. Embryo axes were excised from the cotyledons and washed four to five times in tap water and surface

[1]Res. Assoc., Agronomy Dept., and Assoc. Prof., Agronomy Dept., Plant Molecular and Cellular Biology Program and The Genetics Institute, respectively, Univ. of Florida, Gainesville, FL 32611-0300.

*Corresponding author (email: mgmea@ifas.ufl.edu).

Table 1. Peanut genotypes used to study embryogenesis and organogenesis, their market types, and relevant agronomic characteristics.

Genotype[a]	Market type	Important features
Andru 93	Runner	Early maturing, larger seed size, very high in taste
F1334	Runner	A component line of SunOleic® 97R, high oleic oil chemistry
Fla MDR 98	Runner	Multiple disease resistance, mid-oleic oil chemistry
Florunner	Runner	Widely grown until late 1980s due to its combination of commercially desirable characteristics
Georgia Green	Runner	Currently dominates southeastern U.S. production for its combination of high yield potential and resistance to tomato spotted wilt
Southern Runner	Runner	Late maturing, parent of Georgia Green, moderate resistance to late leaf spot, white mold, and tomato spotted wilt
SunOleic® 95R	Runner	High oleic oil chemistry with superior shelf life
UF 81206	Runner	Multiple disease resistance, Univ. of Florida breeding line, parent of Fla MDR 98, mid-oleic oil chemistry
African Giant	Virginia	Old land race variety with large seed
Jenkins Jumbo	Virginia	Old land race variety with large seed
NC 7	Virginia	Large seeded and high yielding with mid-oleic oil chemistry
NC 10C	Virginia	Resistant to Cylindrocladium black rot

[a]All genotypes are *Arachis hypogaea* spp. *hypogaea* var. *hypogaea*.

sterilized with 70% alcohol for 1 min followed by 8 min in 10% commercial bleach (0.5% sodium hypochlorite). Excess bleach was removed aseptically by repeated washings with sterile distilled water and soaked 12 to 16 hr in sterile distilled water. MZELs were isolated as previously described (Chengalrayan *et al.*, 1994). Thirty MZELs per Petri plate and five replicates per cultivar were cultured in each of two duplicate experiments.

Somatic Embryogenesis. The MZELs were cultured for 4 wk on a Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) containing 90.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 6% sucrose for embryogenic mass induction (Chengalrayan *et al.*, 1994). For further development, embryogenic masses were transferred to a MS basal medium with 13.6 μM 2,4-D and 6% sucrose. After 4 wk of incubation, clusters of embryos were transferred to MS basal medium for germination. The germinated somatic embryos were transferred to a conversion medium that consisted of MS basal salts and vitamins with 22.7 μM thidiazuron (TDZ) and 2% sucrose.

After 4 wk of incubation for each of three passages (embryogenic mass induction, embryo development, and germination), the percentage of these responses was scored and the number of embryos per explant was determined. The percent conversion was calculated after 8 wk of culture. Earlier results with J.L. 24 showed that 85% of the plants developed by these methods survived in sand:soil (2:1) mixture and grew to maturity (Chengalrayan, 1997).

Organogenesis. The protocol for organogenesis described by Chengalrayan *et al.* (1995) was used in this study. The MZELs were cultured on MS medium supplemented with 17.83 μM α-naphthylacetic acid (NAA), 22.19 μM 6-benzylamino purine (BAP), and 2% sucrose. The percentage of bud induction and the number of buds per explant were counted after 4 wk.

Culture Conditions. The pH of all media was adjusted to 5.6 to 5.8 and gelled with 0.7% Agargel™ (Sigma-Aldrich Co., St. Louis, MO). All the cultures were incubated in a growth chamber at 25 ± 1 C, under cool white fluorescent light at 60 $\mu Mm^{-2}s^{-1}$ with a 16-hr photoperiod. Statistical analysis was carried out using analysis of variance, and treatment means were separated using Duncan's Multiple Range Test (SAS Institute, 1996).

Results and Discussion

Embryogenesis. Somatic embryogenesis in peanut from MZELs involves four stages including (a) embryogenic mass formation, (b) embryo development, (c) germination, and (d) conversion. Embryogenic masses appeared within 2 wk of culture initiation for all genotypes. However, significant differences ($P < 0.05$) in the percentage of embryogenic response at each stage of development were observed among the 12 genotypes (Table 2). The percentage of embryogenic mass induction ranged from a low of 5% for African Giant to a high of 83% for SunOleic® 95R. Except for NC 10C (61%), the virginia market types were significantly less responsive to embryogenic mass induction (5 to 13%) than the runners (17 to 83%; $P = 0.0029$). These results support the findings of Ozias-Akins *et al.* (1992) which suggested that all peanut genotypes are competent for embryogenesis although to different degrees. The magnitude of *in vitro* response can likely be improved by media manipulations. In the current study, the embryogenic mass induction response for NC 7 and Florunner was comparable to that observed by Baker *et al.* (1995) where 18% and 10% of embryo axes responded, respectively.

In contrast, McKently (1995) obtained 42% and 38% embryo induction from the R8 stage of mature embryo axes of these same cultivars on MS basal medium containing 12.42 μM picloram and 2.5% sucrose.

Upon reducing the 2,4-D concentration from 90.5 μM to 13.6 μM, somatic embryos developed from the embryogenic masses. The percentage of embryo development also varied greatly with a low of 23% for NC 7 and high of 74% for NC 10C (Table 2). SunOleic® 95R, which induced the highest percentage of embryogenic masses, was ranked second (70%) and was not significantly different from NC 10C for embryo development (Table 2). Similar to embryogenic mass induction, embryo development was numerically, but not significantly, higher for runners compared to virginia types (with the exception of NC 10C). All genotypes produced somatic embryos that were initially abnormal and fascinated, which is the response observed previously for other genotypes (Wetzstein and Baker, 1993; Chengalrayan *et al.*, 1994, 1997, 1998); and consequently it was not feasible to count the number of viable embryos per explant. However in the present study, once fasciated embryos were transferred to a MS medium for germination, then hypocotyls elongated, rooting was induced, and the number of viable embryos per explant could be scored. Results showed that the number of embryos per explant was similar for all cultivars with an average of approximately two (Table 2).

With the exception of Andru 93 where embryos failed to germinate, all embryos germinated in hormone-free MS basal (Table 2). Shoots and roots of SunOleic® 95R differentiated simultaneously and produced plantlets by the usual process of meristem differentiation as observed in zygotic embryos. However, the percentage of conversion was relatively low (4%). For the remaining 10 genotypes that produced embryos, only root differentiation was achieved on the germination medium (Table 2). Similar results were also observed earlier when only 10 of 16 genotypes responded with 0.5 to 24% of the embryos induced producing both roots and shoots on MS basal medium (Chengalrayan *et al.*, 1998).

Rooted somatic embryos that developed from MZELs of the 11 genotypes were exposed to MS medium containing 5 μM TDZ for 8 wk to differentiate shoot meristems. Similar to the earlier stages of embryogenesis, conversion percentage was also genotype-dependent. Only seven of the 11 genotypes converted into plantlets (6 to 38%) in the conversion medium (Table 2), with SunOleic® 95R producing the greatest number of plantlets. These results were in contrast to earlier results in which embryos of all 16 genotypes induced shoots (Chengalrayan *et al.*, 1997).

The ability to form somatic embryos is considered to be under genetic control and individual genotypes within a species can differ in their ability to undergo somatic embryogenesis (Parrott *et al.*, 1991). The process of somatic embryogenesis is controlled by different sets of genes (Reinbothe *et al.*, 1992; Ma *et al.*, 1994; Lin *et al.*, 1996). The current study with 12 peanut genotypes also indicates that multiple gene sets condition embryogenesis because the relative response ranking was altered at each step for a given genotype, except for SunOleic® 95R, which was consistently a highly responsive genotype for somatic embryogenesis at all stages.

Organogenesis. Shoot regeneration also has been shown to be controlled by multiple genetic factors (Bullock *et al.*, 1982; Lin *et al.*, 1996). Consequently, significant differences ($P < 0.05$) were observed among the peanut genotypes for organogenic bud induction and the number of buds per explant (Table 3). All 12 genotypes had the ability to regenerate shoot buds. In general, runner market types responded better than virginia market types ($P \leq 0.0001$). Among runner market types, the organogenic response ranged from 19% for Andru 93 to 80% for Southern Runner. However, as was the case for embryogenic mass induction, NC 10C responded better

Table 2. Genotypic effect on different stages of somatic embryogenesis from mature zygotic embryo-derived leaflets of peanut.

Genotype	Embryogenic mass induction[a]	Embryo development	Embryos per explant	Germination	Conversion on basal MS	Conversion on MS + TDZ
	% ± SD	% ± SD	no.	%	%	% ± SD
SunOleic® 95R	83.4 ± 11.4a	70.2 ± 7.5ab	1.7 ± 0.8a	100	3.7	37.9 ± 11.8a
UF 81206	63.4 ± 14.0b	45.1 ± 6.9bcd	1.5 ± 1.9a	100	0	15.8 ± 2.9cd
NC 10C	61.3 ± 12.4b	73.6 ± 8.4a	1.8 ± 1.1a	100	0	25.0 ± 10.8bc
F1334	57.5 ± 16.9b	54.3 ± 14.5abc	1.7 ± 1.0a	100	0	31.4 ± 7.4ab
Southern Runner	49.1 ± 6.3bc	60.7 ± 11.1abc	1.5 ± 0.7a	100	0	20.8 ± 5.9c
Fla MDR 98	37.2 ± 20.9cd	51.0 ± 11.0abc	1.8 ± 1.0a	100	0	16.2 ± 0.6cd
Florunner	21.1 ± 9.5de	61.3 ± 0.8abc	1.5 ± 0.7a	100	0	5.9 ± 0.0d
Andru 93	20.7 ± 11.1de	50.0 ± 14.7abc	1.3 ± 0.8a	0	–	–
Georgia Green	17.1 ± 1.8e	63.0 ± 15.5abc	1.7 ± 0.8a	100	0	0e
NC 7	13.5 ± 2.2e	22.7 ± 0.0d	1.0 ± 0.0a	100	0	0e
Jenkins Jumbo	5.3 ± 3.7e	47.4 ± 14.1abcd	1.5 ± 0.8a	100	0	0e
African Giant	4.7 ± 4.5e	41.7 ± 17.6abc	1.4 ± 0.6a	100	0	0e

[a]Values within a column followed by the same letter are not significantly different at $P < 0.05$.

Table 3. Effect of genotype on organogenesis from mature zygotic embryo-derived leaflets of peanut.

Genotype	Bud induction[a]	Buds per explant
	% ± SD	no.
Southern Runner	80.0 ± 6.7a	10.3 ± 4.8a
UF 81206	75.5 ± 2.7abc	8.4 ± 4.0a
SunOleic® 95R	74.0 ± 6.0abc	8.1 ± 4.7a
F1334	68.5 ± 11.4abc	8.3 ± 8.1a
NC10C	61.6 ± 9.8bc	4.7 ± 2.3ab
Fla MDR 98	59.7 ± 3.7c	6.5 ± 3.8ab
Greorgia Green	36.5 ± 7.8d	5.0 ± 0.9ab
Florunner	28.8 ± 16.3de	4.0 ± 0.0ab
Andru 93	19.4 ± 10.9ef	6.1 ± 3.9ab
Jenkins Jumbo	16.0 ± 13.0ef	6.5 ± 3.8ab
NC 7	10.0 ± 8.6fg	5.7 ± 2.5ab
African Giant	8.6 ± 6.4fg	1.0 ± 0.0b

[a]Values within a column followed by the same letter are not significantly different at $P < 0.05$.

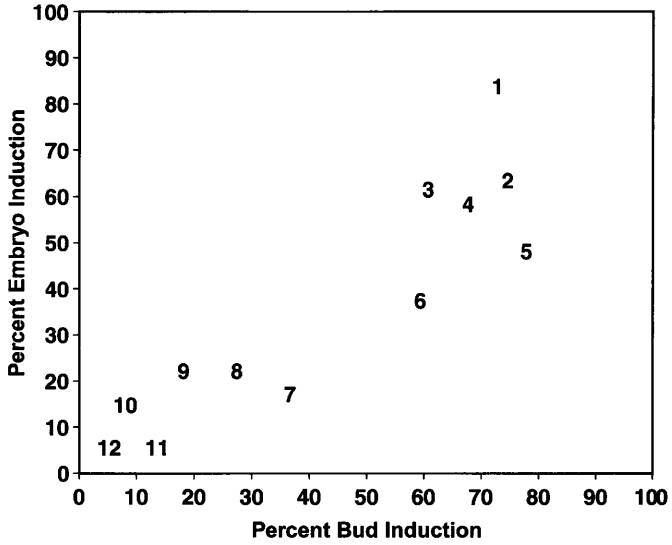

Fig. 1. Comparison of embryogenic and organogenic responses for different peanut genotypes. 1 = SunOleic® 95R; 2 = UF 81206; 3 = NC 10C; 4 = F1334; 5 = Southern Runner; 6 = Fla MDR 98; 7 = Georgia Green; 8 = Florunner; 9 = Andru 93; 10 = NC 7; 11 = Jenkins Jumbo; 12 = African Giant.

than the other virginia genotypes with the 4th highest bud induction response overall (62%). For the remaining virginias, the response was 9% for African Giant, 10% for NC 7, and 16% for Jenkins Jumbo. Cheng *et al.* (1992) evaluated a wide range of peanut genotypes for organogenic responsiveness using petiolule with blade-attached explants. Even though all genotypes produced callus, only valencia types produced buds and shoots. In contrast, our results using MZELs showed that both runner and virginia market types induced organogenic buds, although to varying degrees. The number of buds produced per explant ranged from four (Florunner) to 10 (Southern Runner) for runner market types and one (African Giant) to six (Jenkins Jumbo) for virginia market types. With the exception of the best responders (Southern Runner and UF 81206) as well as the worst responder (African Giant), the relative rankings for percentage of bud induction and number of buds per explant varied among genotypes (Table 3).

To better understand the morphogenic process, the percent of embryogenic and organogenic responses were compared for each genotype (Fig. 1). In general, genotypes were fairly consistent in their ability to regenerate *in vitro*. Genotypes that were highly responsive for embryogenesis also tended to be highly responsive for organogenesis (SunOleic® 95R, UF 81206, NC 10C, F1334, Southern Runner, and Fla MDR 98), while those with a relatively low embryogenic response were also not as good at regenerating via organogenesis (Florunner, Andru 93, Georgia Green, NC 7, Jenkins Jumbo, and African Giant). However, only four genotypes (UF 81206, F1334, Fla MDR 98, and African Giant) maintained the same relative rankings for both processes. This is the first report to compare genotypes for both somatic embryogenesis and organogenesis derived from the same peanut explant.

On the basis of the above data, results can be summarized as follows: (a) the protocol optimized for embryogenesis and organogenesis of MZELs of J.L. 24 is effective to varying degrees for U.S. cultivars and breeding lines; (b) variation in response at each stage of embryogenesis is possibly influenced by genetic constitution; (c) the root meristem of the MZEL-derived somatic embryos was generally well differentiated, although the embryos appeared morphologically abnormal; and (d) irrespective of abnormal morphology, somatic embryos in some cultivars converted into normal plantlets—which suggests that the plumule of these embryos was well differentiated. Thus, the failure of somatic embryos to convert into plantlets, often attributed to malformation of the plumule or to a lack of maturation, may be under genetic control. This study demonstrates that the percentage of embryogenic mass induction, embryo development, and conversion is genotype-dependent in peanut. However, the number of embryos produced per explant and germination seem to be genotype-independent. For this protocol, an obstacle to the establishment of conversion of somatic embryos remains to be the formation of abnormal embryos.

Similar to embryogenesis, all 12 genotypes induced shoot buds. Comparison of embryogenic and organogenic responses showed that although the relative rankings varied between the two types of responses for most genotypes, those cultures which showed a high embryogenic response also were highly responsive for organogenesis. Further refinements will be required for each genotype to obtain its optimal *in vitro* response. Because maximum response for both embryogenesis and organogenesis was observed for SunOleic® 95R, this genotype may serve as a model to identify genes involved in peanut somatic embryogenesis and organogenesis.

Acknowledgments

The authors thank Drs. K.H. Quesenberry and B. Rathinasabapathi for critical review of the manuscript. This research was supported by the Florida Agric. Exp. Sta., and approved for publication as Journal Series No. R-09649.

Literature Cited

Akasaka, Y., H. Daimon, and M. Mii. 2000. Improved plant regeneration from cultured leaf segments in peanut (*Arachis hypogaea* L.) by limited exposure to thidiazuron. Plant Sci. 156:169-175.

Baker, C.M., R.E. Durham, J.A. Burns, W.A. Parrott, and H.Y. Wetzstein. 1995. High frequency somatic embryogenesis in peanut (*Arachis hypogaea* L.) using mature, dry seed. Plant Cell Rep. 15:38-42.

Bullock, W.P., P.S. Baenziger, G.W. Schaeffer, and P.J. Bottino. 1982. Anther culture of wheat (*Triticum aestivum* L.) F_1s and their reciprocal crosses. Theor. Appl. Genet. 62:155-159.

Cheng, M., D.C.H. His, and G.C. Phillips. 1992. *In vitro* regeneration of valencia-type peanut (*Arachis hypogaea* L.) from cultured petiolules, epicotyl sections and other seedling explants. Peanut Sci. 19:82-87.

Chengalrayan, K. 1997. Somatic embryogenesis and organogenesis in peanut and their applications. Ph.D dissertation. Univ. of Pune, India.

Chengalrayan, K., S. Hazra, and M. Gallo-Meagher. 2001. Histological analysis of somatic embryogenesis and organogenesis induced from mature zygotic embryo-derived leaflets. Plant Sci. 161:415-421.

Chengalrayan, K., V.B. Mhaske, and S. Hazra. 1995. *In vitro* regulation of morphogenesis in peanut (*Arachis hypogaea* L.). Plant Sci. 110:259-269.

Chengalrayan, K., V.B. Mhaske, and S. Hazra. 1997. High frequency conversion of abnormal peanut somatic embryos. Plant Cell Rep. 16:783-786.

Chengalrayan, K., V.B. Mhaske, and S. Hazra. 1998. Genotypic control of peanut somatic embryogenesis. Plant Cell Rep. 17:522-525.

Chengalrayan, K., S.S. Sathaye, and S. Hazra. 1994. Somatic embryogenesis from mature embryo-derived leaflets. Plant Cell Rep. 13:578-581.

Gill, R., and P. Ozias-Akins. 1999. Thidiazuron induced highly morphogenetic callus and high frequency regeneration of fertile peanut (*Arachis hypogaea* L.) plants. *In Vitro* Cell. Dev. Biol.-Plant. 35:445-450.

Hazra, S., S. Sathaye, and A.F. Mascarenhas. 1989. Direct somatic embryogenesis of peanut (*Arachis hypogaea* L.). BioTechnol. 7:949-951.

Lakshmanan, P., and A. Taji. 2000. Somatic embryogenesis in leguminous plants. Plant Biol. 2:136-148.

Lin, X., G.J.H. Hwang, and J.L. Zimmerman. 1996. Isolation and characterization of diverse set of genes from carrot somatic embryos. Plant Physiol. 112:1365-1374.

Little, L.E., Z.V. Maganua, and W.A. Parrott. 2000. A protocol for repetitive somatic embryogenesis from mature peanut epicotyls. Plant Cell Rep. 19:351-357.

Ma, H.C., M.D. McMullen, and J.J. Finer. 1994. Identification of a homeobox-containing gene with enhanced expression during soybean (*Glycine max* L.) somatic embryo development. Plant Mol. Biol. 24:465-473.

McKently, A.H. 1995. Effect of genotype on somatic embryogenesis from axes of mature peanut embryos. Plant Cell Tiss. Org. Cult. 42:251-254.

McKently, A.H., G.A. Moore, and F.P. Gardner. 1991. Regeneration of peanut and perennial peanut from cultured leaf tissue. Crop Sci. 31:833-837.

Mroginski, L.A., K.K. Kartha, and J.P. Shyluk. 1981. Regeneration of peanut (*Arachis hypogaea* L.) plantlets by *in vitro* culture of immature leaves. Can. J. Bot. 59:826-830.

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

Ozias-Akins, P. 1989. Plant regeneration from immature embryos of peanut. Plant Cell Rep. 89:217-218.

Ozias-Akins, P., W.F. Anderson, and C.C. Holbrook. 1992. Somatic embryogenesis in *Arachis hypogaea* L.—genotype comparison. Plant Sci. 83:103-111.

Parrott, W.A., W.S. Merkle, and E.G. Williams. 1991. Somatic embryogenesis: Potential for use in propagation and gene transfer systems, pp. 158-200. *In* R.D. Murray (ed.) Advanced Methods in Plant Breeding and Biotechnology. CAB Intl., Wallingford, UK.

Pestana, M.C., C. Lacorte, V.G. De Freitas, and E. Mansur. 1999. *In vitro* regeneration of peanut (*Arachis hypogaea* L.) through organogenesis: Effect of culture temperature and silver nitrate. *In Vitro* Cell. Dev. Biol.-Plant. 35:214-216.

Reddy, R.L., and G.M. Reddy. 1993. Factors affecting direct somatic embryogenesis and plant regeneration in groundnut, *Arachis hypogaea* L. Indian J. Exp. Biol. 31:57-60.

Reinbothe, C., A. Tewes, and S. Reinbothe. 1992. Altered gene-expression during somatic embryogenesis in *Nicotiana plumbaginifolia* and *Digitalis lanata*. Plant Sci. 82:47-58.

SAS Institute. 1996. SAS/STAT guide for personal computers. Ver 6.12. Cary, NC.

Wetzstein, H.Y., and C.M. Baker. 1993. The relationship between somatic embryo morphology and conversion in peanut (*Arachis hypogaea* L.). Plant Sci. 92:81-89.