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Somatic embryogenesis in *Arachis hypogaea*: revisited

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Abstract. Direct somatic embryogenesis is an efficient method of plant regeneration, allowing rapid multiplication of plants in a short period. Six experiments were conducted to study the influence of auxin level on somatic embryogenesis and to optimise the concentration of auxins. Immature embryo axis was the ideal explant and 20–40 mg L^{-1} of 2,4-dichlorophenoxyacetic acid was the best concentration range for obtaining the maximum number of free somatic embryos. Significant differences were observed between the genotypes for induction and the number of somatic embryos per explant. The cv. Girnar 1 produced the maximum number of somatic embryos per explant, the number of secondary somatic embryos ranging from 1.5 to 9.4. The overall germination of somatic embryos was 42.8%, and 65% of the plantlets transferred to the field survived. The development of somatic embryos was from the apical region of the embryo axes without undergoing dedifferentiation. The initial cell divided to form a tier of four cells and subsequent anticlinal and periclinal division resulted in the development of globular somatic embryos with small suspensors, followed by heart-shaped, torpedo-shaped and 'cotyledonary' stages.

Introduction

Peanut (*Arachis hypogaea* L.) is one of the most important seed sources of oil and protein in both developed and developing countries. The crop is vulnerable to an array of pests and diseases, for which there is not much information on the variability within the species. The wild relatives are often studied for incorporation of pest and disease resistance. However, inherent breeding barriers result in poor exchange of desirable traits from the wild taxa (Murthy *et al.* 1980), and the success of conventional breeding programs is very low. Genetic engineering by direct gene transfer offers an alternative, provided a feasible method and regeneration of transgenics are possible. Gene manipulation methods have not been successful in peanut because of low regeneration frequency of cultivated and wild species (Rani and Reddy 1996). Direct somatic embryogenesis (SEG) is an efficient method of plant regeneration as it allows rapid production of large numbers of plants in a short period, without somaclonal variation.

In peanut, direct SEG has been reported from immature zygotic embryos (Sellars *et al.* 1990; George and Eapen 1993; Reddy and Reddy 1993), immature cotyledons (George and Eapen 1993), mature cotyledons (Venkatachalam *et al.* 1999) and leaflets (Baker and Wetzstein 1992). The frequency of SEG varied from 0 to 87% and the number of somatic embryos per explant (NSE) varied from 0.8 to 4.7 per responding explant (Sellars *et al.* 1990; Ozias-Akins *et al.* 1992). George and Eapen (1993) reported 100% response with immature embryo axis as explant but average NSE was only 10.2. Among the growth regulators, although 2,4-dichloro-phenoxyacetic acid (2,4-D) and 4-amino-3,5,6-trichloro-picolinic acid (picloram) have been reported to be equally effective for induction of SEG in peanut, 2,4-D is the most widely used. The level of 2,4-D used for the induction of somatic embryos varies widely from a low level of 3 mg L^{-1} (Hazra *et al.* 1989) to a very high level of 40 mg L^{-1} (Durham and Parrot 1992), with similar responses from similar explants in different experiments. In most of the *in vitro* experiments for regeneration, growth regulators are generally kept at a low level because high doses have weedicidal effects. No systematic efforts have been made to understand the influence of 2,4-D levels on the induction process *per se* and on the NSE in peanut. Here, we report a comparative account of the somatic embryogenesis at low to high levels of 2,4-D and the influence of the level of auxin on the NSE. A detailed account of the role of 2,4-D in primary and secondary somatic embryogenesis across genotypes is provided, along with the histological investigations of the somatic embryogenesis.

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Materials and methods

Induction of somatic embryos

To deduce the effect of low 2,4-D concentrations on somatic embryogenesis and to identify the best responding explant, immature de-embryonated cotyledons (IDC) and the immature zygotic embryo axes (IEA) from 6–10-mm long pods of the cv. J 11 were collected from 110-day-old field-grown plants and used as explants. Pods were first washed thoroughly with tap water and then cleaned with distilled water containing a few drops of Tween 80 (polyoxyethylene sorbitan monooleate, a wetting agent) to remove the adhered particles. These pods were then surface-sterilised in a laminar airflow chamber with 70% ethanol for 1 min, followed by a 0.1% aqueous solution of mercuric chloride for 5 min under constant agitation. This was followed by thorough washing with five changes of sterile distilled water and the pods were aseptically dissected to obtain the IDC and IEA explants. The culture medium used was Murashige and Skoog (1962) basal medium (MS) containing the vitamins of B5 medium (Gamborg *et al.* 1968). The culture medium was supplemented with 1 mg L^{-1} of naphthaleneacetic acid (NAA) and 6, 8, 10, 12, 14, 16, 18 or 20 mg L^{-1} of 2,4-D.

In the next experiment, the culture medium supplemented with 1 mg L^{-1} of NAA and 20, 40, 60, 80 or 100 mg L^{-1} of 2,4-D was used to evaluate the role of higher levels of 2,4-D in somatic embryogenesis. Since embryonal explant was the better responding in the previous experiment, embryo axes of two maturity level, IEA and mature embryo axes (MEA), of cv. J 11 were used as explants in this experiment. Mature embryo axes were obtained from mature dried pods after surface sterilisation and dissection as described above.

On the basis of the results of the second experiment, another experiment was set up to fine tune the level of auxin required for the maximum response. Since a high response (75–100%) was obtained for 20–40 mg L^{-1} 2,4-D, concentrations of 25, 30, 35 and 40 mg L^{-1} were selected along with 0, 1 or 2 mg L^{-1} of NAA. In a departure from the first two experiments, one more concentration of NAA was also included in this experiment as it was aimed at optimising auxin levels required for somatic embryogenesis. Both IEA and MEA of cv. J 11 were used as explants.

To understand the genotypic differences, the best responding explant, IEA, of five common cultivars, Kadiri 3, GG 2, J 11, M 13 and Girnar 1, were tested in culture media containing 25, 30, 35 or 40 mg L^{-1} of 2,4-D with 1 mg L^{-1} of NAA in a subsequent experiment.

The somatic embryos produced from cv. Girnar 1 were further utilised for the induction of secondary somatic embryogenesis. The fully grown individual somatic embryos separated from the mother explants were cultured on media supplemented with 18, 20, 22, 24, 26 or 28 mg L^{-1} of 2,4-D as well as 1 or 2 mg L^{-1} of NAA. As the induction process (primary somatic embryogenesis) was already over, more increments in 2,4-D concentration than in earlier experiments were used to verify the possibility of a more precise level of 2,4-D being required for secondary somatic embryogenesis.

In all of the above experiments, cultures were incubated at 26 ± 1°C in the dark. The number of embryogenic explants and the NSE were measured after 30 days. All the experiments were repeated at least three times for confirmation of the observations.

Maturation and germination of somatic embryos

Somatic embryos along with mother explants of cv. J 11 were cultured for maturation for up to 30 days on culture medium containing 0.2% activated charcoal, at 16 h photoperiod with a light intensity of 88 µmol m^{-2} s^{-1}. Then the individual somatic embryos were transferred to culture medium containing 0.2% activated charcoal and 200 mg L^{-1} casein hydrolysate (germination medium). For inducing roots in germinated somatic embryos that only had the plumule elongated unilaterally, after 3 weeks the somatic embryos were transferred from the germination medium to culture medium with 1 mg L^{-1} NAA (rooting medium). The root-induced plantlets from all the experiments were transplanted to potting mixture (soil, sand and vermiculite; 3 : 2 : 1 by volume) in a thermocol cup and hardened in a plant growth chamber at 30°C, 195 µmol m^{-2} s^{-1} illumination and 80% relative humidity for 10 days and then transplanted to the field.

Histology

The somatic embryogenic IEA of the three cultivars Kadiri 3, GG 2 and Girnar 1 were used for the histological studies. The tissue was fixed in freshly prepared FAA (formaldehyde : glacial acetic acid : 70% ethanol; 5 : 5 : 90 by volume), dehydrated by passing it through a *t*-butyl alcohol series and then embedded in paraffin. Sections 10 µm thick were cut on a rotary microtome and stained with safranin followed by fast Green as described by Dwivedi and Singh (1985).

Statistical design and analysis

All the experiments except the embryo germination were done in completely randomised design with two replications of 30 tubes each. Frequencies of responding explants were converted to percentage (PSE) and analysed after arcsin transformation. The NSE was counted on each responding explant and was analysed in a two-factor non-orthogonal analysis of variance as the frequency of responding explants varied in different treatments.

Results

Low levels of 2,4-D

Somatic embryogenesis was observed in cv. J 11 in both IDC and IEA cultured in all concentrations of 2,4-D. The mean response varied from 3.9 to 48.3% in IDC and from 40 to 100% in IEA (Table 1). There was a significant difference in somatic embryogenesis between the two explants ($P = 0.01$) and IEA was better responding. Although the overall differences in response to the level of 2,4-D were significant, in most cases there was 100% response in IEA. The

Table 1. Mean percentage of somatic embryogenesis, mean and normal somatic embryos per explant induced from the immature de-embryonated cotyledons and immature embryo axes of cv. J 11 in 1 mg L^{-1} naphthaleneacetic acid and low concentrations of 2,4-dichlorophenoxyacetic acid

2,4-D = 2,4-dichlorophenoxyacetic acid, IDC = immature de-embryonated cotyledons, IEA = immature embryo axes, PSE = percentage of somatic embryogenesis, NSE = number of somatic embryos per responding explant, %N = percentage of normal somatic embryos. CV (PSE) = 7.02%, CV (NSE) = 34.1%

[2,4-D] (mg L^{-1})	IDC		IEA			
	PSE	NSE	PSE	NSE		
				Mean	Normal	%N
6	33.3	1.3	63.4	6.8	5.9 ± 2.6	86.8
8	48.3	11.6	40.0	10.7	3.6 ± 3.0	33.6
10	17.9	13.3	100.0	8.2	6.9 ± 2.3	84.2
12	25.0	4.2	100.0	9.3	7.1 ± 6.5	76.3
14	4.2	3.0	68.8	2.9	2.3 ± 0.7	79.3
16	3.9	9.5	100.0	7.3	6.8 ± 3.4	93.2
18	8.1	6.6	100.0	6.6	6.5 ± 4.7	98.5
20	17.2	3.0	100.0	8.9	8.4 ± 5.4	94.4

Table 2. Mean percentage of somatic embryogenesis, mean and normal somatic embryos per explant induced from the immature and mature embryo axes of cv. J 11 in 1 mg L^{-1} naphthaleneacetic acid and high concentrations of 2,4-dichlorophenoxyacetic acid

2,4-D = 2,4-dichlorophenoxyacetic acid, PSE = percentage of somatic embryogenesis, NSE = number of somatic embryos per responding explant, %N = percentage of normal somatic embryos, Min. = minimum number of normal somatic embryos, Max. = maximum number of normal somatic embryos

[2,4-D]	Immature embryo axes						Mature embryo axes					
(mg L^{-1})	PSE	NSE					PSE	NSE				
		Mean	Normal	%N	Min.	Max.		Mean	Normal	%N	Min.	Max.
20	75	5.0	4.5 ± 2.9	90.0	1	10	60	3.1	3.0 ± 1.1	96.8	1	4
40	100	9.5	7.3 ± 5.2	76.8	2	50	60	4.5	2.6 ± 0.6	57.8	2	3
60	80	9.7	7.9 ± 5.2	81.4	3	18	35	3.3	3.0 ± 1.6	90.9	1	6
80	70	4.5	4.0 ± 2.1	88.9	3	8	40	4.0	4.0 ± 1.6	100.0	1	6
100	80	2.3	2.1 ± 0.6	91.3	1	6	30	4.6	4.6 ± 2.7	100.0	2	11

interaction between the explants and 2,4-D concentration was also significant ($P = 0.01$).

The mean NSE varied from 1.3 to 13.3 in IDC and 2.9 to 10.7 in IEA. 2,4-Dichlorophenoxyacetic acid at concentrations of 8 and 10 mg L^{-1} induced higher NSE; however, the difference was not significant. The interaction of the explants with the level of 2,4-D was also not significant. In IDC all the somatic embryos produced were normal and free (Fig. 1), whereas in IEA some of the embryos were fused (Figs 2, 3) and the percentage of normal embryos ranged from 33.6 to 98.5% (Table 1).

High levels of 2,4-D

Somatic embryogenesis was induced in both MEA and IEA at all levels of 2,4-D tested. The difference in response to 2,4-D was not significant. Somatic embryogenesis varied from 30 to 60% in MEA and from 70 to 100% in IEA (Table 2). Although IEA showed a slightly higher response, the difference in the maturity of the explant did not significantly influence the mean percentage of somatic embryogenesis.

There was no significant difference between the two explants in NSE. The mean NSE varied from 2.3 to 9.7 in IEA and from 3.1 to 4.6 in MEA. Although not statistically significant, it was found that IEA were capable of producing more embryos per explant. Both the explants produced normal and fused somatic embryos in varying frequencies. The relative frequency of normal embryos over the fused ones was higher in both explants. The NSE was not consistent and ranged between 2 and 50 per explant for immature embryo axes in the same treatment. The frequency of fused somatic embryos increased with the increase in NSE.

Optimisation of auxin levels

Despite very high levels of response, the overall differences in somatic embryogenesis due to 2,4-D were significant ($P = 0.05$). The contribution of NAA concentration to the induction of somatic embryogenesis was not significant whereas the interaction between the two auxins was significant ($P = 0.01$). The difference in the response of the two explants in somatic embryogenesis was not significant. However, the interaction of the explants and the two auxins was significant ($P = 0.01$). In the combinations of 2 mg L^{-1} NAA with all concentrations of 2,4-D, the response was 100% for IEA (Table 4). As in the previous experiments, IEA gave a better response than MEA. Across combinations of auxins IEA gave a 94–100% response (Table 3).

The mean NSE varied from 2.5 to 22.6 across the treatments. Immature zygotic embroyo axes produced more embryos than MEA. The influence of the auxins on the induction of higher numbers of somatic embryos was not significant. As in the previous experiments, both the explants produced normal as well as fused embryos in varying frequencies and there were more normal embryos than fused ones. The mean number of normal embryos per explant varied from 6.3 to 21.3 in IEA and from 2.5 to 8.5 in MEA.

Genotypic variation

Significant genotypic differences were observed among cultivars ($P = 0.01$) for somatic embryogenesis at the four levels of 2,4-D. The cultivars had a 100% response in the induction of somatic embryogenesis (Table 4) except for GG 2 and M 13. However, 2,4-D concentrations did not significantly influence the induction percentage. In cv. M 13 fewer than 50% of the explants responded, whereas GG 2 had 75–100% response. The interaction of genotypes with media was significant ($P = 0.01$).

The NSE also varied significantly ($P = 0.01$) with the genotypes used. More embryos were produced by Kadiri 3 and Girnar 1. The mean NSE ranged from 3.1 to 19.4. The percentage of normal embryos ranged from 23.1 to 96.8, with a maximum of 38 embryos per explant in Girnar 1.

Secondary somatic embryogenesis

The mean of secondary somatic embryogenesis varied from 30 to 90%. In cv. Girnar 1 in secondary somatic

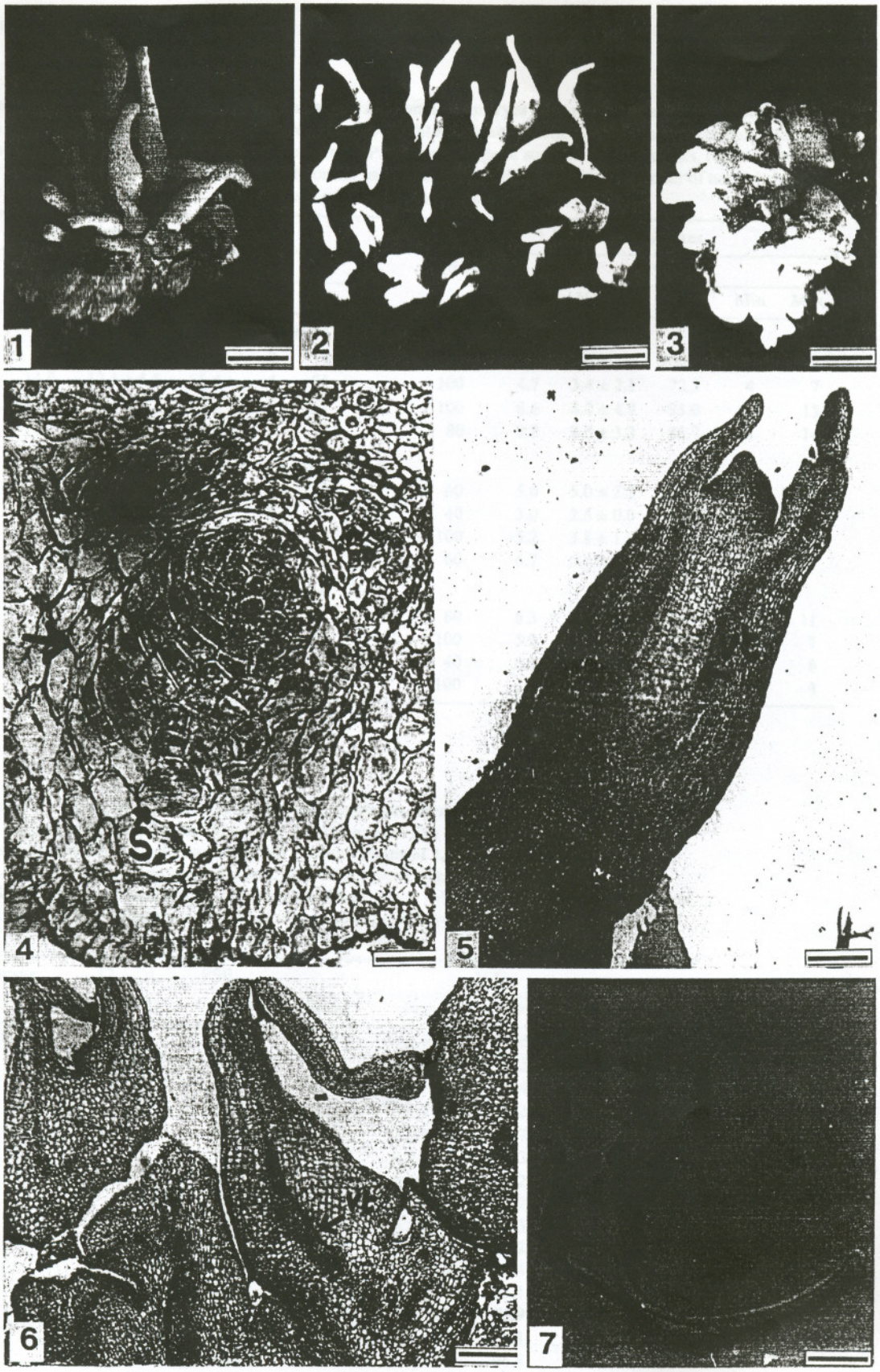

Fig. 1. A cluster of normal somatic embryos on immature de-embryonated cotyledons (scale bar = 2.0 mm). **Fig. 2.** Normal and fused somatic embryos of different shapes detached from explant (scale bar = 3.2 mm). **Fig. 3.** A cluster of somatic embryos showing fused and normal somatic embryos on immature zygotic embryo axes (scale bar = 3.2 mm). **Fig. 4.** Longitudinal section (LS) of somatic embryo. The arrow shows the early globular stage. S = suspensor (scale bar = 105 µm). **Fig. 5.** LS of somatic embryo showing the cotyledonary stage; vt = vascular tissue (scale bar = 350 µm). **Fig. 6.** LS of explant with a cluster of somatic embryos, showing independent vascular tissue; vt = vascular tissue (scale bar = 330 µm). **Fig. 7.** Germination of somatic embryos (scale bar = 5 mm).

Table 3. Mean percentage of somatic embryogenesis, mean and normal somatic embryos per explant induced from the immature and mature embryo axes of cv. J 11 in different concentrations of 2,4-dichlorophenoxyacetic acid and naphthaleneacetic acid

2,4-D = 2,4-dichlorophenoxyacetic acid, NAA = naphthaleneacetic acid, PSE = percentage of somatic embryogenesis, NSE = number of somatic embryos per responding explant,%N = percentage of normal somatic embryos, Min. = minimum number of normal somatic embryos, Max. = maximum number of normal somatic embryos

[2,4-D]	Immature embryo axes						Mature embryo axes					
(mg L^{-1})	PSE	NSE					PSE	NSE				
		Mean	Normal	%N	Min.	Max.		Mean	Normal	%N	Min.	Max.
					0 mg L^{-1} NAA							
25	100	8.3	6.3 ± 4.8	75.9	2	18	40	4.0	4.0 ± 1.0	100.0	3	5
30	95	14.0	12.1 ± 5.5	86.4	4	25	100	4.7	3.4 ± 2.1	72.3	4	7
35	100	14.0	9.2 ± 6.3	65.7	3	27	100	8.6	8.0 ± 4.9	93.0	2	15
40	100	16.5	15.0 ± 6.3	90.9	5	31	80	9.8	8.5 ± 3.3	86.7	3	14
					1 mg L^{-1} NAA							
25	94	11.1	9.3 ± 5.5	83.8	4	19	60	5.0	5.0 ± 2.2	100.0	4	5
30	100	22.6	21.3 ± 9.0	94.2	5	43	40	3.0	2.5 ± 0.0	83.3	3	32
35	100	16.7	13.8 ± 7.3	82.6	4	30	100	5.2	3.8 ± 1.9	73.1	2	7
40	100	15.7	13.4 ± 6.4	85.4	4	22	60	6.7	5.0 ± 2.5	74.6	2	8
					2 mg L^{-1} NAA							
25	100	17.1	14.3 ± 6.7	83.6	12	31	60	6.3	5.7 ± 4.0	90.5	5	11
30	100	16.1	14.1 ± 7.1	87.6	2	27	100	5.0	3.5 ± 2.0	70.0	3	7
35	100	16.1	13.9 ± 6.8	86.3	4	27	40	6.5	5.0 ± 1.0	76.9	4	6
40	100	15.9	13.0 ± 6.8	81.8	6	24	100	3.5	2.5 ± 1.5	71.4	1	4

Table 4. Mean percentage of somatic embryogenesis, mean and normal somatic embryos per explant induced from the immature embryo axes of five cultivars in media with 1 mg L^{-1} naphthaleneacetic acid and different concentrations of 2,4-dichlorophenoxyacetic acid

2,4-D = 2,4-dichlorophenoxyacetic acid, PSE = percentage of somatic embryogenesis, NSE = number of somatic embryos per responding explant, %N = percentage of normal somatic embryos, Min. = minimum number of normal somatic embryos, Max. = maximum number of normal somatic embryos

Cultivar	[2,4-D]	PSE	NSE				
	(mg L^{-1})		Mean	Normal	%N	Min.	Max.
Kadiri 3	25	100	7.2	5.8 ± 3.0	80.6	4	14
	30	100	11.1	8.6 ± 7.9	78.2	1	29
	35	100	12.3	11.0 ± 1.2	89.4	4	32
	40	100	7.7	7.1 ± 4.8	92.2	1	17
GG 2	25	100	8.6	7.8 ± 3.6	90.7	3	15
	30	88	7.1	6.2 ± 3.7	87.3	2	14
	35	88	6.9	6.4 ± 3.3	94.2	3	13
	40	75	10.2	8.8 ± 5.7	82.2	2	19
J 11	25	100	7.1	6.2 ± 5.4	87.3	1	25
	30	100	6.5	5.8 ± 3.9	89.2	2	14
	35	100	8.2	7.0 ± 1.2	85.4	2	12
	40	100	7.4	6.3 ± 3.0	85.1	1	12
M 13	25	43	5.2	4.0 ± 2.3	76.9	2	8
	30	46	11.7	2.7 ± 0.9	23.1	1	4
	35	46	3.1	3.0 ± 1.4	96.8	1	5
	40	40	5.0	3.8 ± 2.8	76	2	9
Girnar 1	25	100	11.7	11.3 ± 5.5	96.6	3	22
	30	100	12.9	11.9 ± 4.1	92.3	1	20
	35	100	13.6	8.7 ± 6.0	64.0	3	33
	40	100	19.4	18.5 ± 9.4	95.4	4	38

Table 5. Mean percentage of secondary somatic embryogenesis and the number of secondary somatic embryos per responding primary somatic embryos of cv. Girnar 1

[2,4-D] (mg L^{-1})	Secondary somatic embryogenesis (%) at [NAA]		No. of secondary somatic embryos per explant at [NAA]	
	1 mg L^{-1}	2 mg L^{-1}	1 mg L^{-1}	2 mg L^{-1}
18	50.0	70.0	5.2 ± 3.0	3.1 ± 1.6
20	60.0	40.0	4.0 ± 2.2	3.3 ± 2.1
22	70.0	90.0	4.5 ± 3.6	9.4 ± 3.6
24	70.0	30.0	7.4 ± 4.1	1.5 ± 0.5
26	40.0	60.0	4.0 ± 1.6	8.5 ± 1.7
28	50.0	80.0	3.8 ± 0.4	5.0 ± 1.4

Table 6. Germination of somatic embryos from cv. J 11 on medium containing 0.2% activated charcoal and 200 mg L^{-1} casein hydrolysate

Response	No. of embryos responded
Total no. of embryos cultured	298.0
Total germinated	128.0 ± 12.5
Germinated to normal plantlets	37.0 ± 5.4
Germinated to radicle only	47.0 ± 3.9
Germinated to plumule only	44.0 ± 9.6

embryogenesis, no difference due to NAA concentrations was evident (Table 5). The mean number of secondary somatic embryos per explant varied from 1.5 to 9.4. Differences in the secondary somatic embryogenesis due to NAA and 2,4-D concentrations were not statistically significant ($P = 0.7$ and 0.6, respectively).

Histological studies

The development of somatic embryos was from the apical region of the embryo axes without dedifferentiation. There was no difference in the mode of development of somatic embryos between the genotypes. Although callus formation was observed in the explants, no embryonic cells could be located in these tissues. The callus might have been produced as a result of physical injury to the explant during dissection. The somatic embryo initial cell divided to form a tier of four cells and subsequent anticlinal and periclinal divisions resulted in the development of a globular somatic embryo with a small suspensor (Fig. 4), followed by heart-shaped, torpedo-shaped and 'cotyledonary' (Fig. 5) stages. The structural identity of the somatic embryos was further confirmed by the absence of the vascular connection to the mother explant (Fig. 6). The induction of somatic embryos was confined to the apical end of the immature embryo axes.

Germination of somatic embryo

The overall frequency of germination was only 43% (128 of 298 embryos cultured). Of this the recovery of fully formed plantlets with both plumule and radicle elongated (Fig. 7) was about 12.4%. An almost equal percentage of somatic embryos developed only unilaterally, either with elongation of plumule or with radicle alone (Table 6). All somatic embryos developed into shoot produced roots in the rooting medium within 3 weeks. Of the 93 normal plantlets replanted in the field after hardening, 61 (65.6%) established in the field.

Discussion

Among the different auxins studied in somatic embryogenesis of peanut, picloram is reported to be the most effective (Ozias-Akins 1989; Sellars *et al.* 1990) and 2,4-D is used most widely. The role of NAA is controversial: Hazra *et al.* (1989) reported failure of induction when used alone, whereas Reddy and Reddy (1993) reported 30–40% induction. However, McKently (1991) reported that concentrations of NAA up to 10 mg L^{-1} have no significant role. Our experiments revealed that, although NAA was not essential for induction, an increase in NSE was apparent when different levels of 2,4-D were supplemented with NAA. Use of higher doses of auxin for induction of embryogenesis in peanut was first reported in suspension cultures of IDC (Durham and Parrot 1992). In the present study, 1 mg L^{-1} NAA and 6 mg L^{-1} 2,4-D were sufficient for induction; however, 2,4-D levels in the range 20–40 mg L^{-1} were better. Increasing the concentration beyond 40 mg L^{-1} was not advantageous either in the percentage of induction or in the NSE.

Somatic embryogenesis in peanut has been reported from late heart-shaped stage of zygotic embryos (Sellars *et al.* 1990) to mature embryos (Baker *et al.* 1995). Cotyledons were reported to be less responsive than embryo axes (Ozias-Akins *et al.* 1992) and often produced abnormal shaped and fused somatic embryos (Wetzstein and Baker 1993). However, in the present study more NSE was produced on IDC at 8–10 mg L^{-1} 2,4-D and all the somatic embryos were of normal morphology. Embryo axes have been utilised for the somatic embryogenesis in a large number of legumes and the developmental ages of the explant have proven to be important for most of the species (Parrot *et al.* 1992). Although IEA produced more NSE than MEA, our results did not show any significant difference in the potential for inducing somatic embryogenesis in IEA and MEA, confirming the earlier reports of Ozias-Akins *et al.* (1992) and McKently (1991). Among the three explants used, IEA was the most responsive at all levels of 2,4-D used.

The maximum frequency of induction and the number of normal embryos per explant were observed at 20–40 mg L^{-1} 2,4-D and a few laterally fused multiple embryos were found when NSE was higher. Higher levels of 2,4-D have been reported to be responsible for such responses in different plant species (Li *et al.* 1985; Ranch *et al.* 1985; Lazzeri *et al.*

1985, 1988; Geneve and Kester 1990). Detailed evaluations of the auxin levels resulting in fusion of peanut somatic embryos and the genetic nature of fused somatic embryos as compared with the normal are areas for further investigation. Fingerprinting methods like restricted fragment length polymorphism or amplified fragment length polymorphism may be useful in finding the probable genetic dissimilarities. The number of somatic embryos and the frequency of the normal embryos per explant appeared to also vary with genotype. The significant difference observed between the five genotypes for somatic embryogenesis confirms earlier reports (George and Eapen 1993; Chengalarayan *et al.* 1998). Secondary somatic embryogenesis was observed in all the auxin combinations selected, indicating that the combinations capable of primary somatic embryogenesis may also be suitable for secondary somatic embryogenesis.

Anatomical studies revealed that somatic embryogenesis is induced at the non-callusing tissues following similar stages of zygotic embryo development as reported earlier in mature cotyledons (Venkatachalam *et al.* 1999).

In germination of the somatic embryos, high frequency could not be obtained, as some of them developed either radicle or plumule. Although embryos with elongated plumule and without roots were induced to produce roots, these plantlets were not as healthy as the directly germinated ones with both radicle and plumule. The low conversion rate of somatic embryos may be ascribed to the failure of switching to germination program despite having 'normal' shoot and root apices (Faure 1990). A desiccation treatment or inclusion of gibberellic acid in the germination medium could probably have overcome this to some extent.

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