Trop. Sci. 2002, **42**, 178–184

Somatic embryogenesis in groundnut: a comparison of sixty-nine Indian genotypes

T Radhakrishnan, K Chandran, P Paria, NR Ghetia and A Bandyopadhyay
National Research Centre for Groundnut, PO Box 5, Junagadh 362001, Gujarat, India
(radhakrishnan@nrcg.guj.nic.in)

Abstract *Immature zygotic embryos were cultured on modified MS medium with 1 mg/l of NAA and 15, 20 or 25 mg/l of 2,4 D. Somatic embryogenesis was observed in all the genotypes studied. It and the number of somatic embryos per explant were significantly influenced by the difference in genotypes, level of 2,4-D and the interaction between the two. The number of somatic embryos per explant varied from 1 to 15.8 and the frequency of shoot regeneration from 45 to 100%. Significant differences were observed between the genotypes in the percentage of shoot induction from somatic embryos and the number of shoots per embryo. The mean number of shoots per somatic embryo varied between 1 and 5.9. Of 17 genotypes studied for rooting, 14 showed 100%. The number of roots per shoot varied between 1 and 15. It appears that strong genotypic regulation exists for the somatic embryogenesis and shoot regeneration in groundnut. The high overall response confirms the suitability of the protocol over a wide range of genotypes.*

Keywords: groundnut, genotypes, somatic embryogenesis, shoot regeneration.

Introduction

Direct somatic embryogenesis is an efficient means of plant regeneration, often with the advantage of obtaining genetically uniform plants. The embryogenic response of somatic tissues may be highly variable, influenced mainly by the genotype. Identifying genotypes with high frequency of uniform regeneration is necessary if somatic embryogenesis is to be used in crop improvement like genetic transformation. Genotypic differences in somatic embryogenesis in groundnut have been reported by Sellars et al. (1990), Ozias-Akins et al. (1992), George and Eapen (1993), Reddy and Reddy (1993), McKently (1991), Chengalarayan et al. (1998) and Radhakrishnan et al. (2001), but for a few genotypes only. This report assesses the genotypic differences for somatic embryogenesis in 69 Indian groundnut genotypes.

Materials and methods

Immature pods of 69 genotypes (Table 1) of cultivated groundnut of four botanical groups (15 Virginia bunch; 17 Virginia runner, ssp. *hypogaea* var. *hypogaea*; 36 Spanish bunch, ssp. *fastigiata* var. *vulgaris*; and one Valencia, ssp. *fastigiata* var. *fastigiata*) were obtained

Accepted 10 February 2002

from 110 day-old field grown plants. The pods (6–10 mm) were washed thoroughly with tap water until all the adhered particles were removed and then with distilled water containing Tween 80. The surface of the pods was sterilized in 70% ethanol for 1 min followed by 0.01% $HgCl_2$ for 5 min with constant agitation. The disinfected pods were rinsed three times with sterile distilled water and cut open to remove the seeds. The zygotic embryos were excised aseptically from the seeds and used as explants.

The culture medium used was modified MS (Radhakrishnan et al. 1999). For induction of somatic embryogenesis, the culture medium was supplemented with 1 mg/l of naphthalene acetic acid (NAA) and 15, 20 or 25 mg/l of 2,4 dichlorophenoxyacetic acid (2,4-D) (based on studies by Radhakrishnan et al. 2001). The frequency of the embryogenic explants and the number of somatic embryos per responding explant were recorded 30 days after incubation.

Of the genotypes, 54 were taken at random to test shoot regeneration, and for each genotype the embryos induced from the three different induction media were pooled. Regeneration was used instead of direct germination to get more than one shoot per embryo. The embryos were separated by gently tapping the explant and cultured on modified MS media supplemented with 1 mg/l gibberellic acid + 3 mg/l benzyl adenine + 1 mg/l NAA and incubated at 16 h photoperiod, irradiance of 88 μmol/m²/s and 26 ± 1°C. The number of somatic embryos producing a shoot and the number of shoots per explant were recorded.

The shoots of 30–40 mm length were transferred to a rooting medium, the culture medium supplemented with 1 mg/l NAA. Shoots from 17 cultivars taken at random were tested for the rooting, with at least 25 shoots from each. The number of responding shoots and the number of roots per shoot was recorded after 20 days. The plantlets were hardened for 15 days in a growth chamber at 35°C and 90% RH and planted in the field.

All the experiments were in three replications of 20 tubes each. Frequencies of responding explants were converted to percentage (% SE) and analysed after arcsin transformation. The different responses for the habit types were tested. Means were ranked by LSD.

Results and discussion

Somatic embryogenesis was observed in all the genotypes, and the difference in response was significant ($P < 0.01$). It varied from 27 (ALR 1 at 20 mg/l) to 100% in 22, 20 and 17 genotypes at 15, 20 and 25 mg/l respectively. Several genotypes did not respond at some levels of 2,4-D. The differences due to the three levels of 2,4-D and their interaction with genotypes were also significant ($P < 0.01$), but the differences in response between the habit types were not significant ($P > 0.05$). The overall response across the genotypes was high, 81% at 15 mg/l 2,4-D, indicating that the protocol applies to a wide range of genotypes. The magnitude of response varied significantly, but four genotypes, Chitra, ICGS 21, Spanish improved and TG 26, had 100% response at all levels of 2,4-D. Some soyabean genotypes have superior embryogenic capabilities over a range of culture conditions (Bailey et al. 1993). The number of somatic embryos per explant was independent of the frequency of response ($r = 0.445, 0.439, 0.526$ at 15, 20 and 25 mg/l respectively), as observed by Baker et al. (1995). However, Ozias-Akins et al. (1992) found a strong correlation between the

Table 1. Somatic embryogenesis in groundnut genotypes

Genotype	Habit	Somatic embryogenesis with 2,4-D						Regeneration	
		15 mg/l		20 mg/l		25 mg/l			
		%SE	NSE	%SE	NSE	%SE	NSE	%	MNS
ALR 1	HYB	83	7.5 ± 1.8	27	11.3 ± 2.4	67	10.0 ± 3.0	85	1.9 ± 0.7
B 95	HYB	100	6.0 ± 2.1	100	4.0 ± 2.1	75	8.0 ± 2.5	100	3.5 ± 0.9
BAU 13	HYB	100	6.0 ± 2.7	43	2.6 ± 1.2	0	0.0 ± 0.0	95	3.9 ± 0.7
BG 2	HYB	100	10.1 ± 1.8	100	8.0 ± 2.4	50	6.7 ± 1.7	–	–
HNG (HPS) 2	HYB	55	5.8 ± 2.6	65	10.3 ± 1.9	100	9.0 ± 1.4	94	2.8 ± 0.6
ICGS 5	HYB	100	12.8 ± 2.6	75	6.3 ± 2.8	100	4.0 ± 1.9	95	5.1 ± 0.7
ICGS 76	HYB	88	7.4 ± 1.6	75	4.7 ± 1.9	60	7.0 ± 1.7	–	–
ICGV 86325	HYB	73	11.6 ± 1.7	98	7.1 ± 1.8	42	11.4 ± 1.5	97	4.2 ± 0.6
Kadiri 3	HYB	80	8.8 ± 1.8	100	9.2 ± 1.6	100	14.5 ± 1.4	80	2.7 ± 0.6
M 522	HYB	65	5.2 ± 2.1	100	5.7 ± 2.8	55	5.0 ± 2.1	–	–
M 145	HYB	80	9.0 ± 2.0	88	10.5 ± 1.7	80	13.4 ± 1.5	87	3.0 ± 0.6
RS 138	HYB	80	9.5 ± 1.8	90	8.9 ± 1.6	100	10.1 ± 1.6	85	2.8 ± 0.7
RSB 87	HYB	90	12.2 ± 1.7	75	8.1 ± 1.8	38	6.3 ± 2.1	85	2.3 ± 0.7
T 64	HYB	68	4.7 ± 2.1	85	2.5 ± 1.7	42	3.5 ± 0.3	100	3.0 ± 0.9
TMV 10	HYB	75	4.0 ± 3.0	100	3.3 ± 2.4	63	7.5 ± 3.0	–	–
Chandra	HYR	60	4.8 ± 2.1	70	3.6 ± 1.8	50	4.4 ± 1.9	97	2.5 ± 0.5
Chitra	HYR	100	9.0 ± 3.7	100	10.0 ± 4.8	100	10.0 ± 4.3	100	4.2 ± 0.7
DRG 101	HYR	80	11.0 ± 1.8	90	10.9 ± 1.6	50	9.1 ± 1.6	90	3.4 ± 0.7
DRG 12	HYR	80	7.5 ± 1.8	80	10.4 ± 1.8	70	12.6 ± 1.6	93	4.1 ± 0.5
DRG 17	HYR	70	7.6 ± 2.0	70	6.9 ± 1.7	30	7.3 ± 2.5	97	3.6 ± 0.6
GAUG 10	HYR	75	12.0 ± 2.1	88	9.0 ± 1.7	100	9.1 ± 1.4	75	1.7 ± 0.1
GG 11	HYR	90	11.7 ± 1.7	100	10.7 ± 1.5	100	13.2 ± 1.4	90	4.2 ± 0.7
GG 12	HYR	88	5.4 ± 2.3	60	3.5 ± 1.4	60	4.7 ± 1.6	100	2.6 ± 0.6
GG 13	HYR	70	6.1 ± 2.0	100	9.7 ± 1.5	90	8.0 ± 1.4	–	–
Karad 4-11	HYR	100	7.3 ± 2.0	88	11.3 ± 1.7	80	8.7 ± 1.6	85	3.3 ± 0.7
Kaushal	HYR	67	7.6 ± 2.3	100	5.4 ± 1.8	63	6.6 ± 1.9	90	4.4 ± 0.7
M 197	HYR	70	10.0 ± 2.0	90	12.8 ± 1.4	100	5.7 ± 1.4	77	2.2 ± 0.6
M 335	HYR	90	2.5 ± 1.8	0	0.0 ± 0.0	58	9.0 ± 2.1	–	–
M 37	HYR	100	6.8 ± 2.1	80	10.4 ± 1.8	90	7.8 ± 1.5	80	3.4 ± 0.7
Punjab 1	HYR	71	5.0 ± 2.3	63	8.2 ± 1.9	60	5.6 ± 1.6	95	2.3 ± 0.5
RS1	HYR	90	8.1 ± 1.7	60	5.4 ± 1.8	90	7.1 ± 1.5	100	5.6 ± 0.7
UF-70-103	HYR	100	9.6 ± 1.6	90	12.4 ± 1.6	70	8.0 ± 1.9	93	3.3 ± 0.5
ALR 2	VUL	100	4.5 ± 2.6	83	5.7 ± 1.6	90	4.0 ± 2.1	–	–
CO 1	VUL	83	5.7 ± 2.0	70	8.0 ± 1.5	75	3.8 ± 1.7	95	3.6 ± 0.7

Table 1. Continued

Genotype	Habit	Somatic embryogenesis with 2,4-D						Regeneration	
		15 mg/l		20 mg/l		25 mg/l			
		%SE	NSE	%SE	NSE	%SE	NSE %	MNS	
Dh 3-30	VUL	90	9.4 ± 2.0	75	6.0 ± 2.8	35	8.0 ± 2.5	–	–
Dh 45	VUL	80	7.5 ± 1.8	100	10.0 ± 1.6	80	11.8 ± 1.5	100	3.8 ± 0.5
Dh 8	VUL	70	9.0 ± 2.0	100	11.5 ± 1.7	30	3.3 ± 1.7	–	–
GG 2	VUL	55	12.0 ± 3.0	0	0.0 ± 0.0	0	0.0 ± 0.0	75	5.1 ± 0.8
GG 3	VUL	100	5.5 ± 1.8	0	0.0 ± 0.0	70	4.0 ± 1.3	95	2.1 ± 0.1
GG 4	VUL	58	8.5 ± 2.1	83	3.4 ± 2.1	63	3.2 ± 1.9	–	–
ICGS (FDRS) 4	VUL	60	2.0 ± 1.1	80	6.0 ± 1.7	50	3.4 ± 1.9	100	4.3 ± 0.5
ICGS (FDRS)10	VUL	100	5.6 ± 1.6	70	5.6 ± 1.8	70	3.4 ± 1.6	100	5.1 ± 0.6
ICGS 1	VUL	70	10.7 ± 2.0	35	12.7 ± 2.8	50	12.3 ± 2.1	95	2.4 ± 0.7
ICGS 11	VUL	65	8.5 ± 2.6	88	5.6 ± 2.1	83	8.3 ± 2.1	–	–
ICGS 21	VUL	100	12.4 ± 1.8	100	14.8 ± 1.5	100	20.4 ± 1.4	100	5.7 ± 0.7
ICGS 44	VUL	100	15.3 ± 1.6	50	8.0 ± 2.8	29	5.5 ± 2.0	95	3.2 ± 0.7
ICGV 86590	VUL	60	11.6 ± 2.0	50	5.2 ± 2.1	35	4.7 ± 2.5	45	1.0 ± 1.0
J 11	VUL	100	14.1 ± 1.6	100	10.0 ± 1.5	80	12.3 ± 1.5	70	4.5 ± 0.6
JL 24	VUL	100	12.6 ± 1.7	75	6.2 ± 2.1	100	14.0 ± 1.5	95	3.6 ± 0.7
Jyoti	VUL	80	4.0 ± 2.0	70	5.0 ± 1.8	100	6.9 ± 1.4	94	3.9 ± 0.6
K 1121	VUL	80	6.0 ± 1.8	70	5.3 ± 1.8	50	4.0 ± 1.9	–	–
K 134	VUL	90	14.4 ± 2.0	70	4.0 ± 2.1	70	10.4 ± 1.6	100	2.4 ± 0.9
MH 1	VUL	0	0.0 ± 0.0	78	4.5 ± 2.4	0	0.0 ± 0.0	100	5.9 ± 0.9
RG 141	VUL	70	5.9 ± 2.0	54	2.2 ± 1.9	100	2.8 ± 2.1	–	–
SB XI	VUL	90	6.5 ± 1.8	100	5.3 ± 1.7	45	4.3 ± 2.1	93	2.7 ± 0.6
SG 84	VUL	100	7.6 ± 1.6	100	7.4 ± 1.8	29	2.5 ± 1.2	60	4.3 ± 0.1
Spanish improved	VUL	100	8.8 ± 1.6	100	10.0 ± 1.5	100	9.0 ± 1.4	83	5.2 ± 0.6
TG 17	VUL	90	13.0 ± 1.7	100	3.4 ± 2.1	45	2.0 ± 2.1	90	3.4 ± 0.7
TG 22	VUL	100	15.8 ± 1.7	100	6.9 ± 1.5	100	10.6 ± 1.4	98	2.8 ± 0.5
TG 26	VUL	90	11.4 ± 2.3	100	7.4 ± 1.5	80	7.0 ± 1.5	84	3.3 ± 0.6
TG 3	VUL	100	14.0 ± 1.6	80	10.1 ± 1.7	40	6.0 ± 1.6	90	2.4 ± 0.6
Tirupati 1	VUL	80	7.4 ± 2.3	80	9.3 ± 1.5	65	5.0 ± 1.7	100	3.0 ± 0.7
Tirupati 2	VUL	100	11.9 ± 2.0	75	6.0 ± 3.4	29	9.0 ± 2.3	100	4.0 ± 1.0
TKG 19-A	VUL	100	10.4 ± 1.6	88	7.3 ± 1.7	100	7.3 ± 1.4	97	3.8 ± 0.5
TMV 12	VUL	90	12.0 ± 1.7	75	11.3 ± 1.9	100	9.1 ± 1.6	80	3.8 ± 1.0
TMV 2	VUL	90	7.3 ± 1.7	70	11.1 ± 1.8	30	3.5 ± 1.0	–	–
TMV 7	VUL	0	0.0 ± 0.0	60	2.7 ± 2.8	68	2.9 ± 1.6	85	1.8 ± 0.9
VRI 3	VUL	90	5.1 ± 1.7	70	5.6 ± 1.8	100	5.6 ± 1.4	95	3.2 ± 0.9
Gangapuri	FST	100	2.0 ± 0.3	100	10.0 ± 4.8	0	0.0 ± 0.0	–	–

response and the number of somatic embryos per explant, but this was for a limited number of genotypes only. There were significant differences in the number of somatic embryos per explant ($P < 0.01$), varying from 1 to 15.8. The differences due to the level of 2,4-D and its interaction with the genotypes were also significant ($P < 0.01$). A similar interaction was reported for soyabean (Komatsuda and Ohyama 1989). The differences in the number of somatic embryos per responding explants between different habit types were not significant ($P > 0.05$). Of the three levels of 2,4-D, 15 mg/l had better mean response (81%) than 20 mg/l (76%) and 25 mg/l (65%). With 15 mg/l, 17 genotypes had 100% response and only two failed to respond.

Somatic embryos from all the genotypes used produced shoots *in vitro*, with a frequency of 45–100%. There were significant differences between the genotypes in the percentage of shoot induction and in the number of shoots per embryo ($P < 0.01$). All the embryos from 13 genotypes produced shoots, and the lowest rate was 45% in ICGV 86590. The mean number of shoots per embryo varied between 1 and 5.9, and most of the genotypes had more than three shoots per embryo. However, differences between habit types in shoot regeneration were not significant ($P > 0.05$). Earlier studies (Ozias-Akins et al. 1992; Chengalarayan et al. 1998) on the conversion of somatic embryos also found no genotypic control over regeneration. There was no relation between somatic embryogenesis and the frequency of shoot regeneration ($r = 0.068$) or the number of shoots per responding explant with the percentage of somatic embryogenesis ($r = 0.276$). Contrary to the report of Ozias-Akins et al. (1992), there was no strong correlation between the percentage regeneration and the number of shoots per explant ($r = 0.128$). Both somatic embryogenesis and embryo conversion are believed to be under genetic control (Parrott et al. 1991; Kris and Bringham 1998). For 76 alfalfa cultivars, the highly embryogenic cultivars could be traced to a common origin (Brown and Atanassove 1985). In our study, ICGS 1, ICGS 11, ICGS 44 and Kadiri 3, selections from the genotype Robut 33-1, did not have similar responses in somatic embryogenesis and shoot regeneration. Genetic control over the rate of conversion was ruled out in soyabean (Komatsuda and Ohyama 1989) and in groundnut (Chengalarayan et al. 1998). No influence of habit type on the frequency of somatic embryogenesis, number of somatic embryos per explant, percentage of regeneration and number of shoots per explant was observed. This influence was observed by Reddy and Reddy (1993) but for fewer genotypes. Our six genotypes which had very high responses belonged to two different habit types, but other genotypes of these two types had varying responses.

Of the 17 genotypes studied for rooting, 14 showed 100%, with 1–15 roots per shoot (Table 2). More than 62% of the hardened plantlets survived in the field.

Conclusions

There appears to be strong genotypic regulation for the frequency of somatic embryogenesis and shoot regeneration. In no genotype was the frequency of somatic embryogenesis low enough to be limiting and any genotype could be made to respond by modifying the culture media. Somatic embryogenesis and shoot regeneration are independent of habit type.

Table 2. Rooting of 17 genotypes

Genotype	Rooting (%)	No. of roots per shoot
B 95	100	4.4 × 1.9
Chitra	100	10.5 × 4.5
DRG 101	100	1.0 × 0.0
GG 2	100	15.0 × 0.0
ICGS (FDRS) 4	100	8.0 × 0.0
ICGS 1	100	4.0 × 1.4
J 11	100	6.3 × 0.5
Jyoti	100	6.2 × 4.4
K 134	100	5.9 × 0.3
Kadiri 3	40	5.0 × 3.0
Punjab 1	100	8.0 × 0.0
RS 1	100	6.1 × 3.0
SB X1	88	7.1 × 2.0
SG 84	75	2.3 × 0.5
Tirupati 2	100	2.3 × 1.3
UF -70-103	100	8.0 × 3.4
VRI 3	100	6.3 × 1.9

Acknowledgement

We thank Dr JB Misra for his advice.

References

Bailey MA, Boerma HR and Parrott WA (1993) Genotype effects on proliferative embryogenesis and plant regeneration of soybean. In vitro Cellular and Developmental Biology 29, 102–6.

Baker CM, Durham RE, Burns JA, Parrot WA and Wetzstein HY (1995) High frequency somatic embryogenesis in peanut (*Arachis hypogaea* L.) using mature dry seed. Plant Cell Reports 15, 38–42.

Brown DCW and Atanassove A (1985) Role of genetic background in somatic embryogenesis in *Medicago*. Plant Cell Tissue and Organ Culture 4, 111–22.

Chengalarayan K, Mhaske VB and Hazra S (1998) Genotypic control of peanut somatic embryogenesis. Plant Cell Reports 17, 522–5.

George L and Eapen S (1993) Influence of genotype and explant source on somatic embryogenesis in peanut. Oleagineux 48, 361–3.

Komatsuda T and Ohyama K (1989) Genotypes of high competence for somatic embryogenesis and plant regeneration in soybean (*Glycine max*). Theoretical and Applied Genetics 75, 695–700.

Kris MHS and Bingham ET (1988) Interactions of highly regenerative genotypes of alfalfa (*Medicago sativa*) and tissue culture protocols. In vitro Cellular and Developmental Biology 24, 1047–52.

McKently AH (1991) Direct somatic embryogenesis from axes of mature peanut embryos. In vitro Cellular and Developmental Biology 27, 197–200.

Ozias-Akins P, Anderson WF and Holbrook CC (1992) Somatic embryogenesis in *Arachis hypogaea*, a genotype comparison. Plant Science 83, 103–11.

Parrott WA, Merkle SA and Williams EG (1991) Somatic embryogenesis: potential for use in propagation and gene transfer systems. In: Advanced methods in plant breeding and biotechnology (Murray DR, ed.), pp., 158–200. Wallingford: CAB International.

Radhakrishnan T, Murthy TGK, Desai S and Bandyopadhyay A (1999) Meristem culture of interspecific hybrids of groundnut. Biologia Plantarum 42, 309–12.

Radhakrishnan T, Murthy TGK, Chandran K and Bandyopadhyay A (2001) Somatic embryogenesis in *Arachis hypogaea*: revisited. Australian Journal of Botany 49, 753–9.

Reddy LR and Reddy GM (1993) Factors affecting somatic embryogenesis and plant regeneration in groundnut. Indian Journal of Experimental Biology 31, 57–60.

Sellars MR, Southward GM and Phillips GS (1990) Adventitious somatic embryogenesis from cultured immature zygotic embryos of peanut and soybean. Crop Science 30, 408–14.