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Research Article

SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION IN THREE HIGH YIELDING VARIETIES OF *ARACHIS HYPOGAEA* L.

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ABSTRACT
Somatic embryogenesis and plantlet regeneration using embryonic axes of *Arachis hypogaea* L. have been attempted in three commercial varieties (AK 1224, ICGS 44 and JL 24). Apical portions of the embryonic axes were cultured in Murashige and Skoog's basal media with various concentrations of 2, 4-dichlorophenoxy acetic acid (2, 4-D). After 30 days of incubation, explants were sub-cultured in the same media without 2, 4-D. The optimum concentrations of 2, 4-D in the culture medium for best explant response in terms of the number of somatic embryos induced per explant in the varieties AK 1224, ICGS 44 and JL 24 were different. Application of N^6- benzylaminopurine (BAP) or gibberellic acid (GA_3) showed better germination of somatic embryos than the hormone free medium. The variations of the explant response, the number of somatic embryos generated per explant and the subsequent germination of somatic embryos in these varieties of *Arachis hypogaea* might be due to their varietal difference. All the varieties grew normally and set viable seeds in the experimental garden.

Key Words: *Arachis Hypogaea, In Vitro Regeneration, Somatic Embryogenesis*

INTRODUCTION
The genetic engineering is expected to produce novel groundnut cultivars with qualities hardly thought of at the present time. However, an efficient *in vitro* plant regeneration protocol is essential prerequisite for improvement of crop plants through genetic engineering. Somatic embryogenesis is an extremely elegant *in vitro* technique for rapid regeneration of very high number of uniform clonal plantlets and also useful in experiments on genetic transformation (Batra, 1998). A large number of reports have been accumulated on somatic embryogenesis in *Arachis hypogaea* using various explants viz. immature cotyledon (Eapen and George, 1993; Baker and Wetzstein, 1994), mature cotyledon (Venkatachalam *et al.*, 1999, 2000), mature zygotic embryo derived leaflets (Chengalrayan *et al.*, 1998), immature embryonic axes (Hazra *et al.*, 1989; McKently, 1991; George and Eapen 1993), hypocotyl (Venkatachalam *et al.*, 1997) and immature leaflets (Venkatachalam *et al.*, 1999).
The tissue culture response in groundnut is strongly influenced by the plant genotype, the hormone content of the culture medium, as well as by the age of explant source (Mroginski *et al.*, 1981; McKently *et al.*, 1990; McKently, 1991; McKently *et al.*, 1991, Banerjee *et al.*, 2007, 2011). The type of auxin and their concentrations present in the medium influence the somatic embryogenesis process in groundnut (Baker and Wetzstein, 1994). Therefore, a general protocol for somatic embryogenesis in groundnut is difficult to formulate. In the present investigation attempts have been made to induce and compare somatic embryogenesis process in three commercial varieties using embryonic axes of mature unimbibed seeds and their subsequent development into plantlets.

MATERIALS AND METHODS
Seeds of three high yielding, tikka susceptible groundnut varieties (AK 1224, procured from West Bengal State Seed Corporation, Midnapore; ICGS 44, obtained from BCKV, Jhargram and JL 24, procured from West Bengal State Seed Corporation, Burdwan) were washed with 2% (v/v) liquid detergent (Teepol) for 5 min and surface sterilized with 90% ethanol for 2 min followed by treatment with 0.1% (w/v) mercuric chloride solution for 5-6 min and finally washed repeatedly with sterile distilled water. Embryonic axes were excised from these seeds. Radicle part of the embryo axes were removed carefully and the apical

portion of the embryo axes were used as explant for the induction of somatic embryos. Explants were inoculated in the MS basal media (Murashige and Skoog, 1962) with 6% sucrose and 200 mg l^{-1} casein hydrolysate and supplemented with various concentrations of 2, 4-D (5–40 mg l^{-1}) and solidified with 0.6% agar powder (somatic embryo induction medium). Cultures were incubated in the dark at 25±2°C for 30 days. After 30 days explants were subcultured in the MS basal (sucrose 6%, casein hydrolysate 200 mg l^{-1} and solidified with 0.6% agar powder) medium without 2, 4-D (somatic embryo development medium). Cultures were incubated in the same conditions and responses were recorded after 30 days. For germination of the somatic embryos MS basal media were supplemented with either GA_3 (0.25 mg l^{-1}) or BAP (1 mg l^{-1}). Data were shown along with their respective standard errors (SE), and were analyzed by ANOVA. After obtaining a significant F– value ($\alpha = 0.05$), the treatment means were separated by Duncan's Multiple Range Test (DMRT). All statistical analyses were performed according to Little and Hills (1978).

Plantlets regenerated through somatic embryogenesis were first washed carefully to remove the nutrient agar adhered to the roots and subsequently were transferred to half-strength MS liquid medium without sucrose and incubated in the culture room in unplugged condition. When growth of the plantlets, exhibited by the emergence of new leaves, was observed those were transplanted to plastic pots containing sterile sand-soil mixture (1:1) with adequate water and little amount of half-strength MS salts. The plastic pots with transferred plants were covered with transparent polythene bags to maintain high humidity level and kept in the culture room for 7–10 days under controlled environmental conditions. Survived plants were transplanted to the experimental garden. The process of the transfer and gradual acclimatization to the natural conditions were stringently followed.

RESULTS

The response of the excised embryonic axes of the varieties AK 1224, ICGS 44 and JL 24 in the development medium is presented in Table 1. In the control, somatic embryogenesis was not observed. The cultures in 20 mg l^{-1} 2, 4-D showed higher frequency of somatic embryogenesis (80.94 ± 4.75) in the variety AK 1224 but the mean number of somatic embryos per explant was relatively low (4.52 ± 0.41) (Figure 1A). On the other hand, 10 mg l^{-1} 2, 4-D exhibited moderate explant response (63.88 ± 7.34%) and higher number of somatic embryo formation (7.57 ± 1.74 per explant). In the variety ICGS 44, the highest frequency of somatic embryogenesis (100%) and highest number of somatic embryos (10.45 ± 1.40) were recorded with the induction medium containing 20 mg 1^{-1} 2, 4-D. However, in JL 24, 5 mg l^{-1} 2, 4-D in the induction medium exhibited best explant response (71.99 ± 12.71%), which also yielded maximum number of somatic embryos per explant (5.53 ± 1.58). Supra-optimal level of 2, 4-D in the induction medium proved to be inhibitory.

So far as the *in vitro* germination of somatic embryos is concerned, the frequency of germination of somatic embryo increased significantly with the application of either BAP (1 mg l^{-1}) or GA_3 (0.25 mg l^{-1}) in all the varieties (Table 2). Although the somatic embryos generated in the present study exhibited bipolarity (Figure 1B) with distinct root and shoot meristem, root development occurred readily along with their germination. Regenerated plantlets obtained via somatic embryogenesis (Figure 1C) when transferred to the soil showed 73.33, 80.00, 86.66% survival in the varieties JL 24, ICGS 44 and AK 1224 respectively (Figure 1D). All the varieties grew normally and set viable seeds in the experimental garden.

DISCUSSION

In the present investigation, it was observed that 20 mg l^{-1} 2, 4-D gave the best explant response in the varieties AK 1224 and ICGS 44, whereas 5 mg l^{-1} 2, 4-D was found optimum for the variety JL 24. It was also observed that in the varieties AK 1224 and ICGS 44, the explant response i.e. percent somatic embryogenesis initially increased with the increase in the concentration of 2, 4-D. The percent somatic embryogenesis decreased significantly at a very high level of 2, 4-D (40 mg l^{-1}) in the varieties JL 24 and AK 1224. On the contrary, the variety ICGS 44 showed no significant reduction in the percent of somatic

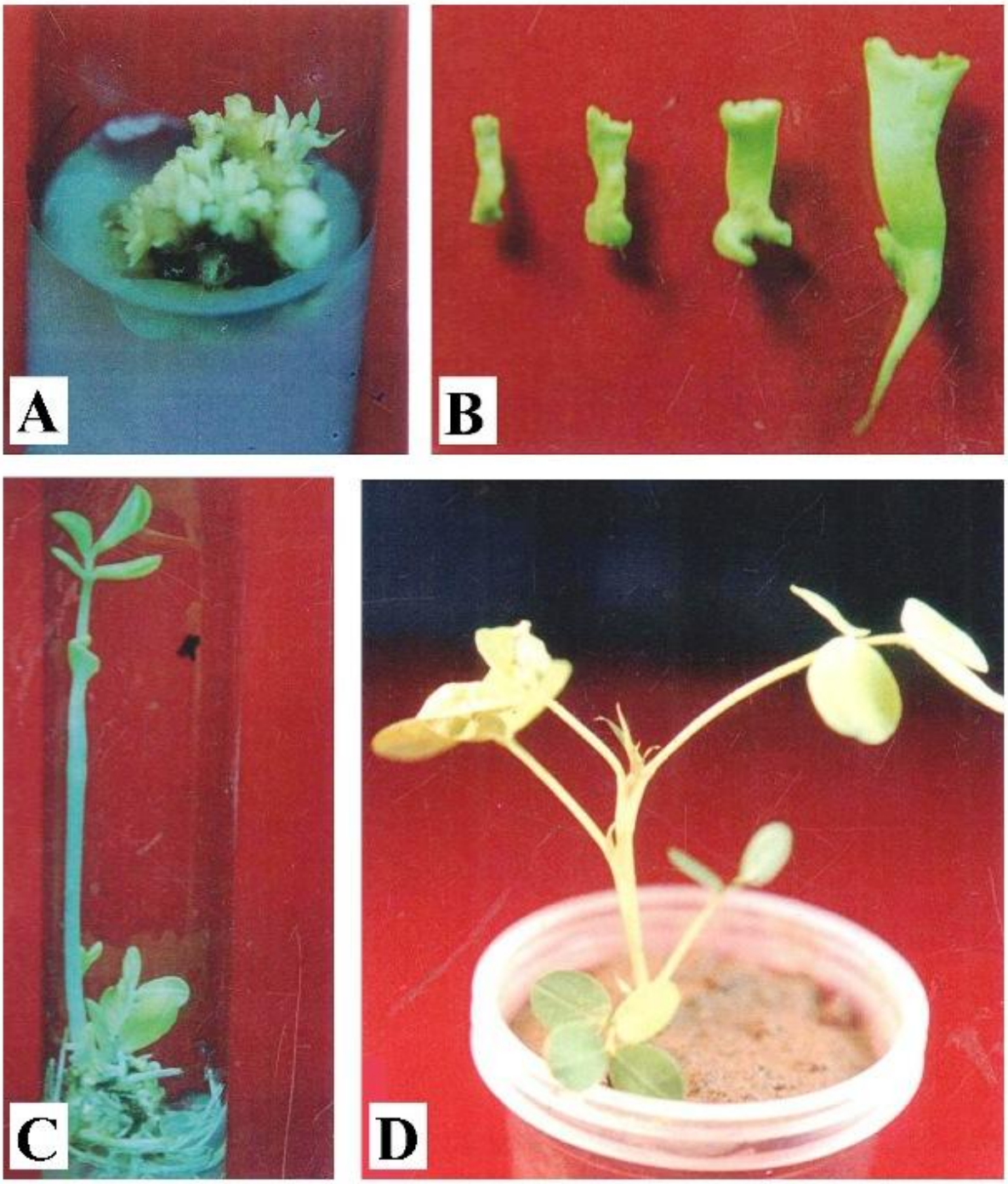

Figure 1: Somatic embryogenesis in *Arachis hypogaea* L. A. A cluster of somatic embryos (× 2); B. Bipolar somatic embryos at different stages of development (× 3); C. Plantlet derived from somatic embryo (× 0.8); D. Plantlet established in pot (× 0.6)

Table 1: Effect of 2, 4-D on direct somatic embryogenesis from excised embryonic axis of *Arachis hypogaea* L. (response recorded after 30 days of culture on development medium)

Variety	Induction medium with 2,4-D (mg l^{-1})	Development medium (PGR-free)	
		Explant response (%) ± SE	Number of embryos per explant ± SE
AK 1224	0	0	0
	5	27.77±5.55^{c}	3.20±0.37^{b}
	10	63.88±7.34^{b}	7.57±1.74^{a}
	20	80.94±4.75^{a}	4.52±0.41^{b}
	40	50.00±0.00^{b}	4.33±0.98^{b}
ICGS 44	0	0	0
	5	86.66±3.33^{b}	5.36±0.79^{b}
	10	88.57±5.94^{b}	6.53±0.84^{b}
	20	100.0±0.00^{a}	4.52±0.41^{b}
	40	95.83±4.16^{ab}	5.47±0.76^{b}
JL 24	0	0	0
	5	71.99±12.71^{a}	5.53±1.58^{a}
	10	71.42±17.26^{a}	4.40±0.19^{b}
	20	50.00±10.95^{ab}	4.21±0.65^{b}
	40	37.77±10.13^{b}	2.41±0.22^{c}

Mean values followed by same letter are not significantly different at 0.05 levels (Duncan's multiple range test).

Table 2: *In vitro* germination of somatic embryos of *Arachis hypogaea* L.

Variety	PGR (mg l^{-1})	Root formation (%) ± SE	Shoot formation (%) ± SE
AK 1224	0	100±0.0	24.0±4.00^{b}
	BAP 1	92.0±8.0	52.0±4.89^{a}
	GA_3 0.25	100±0.0	64.0±4.00^{a}
ICGS 44	0	100±0.0	44.0±7.48^{b}
	BAP 1	100±0.0	56.0±9.79^{ab}
	GA_3 0.25	100±0.0	84.0±4.00^{a}
JL 24	0	100±0.0	16.0±7.48^{b}
	BAP 1	92.0±4.89	28.0±8.00^{ab}
	GA_3 0.25	100±0.00	48.0±4.89^{a}

Mean values followed by same letter are not significantly different at 0.05 levels (Duncan's multiple range test).

embryogenesis at 40 mg l^{-1} 2, 4-D. Reduction in the percent embryogenesis with increase in the concentration of 2, 4-D was in conformity with the results obtained by other workers in groundnut (Baker and Wetzstein, 1994) and also in other plants like tea (Bano *et al.,* 1991), papaya (Fitch and Manshardt, 1990) and in *Bauhinia variegata* (Banerjee *et al.,* 2012). A concentration of 20 mg l^{-1} 2, 4-D gave satisfactory explant response in terms of somatic embryogenesis in different varieties of *Arachis hypogaea* using various explants (Eapen and George, 1993; Venkatachalam *et al.,* 1997). Those findings are in conformity with the results obtained in the present investigation on the varieties AK 1224 and ICGS 44. However, Baker and Wetzestein (1994) reported that 5 mg l^{-1} 2, 4-D gave best response in terms of somatic embryogenesis using cotyledon as explant in the cultivar AT 127 of *Arachis hypogaea.*

The number of somatic embryos per explant in the present study decreased significantly in all the varieties at relatively higher concentrations of 2, 4-D and these findings corroborated with the results of Venkatachalam *et al.,* (1997) and Banerjee *et al.,* (2012). In the present investigation it was also recorded that the requirement of 2, 4-D in the induction medium for achieving best explant response was not uniform in all the varieties.

Ozias-Akins (1989), Hazra *et al.,* (1989) and McKently (1991) used different auxins and obtained variable results. On the basis of their observations Baker and Wetzstein (1994) concluded that a homogeneous and uniform pattern of somatic embryogenic response in groundnut was rather difficult to formulate. The use of different genotypes and or explant sources might have contributed to the divergent results reported (Baker and Wetzstein, 1994; Banerjee *et al.,* 2011). The present observations on three varieties also supported these authors where a non homogeneous embryogenic tissue mass was always obtained which contained particularly somatic embryos of various sizes and shapes along with root formation in certain other structures.

Leaving aside the role of 2, 4-D in somatic embryogenesis in groundnut (Baker and Wetzstein, 1992, 1994; George and Eapen, 1993; Chengalrayan *et al.,* 1994, 1997; Venkatachalam *et al.,* 1997), and the effect of BAP in induction of somatic embryos has also been reported (Venkatachalam *et al.,* 1999). In the present investigation higher sucrose level (6%) had been used which was found to favour somatic embryogenesis in several other plants like *Trifolium* (Maheswaran and Williams, 1986), *Dalbergia latifolia* (Lakshmi Sita and Rao, 1999) and groundnut (Eapen and George, 1993).

Although the somatic embryos possess both root and shoot meristems, simultaneous development of root and shoot was infrequent (Venkatachalam *et al.,* 1999). Root development in the somatic embryos occurred readily in all the varieties in PGR-free as well as in the media supplemented with either BAP (1 mg l^{-1}) or GA_3 (0.25 mg 1^{-1}). The frequency of shoot development also increased in those media. These observations corroborated with those of Bandyopadhyay *et al.,* (1996). BAP (1 mg l^{-1}) was also found more effective in embryo germination of the variety AK 1224 compared to the PGR-free medium. A similar result was reported earlier (Venkatachalam *et al.,* 1997; Chengalrayan *et al.,* 1997). However, in the varieties ICGS 44 and JL 24, germination of somatic embryos in BAP (1 mg l^{-1}) supplemented medium was not markedly different compared to the hormone-free medium.

The conversion of somatic embryos into plantlets depended on the type and concentration of auxin used in the somatic embryo induction medium. The higher concentration of auxin in the induction medium may be responsible for the low conversion frequency (Eapen and George, 1993). However, the difference in explant response, the number of somatic embryos per explant and the germination percentage in three varieties might be due to their varietal difference as reported previously in groundnut (Sellars *et al.,* 1990; Vekatachalam *et al.,* 1997; Radhakrishnan *et al.,* 1999), in *Helianthus anuus* L. (Potdar *et al.,* 1999) and in coffee (Naidu *et al.,* 1999).

In view of these findings, it could be suggested that somatic embryogenesis and subsequent regeneration of plantlets could be an efficient *in vitro* method for propagation of *Arachis hypogaea* apart from the propagation through axillary and adventive shoot formation. The protocol of somatic embryogenesis, therefore, might facilitate germplasm conservation and gene transfer research in groundnut.

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