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Regeneration of Plantlet through Somatic Embryogenesis in a Tikka Immune Variety of *Arachis hypogaea* L. Var. ICG 6284

Sharmistha Maity

Department of Botany, Krishnagar Government College,
Krishnagar, Nadia-741101 (India)
E-mail address: **sharmistha_maity@ rediffmail.com;**
Mobile No. - **9832223933**

Abstract

Somatic embryogenesis and plantlet regeneration using embryonic axes of *Arachis hypogaea* L. variety ICG 6284 had been attempted. Apical portion 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. Application of N6-benzylaminopurine (BAP) or gibberellic acid (GA3) showed better germination of somatic embryos than the hormone free medium. Survival rate of the in vitro regenerants in the field was above 90%, grew normally and set viable seeds in the experimental garden.

Abbreviations: ANOVA- analysis of variance; BAP- N6–benzylaminopurine; DMRT- Duncan's Multiple Range Test; GA3- Gibberellic acid; MS- Murashige and Skoog's medium; 2.4-D- 2,4-dichlorophenoxyacetic acid;

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[9]. A large number of reports have been accumulated on somatic embryogenesis in *Arachis hypogaea* using various explants viz. immature cotyledon[2,13], mature cotyledon[31,32], mature zygotic embryo derived leaflets[12], immature embryonic axes[15,16,20], hypocotyls[30], immature leaflets[31] and mature embryonic axes[4]. 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[4-6,8,11-16,22,33]. The type of auxin and their concentrations present in the medium influence the somatic embryogenesis process in groundnut[2]. Therefore, a general protocol for somatic embryogenesis in groundnut is difficult to formulate. In the present investigation attempts had been made

to induce somatic embryogenesis process in the elite variety ICG 6284 using embryonic axes of mature un-imbibed seeds and their subsequent development into plantlets.

Seeds of the high yielding, tikka susceptible groundnut variety ICG 6284 procured from ICRISAT, Patancheru, India 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[24] 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 sub-cultured 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[18]. 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 plantlets 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.

The response of the excised embryonic axes of the variety ICG 6284 in the development medium is presented in Table 1. In the control, somatic embryogenesis was not observed. The cultures in 15 to 20 mg l^{-1} 2, 4-D, showed 100% frequency of somatic embryogenesis. The mean number of somatic embryos per explant was highest in 15 mg l^{-1} 2, 4-D (17.31 ± 2.11) (Figure 1A & 1B). 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 (Table 2) with the application of either BAP (1 mg l^{-1}) or GA_3 (0.25 mg l^{-1}). Although the somatic embryos generated in the present study exhibited bipolarity (Figure 1C) with distinct root and

shoot pole, root development occurred readily along with their germination. Regenerated plantlets obtained via somatic embryogenesis (Figure 1D) when transferred to the soil showed 92.64% survival (Figure 1E). All the regenerants grew normally and set viable seeds in the experimental garden.

In the present investigation, it was observed that 15 mg l^{-1} 2, 4-D gave the best explant response. It was also observed that percent somatic embryogenesis initially increased with the increase in the concentration of 2, 4-D in the induction medium. The percent somatic embryogenesis decreased significantly at a very high level of 2, 4-D (40 mg l^{-1}). 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[2,4] and also in other plants like tea[18], papaya[24] and in *Bauhinia variegate*[7]. 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[13,30]. Those findings are in conformity with the results obtained in the present investigation in terms of the percent embryogenesis. However, Baker and Wetzestein[2] 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 at relatively higher concentrations of 2, 4-D and these findings corroborated with the results of Venkatachalam *et al.*,[30], Banerjee *et al.*,[7] and Banerjee[4]. Ozias-Akins[26], Hazra *et al.*,[16] and McKently[20] used different auxins and obtained variable results. On the basis of their observations Baker and Wetzstein[2] concluded that a homogeneous and uniform pattern of somatic embryogenic response in groundnut was rather difficult to formulate which was again supported by Banerjee[4]. The use of different genotypes and or explant sources might have contributed to the divergent results reported[3,4,6]. Leaving aside the role of 2, 4-D in somatic embryogenesis in groundnut[1,2,10,15,30] and the effect of BAP in induction of somatic embryos has also been reported[31]. In the present investigation higher sucrose level (6%) had been used which was found to favour somatic embryogenesis in several other plants like *Trifolium*[19], *Dalbergia latifolia*[17] and groundnut[4,13]. Although the somatic embryos possess both root and shoot meristems, simultaneous development of root and shoot was infrequent[11,31]. 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.*,[3]. A similar result was reported earlier[4,11,30]. 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[13]. However, the difference in explant response, the number of somatic embryos per explant and the germination percentage in *Arachis hypogaea* might be due

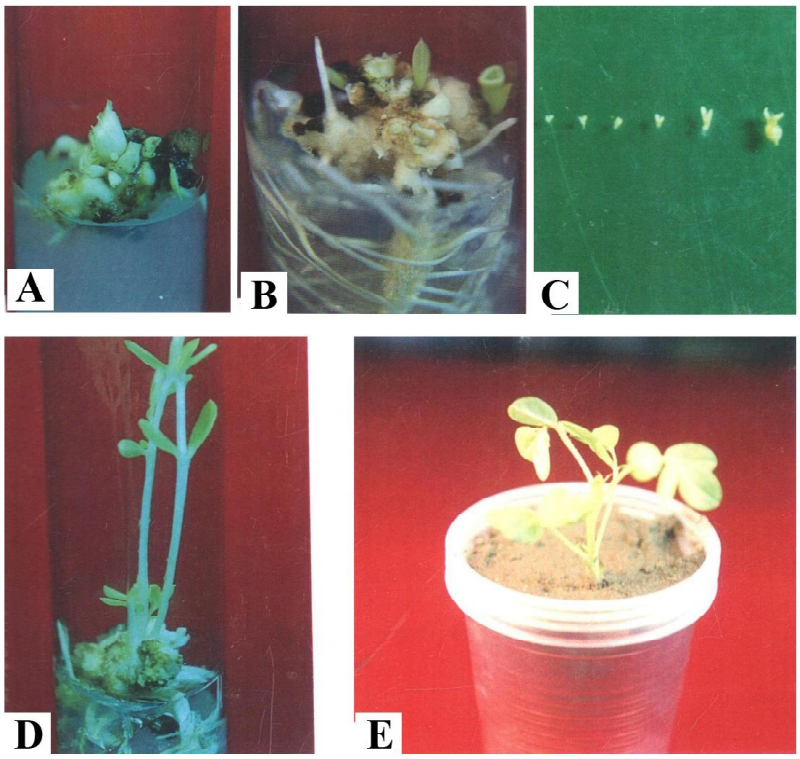

Figure 1

Figure Captions

A. A cluster of somatic embryos, B. Cup shaped somatic embryo
C. Bipolar somatic embryos at different stages of development
D. Plantlet derived from somatic embryo E. Plantlet established in plastic cup

Table-1: Direct somatic embryogenesis from excised embryonic axis of *Arachis hypogaea* L. var. ICG 6284 (response recorded after 30 days)

Induction medium with 2,4-D (mg/l)	Development medium (PGR-free)	
	Explant response (%) ± SE	Number of embryos per explant ± SE
0	0	0
5	75.62 ± 4.43^{d}	8.62 ± 1.49^{d}
10	84.25 ± 2.52^{c}	10.22 ± 1.43^{c}
15	100.0 ± 0^{a}	17.31 ± 2.11^{a}
20	100.0 ± 0^{a}	14.28 ± 1.33^{b}
40	94.26 ± 3.21^{b}	10.22 ± 0.48^{c}

Mean values followed by same letter are not significantly different at 0.05 level (DMRT).

Table-2: *In vitro* germination of somatic embryos of *Arachis hypogaea* L. var. ICG 6284 (response recorded after 30 days of culture)

PGR (mg/l)	Root formation (%) ± SE	Shoot formation (%) ± SE
0	100 ± 0	64.12 ± 3.41^{c}
BAP 1	100 ± 0	77.36 ± 4.72^{b}
GA_3 0.25	100 ± 0	89.21 ± 2.44^{a}

Mean values followed by same letter are not significantly different at 0.05 level (DMRT).

to their varietal difference as reported previously in groundnut[4,30,28,29] in *Helianthus anuus* L.[27] and in coffee[25]. 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* variety ICG 6284. The protocol of somatic embryogenesis, therefore, might facilitate germplasm conservation and gene transfer research in groundnut and more particularly of this tikka immune variety.

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