



Plant growth regulators and their efficacy in promoting direct organogenesis in various groundnut varieties

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Abstract

Projects have been developed to enhance crops and establish a successful method for plant regeneration. This study focused on the regeneration response of cotyledonary node explants from germinating peanut (*Arachis hypogaea* L.) seeds. Sectioned cotyledonary nodes were grown *in vitro* to regenerate whole plants. Mixing 6-benzylaminopurine (BAP) with Murashige and Skoog's media yielded many shoots (1–50mg.L⁻¹), with optimal production for most varieties at 15 mg L⁻¹. Higher BAP concentrations inhibited all genotypes, while lower doses promoted repeated shoot production. Genotypic differences affected shooting potential with BAP presence. Two genotypes showed rooting on MS medium with 1 mg L⁻¹ NAA, whereas GJG 20 required a combination of 1 mg L⁻¹ NAA and 0.5 mg L⁻¹ IBA. Rooted plantlets were acclimatized in plastic cups with a soil, sand, vermicompost, and farmyard manure mix (3:1:1 w/w) for 7-10 days before field transfer. These plants flowered and produced healthy seeds without phenotypic variations, demonstrating a reliable *in vitro* regeneration method for peanut plants.

Keywords: Groundnut, cotyledonary node, 6-benzyladenine (BAP), naphthalene acetic acid (NAA) indole butyric acid (IBA)

Introduction

Grain legumes are an essential class of crops that provide a significant amount of oil and protein for nutrition. *Arachis hypogaea* L., or groundnut, is one of the most significant oil seed crops in the world. In addition to giving farmers a source of money, groundnuts are a cheap and high-quality source of nutrition. On a dry seed basis, groundnut seeds have an oil content of 44-56% and a protein content of 22-30% (Savage and Keenan, 1994) [22]

The main obstacle is that grown groundnuts are not resistant to biotic and abiotic stressors. Despite a vast area being farmed, production is still low. The development of tissue culture techniques for this species is therefore of great importance. It is well known that plant growth regulators increase peanut yield, oil content, and fatty acid content (CP. Malik *et al.*, 1993, 1988) [15, 16]. *In vitro* manipulation of groundnuts has proven to be challenging, and only a limited degree of successful whole plant regeneration has been achieved in certain cultivars (Heatley and Smith, 1996, Ponsamuel *et al.*, 1998) [9, 20]. There have been reports of organogenesis (Cheng *et al.*, 1992; Eapen and George, 1993; Li *et al.*, 1994; Narasimhulu and Reddy, 1983; Venkatachalam *et al.*, 1996) [9, 7, 13, 17, 22] and somatic embryogenesis (Baker and Wetzstein, 1992, 1995; Chengalrayan *et al.*, 1994, 1997; Ikeuchi *et al.*, 2013; Kanyand *et al.*, 1997; Ozias-Akins *et al.*, 1992) [1, 2, 5, 6, 10, 12, 18] methods for plant regeneration using various explants.

The genotype of groundnut varies. Groundnuts require particular techniques for each variety, making their *in vitro* multiplication and upkeep laborious. Groundnut cultivar mass propagation would undoubtedly benefit from the standardization of *in vitro* plant regeneration procedures without the need for a callus phase.

The current work explains how five different groundnut cultivars' cotyledonary node (CN) explants can be used to induce direct shoot organogenesis through the use of plant growth regulators. The goal was to create a straightforward procedure for repeated shooting and plant regeneration that would work for all three varieties of market groundnuts—spreading, semi-spreading, and spanish bunch—without the need for a callus stage in between.

Materials and Methods

GJG 20 and GJG 22 (semi-spreading) groundnut (*A. hypogaea* L.) seeds were acquired from the Main Oil Seeds Research Station at the Junagadh Agricultural University in Junagadh. The seeds were submerged in a liquid detergent solution for five minutes after being rinsed with tap water for ten to fifteen minutes. The seeds were first cleaned with distilled water, then submerged in 70% ethanol for three to five minutes.

This process was repeated three or four times. Following their introduction into the inoculation chamber, the seeds were surface sterilized for eight minutes using 0.1% HgCl₂ and then rinsed three to four more times with sterile distilled water. In a 250 mL wide mouthed conical flask with a sterile wet cotton bed, four to six surface-sterilized seeds were aseptically germinated in the dark. From seedlings that were 10–12 days old, CN was removed and used as explants. These explants were cultivated in MS medium (Murashige and Skoog, 1962) [16], which included different doses of 6-Benzyladenine (BAP; 1, 5, 10, 15, 25, and 50 mg L⁻¹) and 3% (w/v) sucrose. Before autoclaving, the medium's pH was brought to 5.8±0.02, it was solidified with 0.8% agar-agar, and it was autoclaved for 15 minutes at 121°C and 15 psi of pressure. For 4-5 weeks to allow for

repeated shootings, all cultures were cultured at $24\pm 2^{\circ}\text{C}$ with 16 hours per day exposure to fluorescent tubes that produced $80\ \mu\text{Em}^{-2}\ \text{s}^{-1}$ of white light.

In order to produce plantlets of a consistent size using micropropagation, later shoots were divided and moved to other media. Excision and culture of healthy, elongated shoots measuring about 3 cm in length were done on MS medium supplemented with α -Naphthalene acetic acid (NAA) at concentrations of 0.5, 1.0, 1.5, and 2.0 mg L^{-1} alone and in combination with Indole butyric acid (IBA) at concentrations of 0.5, 1.0, 1.5, and 2.0 mg L^{-1} respectively.

Up until the development of secondary roots, rooting shoots were seen. After washing the roots under running water to get rid of any remaining medium, they were moved to plastic cups filled with a 3:1:1 w/w mixture of soil, sand, and farmyard manure or vermicompost and left in the growth chamber for a period of seven to ten days. After being transplanted to the field, the freshly created plantlets flowered and produced healthy seeds. Every experiment was conducted in triplicate, with all parameters held constant during the three repetitions. Two-way ANOVA for unequal observation per cell (Table 2) was performed to infer the significance of multiplication potential at varieties level (Goon, A., M.K. Gupta and B. Dasgupta, 1998^[8])

Results

The groundnut genotypes shown good response to BAP in terms of cotyledonary node (Fig. 1a). Two genotypes in the BAP free control (GJG20 and GJG22) showed signs of shoot development. Le. 17.6 ± 1.18 per CN (Fig. 1b) was the high rate of multiple shoot development that the genotype

GJG20 demonstrated, even in the absence of BAP, or control (Table 1). Shoot buds, axillary branches, and multiple shoot development were common traits among all genotypes.

The multiplication potential decreased in all genotypes when BAP increased from 1 mg L^{-1} to 50 mg L^{-1} . Higher BAP concentrations were often found to impede shoot lengthening, but lower BAP (1 mg L^{-1} and 5 mg L^{-1}) concentrations caused shoots to elongate, as demonstrated in Fig. 1d in the instance of the GJG20 genotype. Additionally, intra-varietal variations were revealed by the CN explant's capacity to multiply in each of the two genotypes of *Arachis hypogaea* L.

The regenerated shoots were subsequently subcultured on MS medium supplemented with different doses of IAA in order to allow root formation, as root formation occurred within three weeks of transfer to root induction media (Fig. C). The effects of different IAA combinations on root initiation were noteworthy in terms of root development. With IAA at 1 mg L^{-1} , the greatest rooting percentage of 93.47 was attained. As the outcome demonstrated, IAA at 1 mg L^{-1} had the longest root, measuring 3.8 cm. The impact of IAA resulted in a notable variation in the quantity of roots.

To remove the culture medium from the roots, root-bearing plantlets were removed from culture tubes and thoroughly cleaned with tap water. Plantlets that had been washed were placed in pot with regular, sterile soil after being sprayed with fungicide (Fig. D). Plantlets were buried in soil after seven days. Reproducible, the procedure described here may enable large-scale micropropagation of this significant new plant in leeb.

Table 1: Multiplication Potential (multipleshoots, axillary branches and shoot buds) of cotyledonary node explant in Two genotypes of *Arachishypogaea* L. after 45 Days of incubation

Multiplication Potential \pm SE		
BAP mg L^{-1}	GJG20	GJG22
0	17.6 \pm 1.18	2 \pm 0.27
1	22.6 \pm 1.18	21 \pm 0.97
5	24.6 \pm 3.2	22.6 \pm 1.44
10	25.3 \pm 9.15	21.3 \pm 1.18
15	25 \pm 1.79	35.3 \pm 1.96
25	25 \pm 1.8	29.6 \pm 0.72
50	18 \pm 0.94	20.3 \pm 1.18

Table 2: ANOVA for multiplication Potential (multiple shoots, axillary branches and shoot buds) of cotyledonary node explants in five genotypes of *Arachishypogaea* L

Tabular F						
Source of variation	Degree of freedom	Sum of squares	Mean squares	Observed F	5%	1%
Replication	2	217.504	108.752	4.74		
Variety	1	1012.438	253.109	11.048	2.5	3.6
Treatments	6	7565.523	1260.920	55.040	2.23	3.07
var.X treatment	6	2882.096	120.087	5.241	1.67	2.07
Error	26	1557.829	22.909			
Total	41	13235.29				

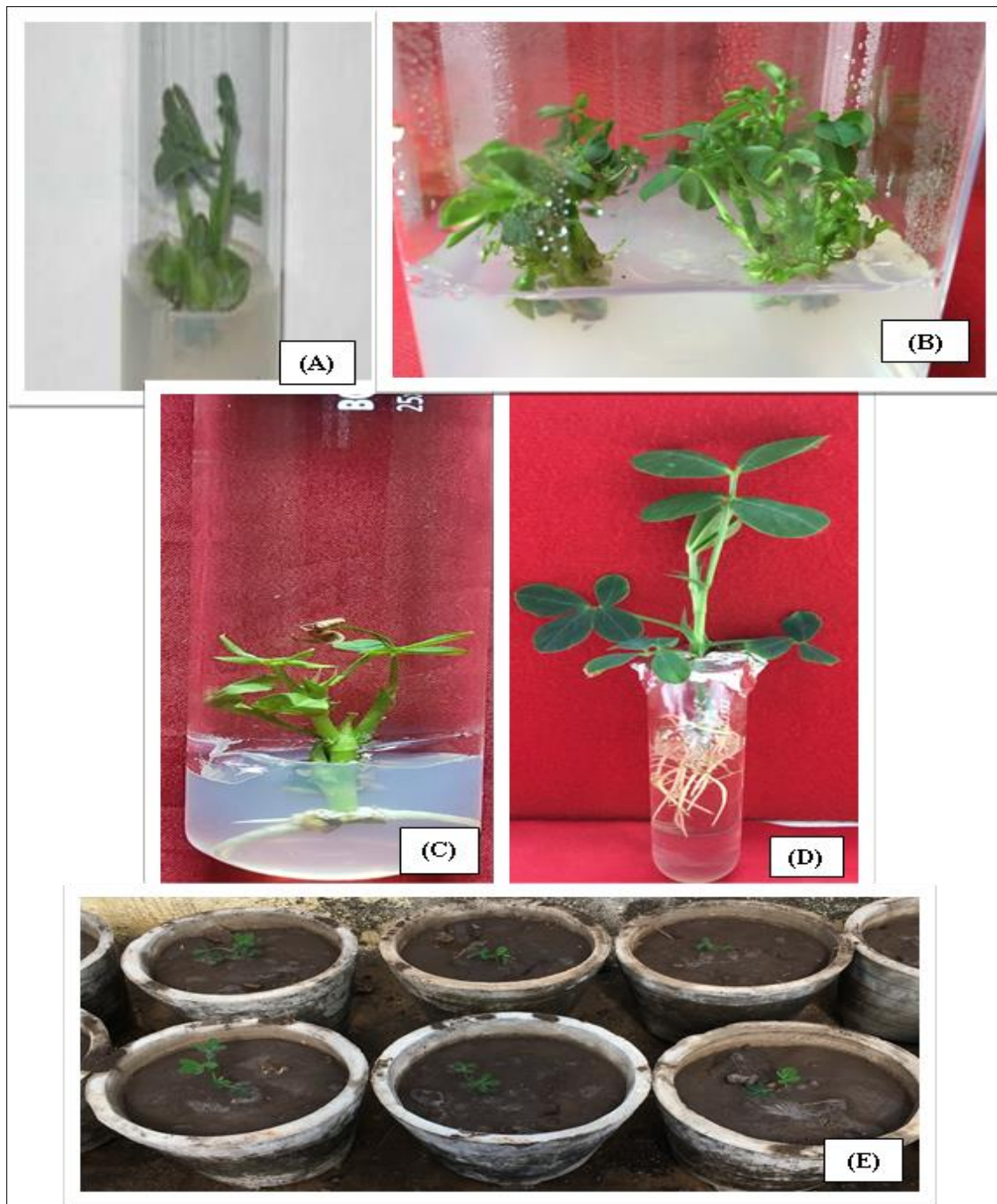


Fig 1: tridirectional response of Cotyledonary node (CN) explant of *Arachis hypogaea* L. (a) Response of CN explant of GJG 20 genotype at 10 mg L^{-1} (b) Response of CN explant of GJG 22 genotype at 15 mg L^{-1} (c) root induction in GJG20 and GJG22 genotype on 1 mg L^{-1} NAA (d) Hoagland media (e) Transfer of plantlets to soil

On MS supplemented with 1 mg L^{-1} NAA, roots of isolated shoots in two genotypes (GJG20 and GJG22) was observed; however, rooting needed a combination of 1 mg L^{-1} NAA and 0.5 mg L^{-1} IBA. The length of root induction or rooting differed throughout genotypes as well. In GJG20 (Figs. 1c and 1d), roots were induced in 7-10 days, whereas in GJG22, it took longer 12-15 days. Under field conditions, the survival percentages (Fig. 1e) of the plantlets of genotypes GJG20 and GJG22 were 80, 82.9, 73, 72.6, and 78%, respectively.

Discussion

The current work describes a straightforward procedure for *Arachis hypogaea* L. regeneration from CN explants. There seems to be a genotype-dependent component to the

multiplication potential, and the current approach may not work with other genotypes. Increased shoot multiplication was the outcome of the CN's good response to varying BAP concentrations. Recorded multiplication rates of 10 mg L^{-1} and 15 mg L^{-1} were the greatest. GJG 20 (25.3 ± 9.15) demonstrated the highest shooting potential, followed by GJG 22 (35.3 ± 1.96), whereas GJG 22 at 15 mg L^{-1} showed a multiplication potential of 35.3 ± 1.96 . (Fig. 1b). Multiplication potential decreased when BAP concentration increased further. Aside from stunted development and improper leaf opening, BAP levels at 50 mg L^{-1} in all genotypes did not show any discernible increase in shooting. These might result from variations in genotype. Our findings concur with those of previous researchers (Banerjee *et al.*; Bernier, 1988; Roy and Banerjee, 2003) [2, 20]

The auxin:cytokinin ratio was essential for groundnut regeneration of numerous shoot buds. In the current investigation, BAP by itself was sufficient to stimulate shoot bud proliferation in each of the five genotypes. Finally, the interaction between plant growth regulators and endogenous growth regulators determines the effect of the regulators in addition to the concentration administered (Joshi and Nadgauda, 1997)^[11].

In summary, the results of this work are highly significant since they have provided a description of a tri-directional (direct shoot development, axillary branching, and shoot bud formation) micropropagation approach in a single medium. Consequently (Cheng et., al.1992)^[4] increasing groundnut productivity may benefit from the current study's findings.

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