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Correlative Effect of Adenine Sulphate and Benzylaminopurine on the Regeneration Potentiality in Cotyledonary Explants of Groundnut (*Arachis hypogaea* L.)

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Abstract

An efficient method of shoot regeneration of peanut is described. *In vitro* shoot organogenesis from the callus of cotyledon explants of *Arachis hypogaea* L. was stimulated by addition of *Adenine sulphate* (Ads) along with 6 - benzylaminopurine (BAP) and - napthalene acetic acid (NAA). Ads (13 μM) had a stimulatory effect on shoot bud differentiation when combined with BAP (13 μM) and NAA (2 μM). Shoot organogenesis was markedly higher (92%) from callus induced on Ads, BAP and NAA combined media than from those formed by the individual supplementation of Ads or BAP with NAA. The shoots elongated on the media with GA_3 (1 μM). Elongated plantlets rooted with MS media containing IBA (9 μM).

Introduction

The major breeding objectives of the groundnut (*Arachis hypogaea* L.) are to develop varieties with high yield and quality, earliness, resistance to major pests, diseases and high protein and oil contents. Plant tissue culture has opened up several new vistas for the induction of genetic variability and the selection of desirable variants.

Sustained *in vitro* regeneration of groundnut via *de novo* organogenesis is important for improving groundnut genotype through somaclonal variation or induced mutation using physical or chemical mutagens. In early studies on groundnut *in vitro* shoot organogenesis was obtained from regenerable tissues (Mroginski et al., 1981; Narasimhulu and Reddy, 1983; Atreya et al., 1984; McKently et al., 1990; Cheng et al., 1992; OziasAkins 1989). In the present study shoot organogenesis was enhanced by physiological manipulation of 6 - benzylaminopurine rich medium coupled with Ads, resulted in high frequency and repetitive production of multiple shoots from cotyledon explants.

Materials and Methods

Plant materials

Seeds of *Arachis hypogaea* cvs VRI-3 and CO-2 were obtained from Tamil Nadu Agricultural University, Coimbatore, India. The surface of seeds were sterilized in a step wise manner by washing and soaking the seeds in soap water for two times, then with 70% ethanol for two times and washed with distilled water. The seeds were again immersed in 1% mercuric chloride for 5 minutes, followed by three washes with sterile distilled water. The seeds were then germinated on moistened cotton.

Explant source

Seven day old seedlings were used as the explant source. Cotyledons were detached from the seedlings and cut into two pieces, nodal areas were removed from the piece. These explants were then placed on media containing different hormonal combinations.

Media source

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Table 1. Effect of Ads on multiple shoot induction on cotyledon derived callus culture of groundnut cv VRI-3 after 60 days of culture

Treatment (μM)	No. of shoot bud per explant	% of shoot bud differentiation
Ads		
3.0	2.26±0.07e	32.27±0.02bc
8.0	5.17±0.12d	35.56±0.19bc
13.0	7.12±0.36c	37.42±0.37bc
19.0	4.69±0.42d	35.44±0.42bc
27.0	3.33±0.09d	31.69±0.22bc
Ads + NAA		
3.0 + 2.0	4.24±0.72d	35.67±0.78bc
8.0 + 2.0	5.37±0.08d	38.48±0.45bc
13.0 + 2.0	10.17±0.61c	40.00±0.94bc
19.0 + 2.0	8.69±0.52c	37.77±0.36bc
27.0 + 2.0	5.66±0.64d	36.10±1.23bc
BAP + NAA + Ads		
13 + 2.0 + 3.0	21.92±0.46b	50.60±2.37ab
13 + 0.5 + 8.0	32.26±0.32a	78.12±1.32a
13 + 0.5 + 13.0	47.50±0.24a	92.20±0.84a
13 + 0.5 + 19.0	43.11±0.78a	86.50±1.98a
13 + 0.5 + 27.0	38.62±0.69a	67.20±1.52ab

* Data were represented in mean±SE. Mean values followed by the same letter are not significantly different at p = 0.01, according to Duncans multiple range test.

All media combinations were based on MS media with 3% sucrose. The pH was adjusted to 5.8. Agar (0.8%) was used as solidyfying agent. To this different hormonal combinations of NAA, BAP and Ads were supplemented in different combinations and concentrations. All medium constituents were sterilized under standard autoclave conditions. The cultures were maintained under light (16h/day photoperiod of 2,000 Lux) at 25±20℃. Each combination had twenty replicates. The callus developed all over the explant and subcultured to a fresh medium after every fifteen days. The shoot buds developed from the green calli after two weeks of culture. Well developed plantlets were transferred to rooting media containing IBA. Rooted plants were transferred to pots containing sterilized sand and vermiculite (1:3).

Statistical analysis

The date were statistically analysed using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1976).

Results

The effect of different cytokinin concentrations, auxin and their interactions were significant for callus induction and proliferation. Two varieties cultured exhibited slight difference regarding their callusing and regeneration capability. Between those varieties cultured, the cultivar VRI-3 responded well than the cultivar CO-2 in terms of multiple shoot induction. Therefore, VRI3 was selected for our experimental studies.

Callus induction

Excised segments of cotyledons started to elongate within a week. The best callus growth was obtained from the explants on the medium supplemented with NAA (2 μM). Rhizogenesis occurred above 2 μM of NAA.

Shoot regeneration

Well developed, green compact calli were transferred to medium fortified with different concentrations of Ads, Ads + NAA and Ads + BAP+ NAA respectively. Bud formation and shoot proliferation occurred on green calli after 2 weeks of culture on media containing combination of Ads, BAP and NAA at different concentrations (Figure 1). Highest frequency of shoot regeneration (47.5) were observed on the media containing Ads (13 μM), BAP (13 μM) and NAA (2 μM) (Figure 2).

Influence of Ads on regeneration potentiality :

When Ads was supplemented to the medium individually, it produced only lesser number of shootlets (Figure 3). Maximum number of shoots (37. 42%) were produced at 13 m Ads, the same when combined with BAP (13 μM) and NAA (2 μM) showed increasing frequency of the shoots (92%) (Figure 4) (Table 1). Elongation of shoots were achieved when the shoots were transferred to medium containing NAA (2 μM) + BAP (13 μM) + GA_3 (1 μM) (Figure 5).

Root induction

The elongated plantlets (5 - 6 cm) were rooted on medium supplemented with IBA 9 um (Figure 6). Rooted plants were transferred to pots containing sand and vermiculite mix (Figure 7).

1

Figure 1. Shoot bud proliferation from cotyledon derived calli (BAP+ NAA).

2

Figure 2. Shoot regeneration on BAP (13 μM) and NAA (2 μM) containing media.

3

Figure 3. Shoot development on supplemented with Ads media.

4

Figure 4. Higher frequency of shoots from BAP (13 μM), NAA (2 μM) and Ads (13 μM) combined media.

5

Figure 5. Elongated plantlets rooted with IBA (9 μM).

6

Figure 6. Rooted plantlets established in plastic cups.

Discussion

Present study shows the role of Ads in combination with BAP and NAA in inducing shoots from cotyledon explants. Banerjee et al. (1988) reported that BAP and NAA combination was very effective in inducing multiple shoots from cotyledonary explants of groundnut. This previously reported result was consistent to our study. About 47.5 shoots developed from cotyledon callus within sixty days on a medium supplemented with Ads (13 μM), BAP (13 μM) and NAA (2 μM). In contrast to this, Atreya et al. (1984) reported that the combination of NAA and BAP helped in development of whole plants but reduced the frequency of shoot formation. Narasimhulu and Reddy (1983) have also reported that cotyledon callus produced 1-3 shoots in the presence of 4.4 μM BAP and 2 μM NAA. In the present investigation, The same calli were obtained on the media containing (8.8 μM) and NAA (2.0 μM). McKently et al. (1990) also reported only a maximum number of 12 shoots per cotyledon explants cultured on 110.98 μM of BAP. Multiple shoot formation by application of cytokinins have been reported in some other legumes such as Vigna radiata (Mathews, 1987), Phaseolus vulgaris (Franklin et al., 1991). While studying the effect of Ads on the regeneration efficiency, individual supplementation to the medium was not effective. Only 7 shoot buds were induced at 13 μM of Ads, at higher concentration the calli turned brown. But the addition of Ads along with BAP (13.0 μM) and NAA (2.0 μM) show a positive influence on shoot production. This is in consistant with the reports of Varisaimohamed et al. (1998) in *Macrotyloma uniflorum* were BAP and Ads promoted maximum shoots per explant. In contrast to this, Gulati and Jaiwal (1990) reported that in Vigna radiata Ads in combination with BAP promoted only root formation. Percentage of shoot production initially increased with 13 μM of Ads and then decreased at higher concentration. After 2-3 leaves stage, further development of shoot was suppressed. Shoots when transferred to medium containing BAP (13 μM), NAA (2 μM) and GA_3 (1 μM) resulted in shoot elongation. Studies on the effect of Ads and BAP combination with respect to regeneration of peanut was limited. The protocol reported here employing Ads with BAP and NAA is a successful repetitive combination for rapid and higher frequency of in vitro regeneration of groundnut.

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