J. Plant Biol. 41(1) : 1~8 (1998)

Influence of Growth Regulators on Plant Regeneration from Epicotyl and Hypocotyl Cultures of Two Groundnut (*Arachis hypogaea* L.) Cultivars

Venkatachalam, Perumal[1]*, Natesan Geetha[1] and Narayanasamypillai Jayabalan

Plant Tissue Culture Unit, Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620 024

[1]Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560 012, India

This study was designed to evaluate the effect of phytohormones on plant regeneration from epicotyl and hypocotyl explants of two groundnut (*Arachis hypogaea*) cultivars. Explants cultured on media with auxins and in combination with cytokinin produced high frequency of callus. After four weeks, callus from these cultures was transferred to medium with cytokinin and reduced auxin, shoot buds regenerated from the cultures. A high rate of shoot bud regeneration was observed on medium supplemented with 2.0 mg/L BAP and 0.5 mg/L NAA. Among the different auxins tested, NAA was found to be most effective, producing the highest frequency of shoot buds per responding cultures. Of the two explants tested, epicotyl was found to be best for high frequency shoot bud regeneration. Multiple shoots arose on MS medium supplemented with BAP or kinetin (1.0-5.0 mg/L) plus IBA (1.0 mg/L), with maximum production occurring at 5.0 mg/L. The elongated shoots developed roots *in vitro* upon transfer to MS medium supplemented with NAA or IBA (0.5-2.0 mg/L) and kinetin (0.5 mg/L) for 15 days. *In vitro* produced plantlets, were transferred to soil and placed in a glasshouse developed successfully, matured, and set seeds.

Keywords: *Arachis hypogaea*, explants, growth regulators, *in vitro*, shoot bud regeneration

Groundnut (*Arachis hypogaea* L.), an important oilseed crop, is susceptible to pests, diseases, salinity and aridity and hence yield levels are low (Vajranabhaiah *et al.*, 1993). The lack of an efficient regeneration system for groundnut has slowed the improvement of this species via tissue culture selection and genetic transformation (Eapen and George, 1994). The cultivated groundnut is known to be relatively recalcitrant in tissue culture (Cheng *et al.*, 1992; Heatly and Smith, 1996). Although some successful plantlet regeneration has been reported either by organogenesis or somatic embryogenesis, it was necessary to standardize this technique for the cultivated groundnuts for experiments on transformation (Eapen and George, 1994). The most widely used explant of cultivated groundnut for shoot bud regeneration is the immature leaflet isolated from very young seedlings (Eapen and George, 1993). Mroginski *et al.* (1981) studied shoot regeneration from primary callus of immature leaves on MS medium supplemented with BAP (1.0 mg/L) and NAA (1.0 mg/L), but no report was given of whole plant re-establishment. Pittman *et al.* (1983), Narasimhulu and Reddy (1983), Seitz *et al.* (1987) and Eapen and George (1993) extended this study to several genotypes of the *Arachis* genus using the same explants. Shoots were recovered from 0% to 20% of the explants. Atreya *et al.* (1984), McKently *et al.* (1990), Dunbar and Pittman (1992), Cheng *et al.* (1992) and Venkatachalam *et al.* (1994), evaluated plant development via organogenesis from different explants of groundnut. Excised segments of the epicotyl, mesocotyl, cotyledon and petiole from *in vitro* grown seedlings have been cultured on different media composition. Buds developed from the original meristem of epicotyl segments. Limited bud development was observed from mesocotyl segments. Only callus formed from excised cotyledons and pe-

*Corresponding author: Fax +91-80-334-1683

tiole segments. Direct shoot bud regeneration also was observed (Daimon and Mii, 1991).

Recently, there has been a great deal of interest in *in vitro* regeneration of peanut plants via somatic embryogenesis (Hazra *et al.*, 1989; Ozias-Akins *et al.*, 1992; Baker and Wetzstein, 1992; Ramdev Reddy and Reddy, 1993; Chengalrayan *et al.*, 1994). These studies provided some information on the regeneration potential of young leaflets and limited information on the regeneration capacity of other seedling explants in commercial genotypes, therefore it was essential to have a system from which plants may be regenerated with high reproducibility. Moreover, the process of regeneration in these studies was slow and produced generally low frequencies of shoot bud regeneration.

There are no reports on efficient plant regeneration from hypocotyl and epicotyl explants of *Arachis hypogaea* (Heatly and Smith, 1996). Commercially available auxins and cytokinins differ in their stability, effectiveness and influence on organogenesis (Eapen and George, 1993). Generally NAA or IAA have been used in the reported regeneration media, whereas 2,4-D has been shown to decrease the shoot formation (Nielsen *et al.*, 1991). Thus the IAA or NAA conjugating system which is induced by the exposure of pea tissues to auxin (Sudi, 1966) will keep the endogenous IAA concentration below a level where shoot induction/ development is not suppressed, provided the added auxin itself is metabolized. We report the influence of auxins (IAA, NAA, IBA and 2,4-D) and cytokinins (BAP and kinetin) on organogenesis from cultured epicotyls and hypocotyls of groundnut.

MATERIALS AND METHODS

Plant Material and Preparation of Explants

Seeds of 2 cultivars (VRI-2 and TMV-7) of *Arachis hypogaea* were obtained from the Tamil Nadu Agriculture University, Coimbatore, India. Seeds were surface sterilized and germinated as previously described by Venkatachalam *et al.* (1994). After 7 days of culture, the seedlings were used as sources of explants. From each seedling one disc of hypocotyl 2. 5-5.0 mm thick was cut from just below the cotyledonary nodes and the hypocotyls were planted on the medium. Epicotyl explant (5.0 mm long) was cut from just above the cotyledonary node of the same explant and cultured on the medium.

Culture Medium and Conditions

All the experiments were conducted using MS medium. The medium containing MS salts (Murashige and Skoog, 1962) B5 vitamins (Gamborg *et al.*, 1968), 3.0% (W/V) sucrose and 0.7% (W/V) agar. Routinely, 15 mL of the molten medium containing all the constitutents was poured into 150 mm × 25 mm rimless Borosil tubes for initial experiments. For shoot bud differentiation and multiple shoot regeneration, 50-75 mL of the medium was poured into 250 mL Erlenmayer conical flasks. The tubes and flasks were plugged with non-absorbant cotton wrapped in one layer of cheese cloth, and sterilized by autoclaving at 1.06 kg cm^2 pressure and 121°C temperature for 15 min. The pH of the medium was adjusted to 5.8 prior to autoclaving. All cultures were maintained at 25±2°C under 16h light of 80 μE $m^{-2}s^{-1}$ irradiance provided by white fluorescent lamps (TL40W/54 cool day light).

Growth Regulators

To assess the influence of various concentrations of IAA, NAA, IBA, 2,4-D, BAP and kinetin on callus initiation, shoot bud differentiation, multiple shoot regeneration and root formation, the hypocotyl and epicotyl explants were excised from 7-day-old seedlings and cultured on MS medium supplmented with IAA, NAA, IBA, 2,4-D, BAP and kinetin (0.5-2.0 mg/L). The shoot multiplication response of BAP (1.0-5.0 mg/L) was also compared with kinetin along with IBA at 1.0 mg/L.

Rooting of Regenerated Plants and Establishment

The larger shoot buds (>3 cm in length) were excised and rooted on MS medium supplemented with NAA or IBA (0.5-2.0 mg/L) in combination with kinetin (0.5 mg/L). Smaller shoot buds were subcultured on medium supplemented with BAP (1.0 mg/L) for further shoot growth and were later transferred to rooting medium. Plantlets with well developed roots were removed from culture tubes, and after washing roots in running tap water, plants were grown in red soil, sand and manure in 1:1:1 ratio in the plastic cups for 15 days and then transferred to the field as previously described by Venkatachalam *et al.* (1994).

Observations of Cultures and Presentation of Results

A minimum of 20-25 cultures was used per treatment and all the experiments were repeated at least thrice. The cultures were examined peridically and the morphological changes were recorded on the basis of visual observations. Wherever possible, the effects of different treatments were quantified on the basis of percent cultures showing the response per culture after 4 weeks. The experimental design was random and factorial with auxin and cytokinin as independent variables. The data pertaining to frequencies of callus induction, shoot bud differentiation, multiple shoot regeneration and rooting were subjected to analysis of variance (ANOVA) test. Mean separation was by using New Duncan's Multiple Range test.

RESULTS

Culture of Epicotyl Explants

Epicotyl explants from both the cultivars were subjected to medium containing different concentrations of IAA, NAA, IBA and 2,4-D (0.5-2.0 mg/L) and in combination with 0.5 mg/L kinetin for callus initiation. The epicotyl explants began to enlarge on medium within a week of culture initiation callus developed from the cut end of the explant and became green in color. Among the different auxins concentrations and combinations tested, NAA (2.0 mg/L) and kinetin (0.5 mg/L) combination was found to be best for highest frequency of callus initiation (90.4%) followed by IAA, IBA and 2,4-D with kinetin combinations. The percentage of callusing was found to be high (90.4%) in VRI-2 cultivar and it was 89.3% in TMV-7 cultivar (Table 1). The callus was non-friable and did not regenerate plants on the same medium. The percentage of explants forming callus var-

Table 1. Comparative effect of various concentrations of NAA, IAA, IBA, 2,4-D and in combination with 0.5 mg/L kinetin on callus induction frequency of two groundnut cultivars

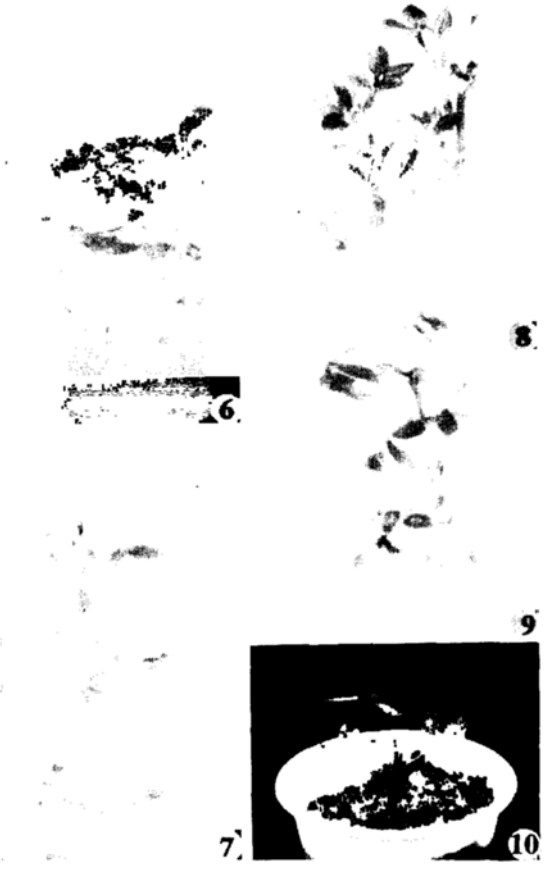
Growth regulators (Conc.mg/L)	Calls initiation (Percentage#mean ± SD)			
	VRI-2		TMV-7	
	Hypocotyl	Epicotyl	Hypocotyl	Epicotyl
IAA				
0.5	61.2±3.4g	58.5±2.9h	57.3±3.1gh	53.8±4.2g
1.0	70.4±5.6e	68.3±4.5f	68.9±4.9f	64.9±5.1d
1.5	77.3±5.3d	74.8±4.6d	75.8±5.1d	70.5±6.0c
2.0	85.5±6.1b	81.3±5.8c	81.5±5.7c	78.2±4.3b
NAA				
0.5	65.9±4.5f	60.5±4.4h	61.4±4.3g	57.4±4.9ef
1.0	79.8±6.1c	73.8±5.7d	74.7±4.9d	69.3±4.5c
1.5	87.7±5.7b	86.3±4.2b	85.3±5.3b	79.9±4.7b
2.0	95.4±7.1a	90.4±6.9a	91.5±5.8a	89.3±5.1a
IBA				
0.5	52.3±3.1j	50.1±3.3j	48.3±2.9k	45.4±3.2h
1.0	59.3±3.9h	57.3±3.1hi	56.6±4.4hi	54.6±3.5g
1.5	65.2±4.1f	64.5±3.7fg	62.2±4.6g	59.3±4.4e
2.0	76.8±4.6d	73.3±5.2de	72.7±6.1de	68.8±4.0c
2,4-D				
0.5	49.5±3.9k	46.3±2.4k	45.3±3.6l	42.3±3.1i
1.0	56.6±3.5i	51.7±4.1j	53.8±4.1j	54.7±3.5g
1.5	62.6±4.9g	58.8±4.2h	59.1±4.7gh	60.2±5.3e
2.0	69.8±5.1e	66.6±4.4f	67.2±4.9f	66.9±4.6cd

Values within column with the same letter are not significantly different at the 1% probability level according to the Duncan's New Multiple Range Test

Figs. 1-5. Plantlet regeneration from epicotyl and hypocotyl explants of groundnut (*Arachis hypogaea* L.). 1. Regeneration of shoot buds from epicotyls cultured on MS+ BAP (2.0 mg/L)+NAA (0.5 mg/L). 2. Shoot buds developing from the epicotyl derived callus. 3. Epicotyl derived callus showing multiple shoot bud regeneration on MS+ BAP (5.0 mg/L)+IBA (1.0 mg/L). 4. A groundnut shoot (4 week old) showing root formation on a medium with 2.0 mg/L IBA+0.5 mg/L kinetin after 2 weeks of culture. 5. A regenerated plantlet acclimatized in plastic cup containing soil.

ied with different auxins concentrations.

After 4 weeks, the calli obtained from 2.0 mg/L NAA and 0.5 mg/L kinetin combination were transferred to shoot bud regeneration medium which was augmented with different concentrations of BAP (0.5-2.0 mg/L) along with 0.5 mg/L of different auxins (IAA, NAA, IBA and 2,4-D). Proliferation of shoot-initials from the callus within 2 weeks of culture initiation, which developed further multiple shoot-initials with little callusing (Fig. 1). Within 4 weeks of culture initiation, shoot buds formed from the epicotyl derived calli (Fig. 2). The best frequency of shoot bud formation was obtained using 2.0 mg/L BAP and 0.5 mg/L NAA combination. The VRI-2 cultivar was found to be best for highest frequency of shooting (96.7%) and the shoot bud differentiation frequency was 89.8% in TMV-7 cultivar (Table 2). The percentage of shooting from epicotyl derived calli varied with different concentrations of BAP along with various auxins combination. After shoot bud differentiation, they were cultured on multiple shoot induction medium which was supplemented with different concentrations of BAP (1.0-5.0 mg/L) and in combination with 1.0 mg/L IBA (Fig. 3). The highest number of multiple shoots (33.2) was observed in VRI-2 cultivar and it was 30.5 in TMV-7 cultivar (Table 3). Multiple shooting was best in the presence of 5.0 mg/L BAP or kinetin along with 1.0 mg/L IBA combination. Elongated shoots (3-5 cm long) were excised and cultured on a medium supplemented with different concentrations of IBA or NAA (0.5-2.0 mg/L) in combination with 0.5 mg/L kinetin for rooting (Fig. 4). Roots emerged from the cut end of the shoots in 80% of the cultures within 15 days of culture. Of the two auxins tested, IBA and kinetin combination was found to be efficient for higher fre-



Figs. 6-10. Plantlet regeneration from epicotyl and hypocotyl explants of groundnut (*Arachis hypogaea* L.). 6. Hypocotyl explant producing calli with shoot bud regeneration on MS+BAP (2.0 mg/L)+NAA (0.5 mg/L). 7. Shoot bud development from hypocotyl derived callus. 8. Multiple shoot bud production from hypocotyl derived callus cultured on MS+BAP (5.0 mg/L)+IBA (1.0 mg/L). 9. Regenerated shoot rooted on MS+IBA (2.0 mg/L)+kinetin (0.5 mg/L). 10. Well developed groundnut plantlet established in plastic cup containing soil.

Table 2. Effect of different concentrations of BAP in combination with IAA, NAA, IBA, and 2,4-D (0.5 mg/L) on shoot bud differentiation in two groundnut cultivars

Growth regulators (Conc.mg/L)	Shoot bud regeneration (Percentage#mean±SD)			
	VRI-2		TMV-7	
	Hypocotyl	Epicotyl	Hypocotyl	Epicotyl
BAP IAA				
0.5 0.5	53.7±3.3ef	56.5±3.6g	51.6±4.1h	53.3±3.3g
1.0 0.5	64.7±4.1d	67.4±4.7e	62.7±4.7f	64.7±5.7e
1.5 0.5	74.7±5.1c	77.9±4.5c	70.5±5.2d	72.5±5.0d
2.0 0.5	80.5±6.5b	85.2±5.6b	77.3±65.b	80.5±5.3b
BAP NAA				
0.5 0.5	55.5±4.4e	60.7±4.3f	52.6±4.2h	56.3±4.3g
1.0 0.5	67.4±56.cd	74.7±5.2cd	66.9±4.7e	71.8±5.7d
1.5 0.5	79.8+5.5b	83.2±5.9b	75.4±6.1c	79.2±5.4b
2.0 0.5	91.3±6.6a	96.7±5.2a	88.4±6.3a	89.8±6.6a
BAP IBA				
0.5 0.5	49.3±2.7f	52.3±3.7h	46.3±3.1i	51.2±3.0gh
1.0 0.5	58.6±4.5e	61.7±5.5f	56.5±4.2g	60.2±4.1f
1.5 0.5	71.3+6.2e	78.3±5.3c	69.3±4.5de	74.7±5.1cd
2.0 0.5	82.7±5.8b	84.4±6.9b	79.4±5.6b	81.5±5.7b
BAP 2,4-D				
0.5 0.5	46.7±3.3fg	50.7±4.2h	45.4±3.1i	49.8±2.5h
1.0 0.5	59.4±4.2e	61.2±4.0f	57.3±3.4g	60.1±4.6f
1.5 0.5	65.3±3.5d	72.3±5.5d	64.8±4.6ef	70.4±5.2d
20 0.5	74.5±6.2c	78.8±6.5c	72.5±4.7d	76.3±5.7bc

Values within column with the same letter are not significantly different at the 1% probability level according to the Duncan's New Multiple Range Test

Table 3. Mean number of shoots recovered per culture after 3-4 weeks on MS medium supplemented with different concentrations of BAP and kinetin in combination with 1.0 mg/L of IBA in two groundnut cultivars

Growth regulators (Conc.mg/L)	Mean number of shoots/culture ± SD			
	VRI-2		TMV-7	
	Hypocotyl	Epicotyl	Hypocotyl	Epicotyl
BAP IAA				
1.0 1.0	7.4±1.1h	9.4±1.3h	6.5±1.5f	7.9±1.2g
2.0 1.0	12.7±1.7f	15.7±1.6f	11.2±1.3e	12.5±1.6e
3.0 1.0	16.7±2.4d	21.2±3.1d	14.5±1.7d	18.5±2.2c
4.0 1.0	24.7±2.9b	29.8±2.7b	22.4±2.4b	24.7±2.8b
5.0 1.0	30.4±3.3a	33.2±3.6a	27.4±3.2a	30.5±3.0a
kinetin IBA				
1.0 1.0	6.2±1.2i	8.2±1.4h	5.7±0.9g	6.9±1.0g
2.0 1.0	9.5±1.4g	13.2±2.0g	8.5±1.1f	9.8±1.2f
3.0 1.0	14.3±2.1e	17.5±2.6e	12.2±1.6e	15.5±1.7d
4.0 1.0	19.2±1.9c	24.7±2.3c	18.4±1.5c	19.4±2.6c
5.0 1.0	25.5±2.5b	28.5±2.7b	21.5±2.0b	23.2±2.2b

Values within column with the same letter are not significantly different at the 1% probability level according to the Duncan's New Multiple Range Test

Table 4. Percentage of root formation on MS medium supplemented with different concentrations of NAA/IBA and in combination with 0.5 mg/L of kinetin for two groundnut cultivars

Growth regulators (Conc.mg/L)	Root induction (Percentage#/mean ± SD			
	VRI-2		TMV-7	
	Hypocotyl	Epicotyl	Hypocotyl	Epicotyl
NAA kinetin				
0.5 0.5	45.4±2.6e	43.5±2.8c	35.4±2.7c	34.9±2.4f
1.0 0.5	56.7±4.4d	55.3±3.4d	42.5±3.6c	41.5±3.6e
1.5 0.5	64.5±4.8c	66.4±4.6b	58.3±3.7ab	55.7±4.8c
2.0 0.5	72.75.3b	70.8±4.9b	69.7±4.5a	66.2±5.6b
IBA kinetin				
0.5 0.5	47.5±3.4e	45.4±3.5e	40.2±3.1c	38.4±2.6e
1.0 0.5	65.4±4.5c	62.7±5.2bc	54.8±2.9b	50.5±3.5d
1.5 0.5	74.3±5.2b	71.3±5.9b	68.5±5.2a	64.5±4.7b
2.0 0.5	81.5±6.0a	79.8±6.3a	76.2±5.6a	73.7±4.9a

Values within column with the same letter are not significantly different at the 1% probability level according to the Duncan's New Multiple Range Test

quency of rooting than NAA and kinetin combination. The percentage of rooting was high (79.8%) in VRI-2 cultivar (Table 4) whereas it was 73.7% in TMV-7 cultivar. Rooted plantlets have been transferred to plastic cups initially (Fig. 5), later established in field and grown to maturity, producing viable seeds under field conditions.

Culture of Hypocotyl Explants

Hypocotyl explants derived from 7-day-old seedlings cultured on MS medium supplemented with different concentrations of IAA, NAA, IBA and 2,4-D (0.5-2.0 mg/L) in combination with kinetin (0.5 mg/L) for callus initiation. The explants enlarged to their original size and also formed greenish callus from the cut end where the shoot bud primordia did not occur. These calluses did not differentiate into shoot bud primordia after one month of culture in the same medium, but 95.4% of the explants induced calli in this medium in the first month of culture. Callus growth was best in the presence of 2.0 mg/L NAA followed by IAA, IBA and 2,4-D. The highest frequency of callus induction (95.4%) was observed on MS medium containing 2.0 mg/L NAA and 0.5 mg/L kinetin combination (Table 1). Callus initiation from hypocotyl explant varied from 49.5 to 95.4% in VRI-2 cultivar whereas it was 45.3 to 91.5% in TMV-7 cultivar (Table 1). The callus was non-friable, greenish and did not regenerate plants on subculture despite several attempts. In both cultivars callus was formed at high frequency.

The calli obtained from 2.0 mg/L NAA and 0.5 mg/L kinetin combination were transferred to shoot bud regeneration medium containing different concentrations of BAP (0.5-2.0 mg/L) and in combination with various auxins individually (0.5 mg/L) for shoot bud differentiation (Fig. 6). Addition of BAP suppressed callus formation and induced shoot bud differentiation (Fig. 7). In both cultivars, shoots were formed at high frequency from the hypocotyl derived callus. Ninety percent of the calli produced shoot bud primordia and these shoot buds developed into normal shoots after 4 weeks of culture. The highest frequency of shoot bud regeneration (91.3%) was observed on MS medium containing BAP (2.0 mg/L) and NAA (0.5 mg/L) combination in VRI-2 cultivar while it was 88.4% in TMV-7 cultivar (Table 2). These shoot buds were cultured on shoot bud multiplication medium containing different concentrations of BAP or kinetin (1.0-5.0 mg/L) and in combination with IBA (1.0 mg/L) (Fig. 8). The highest mean number of shoots (30.4) observed in VRI-2 cultivar and it was 27.4 in TMV-7 cultivar (Table 3). Well-developed shoots (3-5 cm long) were isolated from the culture tubes and subcultured on rooting medium supplemented with different concentrations of NAA or IBA (0.5-2.0 mg/L) in combination with kinetin (0.5 mg/L) for root formation (Fig. 9). The highest frequency of rooting (81.5%) was observed on medium containing 2.0 mg/L IBA and 0.5 mg/L kinetin combination in VRI-2 cultivar whereas it was 76.2%

in TMV-7 cultivar (Table 4). The regenerated plantlets were transferred to plastic cups (Fig. 10) and subsequently established in the field.

DISCUSSION

The classical findings of Skoog and Miller (1957) that organogenesis in tissue cultures is governed by the balance of auxin and cytokinin in the medium can not be demonstrated universally due to the explant sensitivity or the original content of endogenous growth regulators (Sharma *et al.*, 1990). Methods have been developed for reproducible plant regeneration from cultured hypocotyl and epicotyls of groundnut. For callus induction it was essential to have both auxins and cytokinins. NAA in combination with kinetin produced the best results in both the cultivars. The callus induction response of the explants was dependent on the type of auxins used in combination with kinetin. NAA and kinetin combination induced profuse callus growth followed by IAA, IBA and 2, 4-D with kinetin combinations. Hossain *et al.* (1994) reported that the MS medium was supplemented with different concentrations of IAA and NAA, the explants produced callus but the growth of callus was very poor and the frequency of callusing was low. Therefore, a low concentration of kinetin was added to increase the frequency of callusing. NAA was found to be more effecient than other auxins for callus initiation. Similar results were also observed and reported by Mroginski *et al.* (1981), Pittman *et al.* (1983), Narasimhulu and Reddy (1983), McKently *et al.* (1990, 1991), Daimon and Mii (1991), Cheng *et al.* (1992), Dunbar and Pittman (1992), and Eapen and George (1993) in groundnut, Hossain *et al.* (1994) in *Aegle marmelos*, Mendoza and Futsuhara (1990) in mungbean, Pandey and Bansal (1992) in soybean and Barna and Wakhlu (1994) in chickpea. Addition of cytokinins (either kinetin or BAP) was observed to significantly improve the response of auxins for efficient callus induction (Barna and Wakhlu, 1994). The observation confirms the persistance of NAA and apparently supports the assumption that added auxin has to be metabolizable to allow callus formation. In the present study, a large number of shoot buds could be regenerated from both hypocotyl as well as epicotyl derived calli by using BAP and NAA combination. The cytokinins (BAP and kinetin) enhanced shoot bud formation in cultured callus of *Arachis hypogaea* is in accordance with previous reports on groundnut (Mrogniski *et al.*, 1981; Banerjee *et al.*, 1988; McKently *et al.*, 1990; Cheng *et al.*, 1992; Eapen and George, 1993). The mean number of shoots was increased with increase in the concentration of cytokinins (Venkatachalam, 1996).

One of the strategies for obtaining regeneration from undifferentiated callus was to maintain the callus on basal medium with a low concentration of auxin together with various concentrations of BAP. Thus, the concentration of auxin was fixed at 0.5 mg/L which by itself was capable of sustaining a good rate of growth of callus. Under appropriate conditions of auxin and cytokinin, calli showed the differentiation of shoot buds in compact masses. The combinations of auxins and cytokinins at definite proportions are very critical and found to be essential for the induction of shoot bud and root in groundnut (Mroginski *et al.*, 1981; Narasimhulu and Reddy, 1983; Banerjee *et al.*, 1988; McKently *et al.*, 1990; Cheng *et al.*, 1992; Eapen and George, 1993; Venkatachalam *et al.*, 1994). The superior effect of BAP over other cytokinins for shoot bud induction has been attributed to the abilities of plant tissues to metabolize the natural hormone more readily than other synthetic growth regulators or to the ability of BAP to induce endogenous production of zeatin (Barna and Wakhlu, 1994).

Both explant types produced multiple shoots at five levels of BAP tested. The epicotyl produced the highest number of shoot bud primordia (at 5.0 mg/L). In earlier studies, McKently *et al.* (1990) recovered a maximum of 12 shoots per explant using cv. Florigiant cultured on 25 mg/L BAP, while Cheng *et al.* (1992) obtained an average of 1.6 shoots per explant on a medium containing 25 mg/L BAP. Among the several hormonal concentrations and combinations tested for culturing the explants, of groundnut IBA in combination with BAP was most effective where multiple shoot formation was spectacular. So, the auxin:cytokinin ratio was critical for multiple shoot regenereation in groundnut (Banerjee *et al.*, 1988). In the present study, though shoot bud differentiation from hypocotyl and epoicotyl explants was observed on media with BAP, the response was more pronounced on media which contained both auxins (IAA, NAA, IBA and 2,4-D) and cytokinins (BAP and kinetin). Mathews (1987) reported maximum response (80%) of explants for shoot bud differentiation in MS+BAP and IBA, but the worker has not reported the number of shoots regenerated per explant. Shoot multiplication was enhanced by the additional application of an auxin (Gulati and Jaiwal, 1990; Venkatachalam, 1996).

On transfer to media supplemented with either IBA or NAA in combination with kinetin, the growth of

each isolated plantlets was enhanced with simultaneous rooting from the basal part. The percentage of rooting was increased with increase the concentration of auxins. The highest root production was recorded on medium containing IBA and kinetin combination, where 80% of shoots formed roots within 2 weeks. NAA and kinetin combination was also effective for root formation, the root system, however, was qualitatively poorer than that grown in IBA medium. This agrees with a previous report by Ono *et al.* (1994). The differentiated shoot buds required NAA or IBA for rooting (Ganapathi and Nataraja, 1993; Banerjee *et al.*, 1988; Eapen and George, 1993; Barna and Wakhlu, 1994; Venkatachalam, 1996). However, we have used lower concentration of kinetin for enhance the frequency of rooting and profuse growth of shoots. The plants, thus raised, could be successfully transferred to field and produced viable seeds.

CONCLUSION

In conclusion, we describe a method for rapid and high frequency of *in vitro* regeneration of groundnut plants that results in rapid multiple shoot regeneration on a simple culture medium. This regeneration system may be useful in gene transfer research to develop transgenic peanut plants. In a previous report on transformation of groundnut using *Agrobacterium tumefaciens* (Lacorte *et al.*, 1991), only tumors were obtained. Transgenic plants were not produced from callus cultures initiated from such tumors. In the present investigation, a successful attempt has been made to regenerate complete plantlets from both the explants of groundnut via organogenesis. This protocol could be useful for production of high frequency of transformants in groundnut using recombinant DNA technology in the near future.

ACKNOWLEDGEMENTS

Perumal Venkatachalam acknowledges Council of Scientific and Industraial Research (CSIR), New Delhi-12 (Govt. of India), for financial assistance in the form of Senior Research Fellowship.

LITERATURE CITED

Atreya, C.P., J. Papa Rao and N.C. Subramanyam. 1984. *In vitro* plant regeneration of peanut (*Arachis hypogaea* L.) plantlets from embryo axes and cotyledon segments. *Plant Sci. Lett.* **34**: 379-383.

Baker, C.M. and H.Y. Wetzstein. 1992. Somatic embryogensis and plant regeneration from leaflets of peanut, *Arachis hypogaea*. *Plant Cell Rep.* **11**: 71-75.

Banerjee, S., S. Bandyopadhyay and P.D. Ghosh 1988. Cotyledonary node culture and multiple shoot formation in peanut: Evidences for somatic embryogenesis. *Curr. Sci.* **57**: 252-255.

Barna, K.S. and A.K. Wakhlu 1994. Whole plant regeneration of *Cicer arietinum* from callus cultures via organogenesis. *Plant Cell Rep.* **13**: 510-513.

Cheng, M., D.C.H. Hsi and G.C. Phillips. 1992. *In vitro* regeneration of valencia-type peanut (*Arachis hypogaea* L.) from cultured petioles, epicotyl sections and other seedling explants. *Peanut Sci.* **19**: 82-87.

Chengalrayan, K., S.S. Sathaye and S. Hazra. 1994. Somatic embryogensis from mature embryo-derived leaflets of peanut (*Arachis hypogaea* L.). *Plant Cell Rep.* **13**: 578-581.

Daimon, H. and M. Mii. 1991. Multiple shoot formation and plant regeneration from cotyledonary node in peanut (*Arachis hypogaea* L.). *Japan J. Breed.* **41**: 461-466.

Dunbar, K.B. and R.N. Pittman. 1992. Adventitious shoot formation from mature leaf explants of *Arachis species*. *Crop Sci.* **32**: 1353-1356.

Eapen, S. and L. George. 1993. Plant regeneration from leaf discs of peanut and pigeonpea: influence of benzyl adenine, indole acetic acid and indole acetic acid-amino acid conjugates. *Plant Cell Tiss. Org. Cul.* **35**: 223-227.

Eapen, S. and L. George. 1994. *Agrobacterium tumefaciens* mediated gene transfer in peanut (*Arachis hypogaea* L.). *Plant Cell Rep.* **13**: 582-586.

Ganapathi, T.R. and K. Nataraja. 1993. Effect of auxins and cytokinins on plant regeneration from hypocotyls and cotyledons in niger (*Guizotia abyssinica* Cass.). *Biol. Plant.* **35**: 209-215.

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements for suspension culture of soybean root cells. *Exp. Cell Res.* **50**: 151-158.

Gulati, A. and P.K. Jaiwal. 1990. Culture conditions effecting plant regeneration from cotyledons of *Vigna radiata* (L.) Wilczek. *Plant Cell Tiss. Org. Cul.* **23**: 1-7.

Hazra, R.B., S.S. Sathaye and A.F. Mascarenhas. 1989. Direct somatic embryogenesis in peanut (*Arachis hypogaea* L.). *Biotechnology.* **7**: 949-951.

Heatly, M.E. and R.H. Smith. 1996. Whole plant regeneration from the shoot apex of *Arachis hypogaea* L. *In Vitro Cell. Dev. Biol.* **32**: 115-118.

Hossain, M., R. Islam, M.R. Karim, S.M. Rahman and O.I. Joarder. 1994. Production of plantlets from *Aegle marmelos* nucellar callus. *Plant Cell Rep.* **14**: 570-573.

Lacorte, C., E. Mansur, B. Timmerman and A.R. Codeiro. 1991. Gene transfer into peanut (*Arachis hypogaea* L.) by *Agrobacterium tumefaciens*. *Plant Cell Rep.* **10**: 345-357.

Mathews, H. 1987. Morphogenetic responses from *in vitro* cultured seedling explants in mungbean [*Vigna*

radiata (L.) Wilczek]. *Plant Cell Tiss. Org. Cul.* **11**: 233-240.

Mc Kently, A.H., G.A. Moore and F.P. Gardner. 1990. *In vitro* plant regeneration of peanut from seed explants. *Crop Sci.* **30**: 192-196.

Mc Kently, A.H., G.A. Moore and F.P. Gardner. 1991. Regeneration of peanut and perennial peanut from cultured leaf tissue. *Crop Sci.* **31**: 833-837.

Mendoza, A.B. and Y. Futsuhara. 1990. Varietal differences on plant regeneration by tissue culture in mungbean *Vigna radiata* (L.) Wilczek. *Japan J. Breed.* **40**: 457-467.

Mroginski, L.A., K.K. Kartha and J.P. Shyluk. 1981. Regeneration of peanut plantlets by *in vitro* culture of immature leaves. *Can. J. Bot.* **59**: 826-830.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-497.

Narasimhulu, S.B. and G.M. Reddy. 1983. Plantlet regeneration from different callus cultures of *Arachis hypogaea* L. *Plant Sci. Lett.* **31**: 147-153.

Nielsen, S.V.S., G.B. Poulsen and M.E. Larsen. 1991. Regeneration of shoots from pea (*Pisum sativum*) hypocotyl explants. *Physiol. Plant.* **82**: 99-102.

Ono, Y., Y. Takahata and N. Kaizuma. 1994. Effect of genotype on shoot regeneration from cotyledonary explants of rapeseed (*Brassica napus* L.). *Plant Cell Rep.* **14**: 13-17.

Ozias-Akins, P., W.F. Anderson and C.C. Holbrook. 1992. Somatic embryogensis in *Arachis hypogaea* L.: genotype comparison. *Plant Sci.* **83**: 103-111.

Pandey, P. and Y.K. Bansal. 1992. Plant regeneration from leaf and hypocotyl explants of *Glycine wightii* (W.and A.) Verdc. var longicauda. *Japan J. Breed.* **42**: 1-5.

Pittman, R.N., D.J. Banks, J.S. Kirby, E.D. Mitchell and P.E. Richardon. 1983. *In vitro* culture of immature peanut (*Arachis* spp) leaves: Morphogenesis and plantlet regeneration. *Peanut Sci.* **10**: 21-25.

Ramdev Reddy, I. and G.M. Reddy. 1993. Factors affecting direct somatic embryogenesis and plant regeneration in groundnut (*Arachis hypogaea* L.). *Indian J. Exp. Biol.* **31**: 57-60.

Seitz, M.H., H.T. Stalker and C.C. Green. 1987. Genetic variation for regenerative response in immature leaflet cultures of the cultivated peanut *Arachis hypogaea. Plant Breed.* **98**: 104-110.

Sharma, K.K., S.S. Bhojwani and T.A. Thorpe. 1990. Factors affecting high frequency differentiation of shoots and roots from cotyledon explants of *Brassica juncea* (L.) Czern. *Plant Sci.* **66**: 247-253.

Skoog, F. and C.O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissue culture *in vitro. Symp. Soc. Exp. Biol.* **11**: 118-131.

Sudi, J. 1966. Increase in the capacity of pea tissue to form acyl-aspartic acids specifically induced by auxins. *New Phytol.* **65**: 9-21.

Vajranabhaiah, S.N., M.G. Purushotham, P.C. Reddy and A.H. Prakash. 1993. Regeneration potential of hypocotyl-derived long term callus cultures in groundnut (*Arachis hypogaea* L.) cv.TMV- 2. *Curr. Sci.* **65**: 806-807.

Venkatachalam, P., A.S. Pillai and N. Jayabalan. 1994. Plant regeneration from cultured apical meristems of groundnut (*Arachis hypogaea* L.). *Proc. Nat. Acad. Sci.* **64**: 99-103.

Venkatachalam, P. 1996. Tissue culture, mutational and genetic studies in groundnut (*Arachis hypogaea* L.) to develop cultivars for high yield, oil content and disease resistance. Ph.D Thesis, Bharathidasan University, Tiruchirappalli, India.

Received July 18, 1997
Accepted December 15, 1997