Pak.J.Bot., 25(2): 183-188, 1993.

IMPROVEMENT OF REGENERATION CAPABILITIES OF CALLUS DERIVED FROM SHOOT APICES OF PEANUT (*ARACHIS HYPOGAEA* L.) SEEDLINGS

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Abstract

Shoot apices of peanut seedlings inoculated on MS medium containing 1 mg/l BA and 0.01 mg/l NAA showed a prolific development of callus without differentiation after about 5 months of culture period with regular subcultures of one month. The calli regenerated plants when transferred to MS medium supplemented with 0.5 mg/l each of BA, 2 ip, Z and IAA (0.1 mg/l) after one month of culture. At least 10 plantlets/callus were obtained and the plantlets could be grown to adult stage under field conditions.

Introduction

Legumes which are rich in fat, protein, vitamin form important dietry constituents both for human and animal. Considering the shortage of rich diet food researchers are therefore, endeavouring to improve the production and quality of legumes especially the grain seed and fodder species which may be resistant to pests and diseases and also cope with environmental stresses.

Recent advances in cell culture techniques have opened new perspectives. *In vitro* regeneration of different species has been obtained utilizing different tissues as the initial explant (Vasil & Vasil, 1980) for crop improvement. However, tissue and cell culture of the cereals and legumes have often proved to be not satisfactory for plant regeneration (Thomas & Warnicke, 1978; Mroginski *et al.*, 1981) since grain legumes when grown as callus *in vitro* exhibited a low or no competence for plant regeneration. Plant regeneration has been obtained in different cultivars of peanut (*Arachis hypogaea* L.), the number of regenerants produced per callus mass or flask was less and cannot be satisfactorily utilized for breeding and stress tolerance studies (Mroginski *et al.*, 1981; Kartha *et al.*, 1981; Bhattia *et al.*, 1985; Narasimhulu & Reddy, 1983). The present studies were therefore initiated to devise an efficient and quick method of *in vitro* peanut regeneration and to increase the number of regenerants per callus mass.

Materials and Methods

Seeds of *Arachis hypogaea* L. cv NC7 obtained from PARC, Islamabad were surface sterilized with 75% ethyl alcohol for 10 minutes then treated with chlorox containing 2% Tween 20 for about 5 minutes. The seeds were then washed three times in sterilized distilled water. The seeds were placed on 3% sucrose agar medium in plastic boxes (10x6x6 cm). Five seeds per box containing 25 ml of the medium were placed under diffused fluorescent light of about 300 lux. After 10 days of seed germination the shoot apices were excised from the seedlings and placed on Murashige & Skoog (1962) medium containing essential vitamins of modified B5 medium (myo-inositol & pyridoxine-HCl, 100 mg/l each; thiamine-HCl, 10.0 mg/l and nicotinic acid, 1.0 mg/l) with

1.0 mg/l of BA & 0.01 mg/l of NAA solidified with 0.08% Difco Bacto agar. One month old callus was inoculated onto fresh MS medium supplemented with different phytoohormones at various concentrations in order to see their effect on callus induction and organ formation. The medium adjusted at pH 5.8 was sterilized at 15 p.s.i for 15 minutes. Two hundred ml capacity jars each containing 25 ml of the medium was used. Growth hormones were added before sterilization.

The cultures were grown in growth chambers at 251°C and RH around 75% supplied with 4000 lux light from cool fluorescent tubes. In addition to this the growth chamber had two incadescent light tubes of 40 w each sixteen hours of light was supplied to the cultures in each 24 hrs cycle.

Results

A prolific callus formed after one month of culture (Fig.1) on medium supplemented with 1.0 mg/l BA and 0.01 mg/l NAA. The calli were then inoculated onto fresh BM containing the same phytohormones at the same concentrations. Growth of the callus was observed with no differentiation even after 6 months of regular transfer at one month interval. A retardation in growth rate was noticed in these calli after about 4 months.

In another set, the callus induced on MS supplemented with 1 mg/l of BA and 0.01 of NAA for two passages, was inoculated on fresh MS medium supplemented with 0.5

Table 1. Effect of different growth hormones in MS medium on callus induction and proliferation of peanut stem apices. The cultures were grown on 16/8 light and dark cycles respecitvely in growth chambers at 25±1°C.

		NAA			
	mg/l	0	0.01	0.05	0.1
2 ip	0.	-	-	-	-
	0.5	-	-	-	-
	1.0	+	+	+ +	+ +
	2.0	+	+ +	+ +	+
BA	0.1	-	-	-	-
	0.5	-	+	+ +	+ +
	1.0	+	+ +	+ + +	+ +
	2.0	+	+ +	+ + +	+ + +
Z	0.1	-	+	-	-
	0.5	+	+	+	+
	1.0	+	+ + +	+ + +	+ + +
	2.0	+ +	+ +	+ + + +	+ + +
K	0.1	-	-	-	-
	0.5	+	+	+	+
	1.0				

Legends:- (no callus formation), + (slight callus formation), + + (good callus formation), + + + (adequate callus) and + + + + (excellent callus).

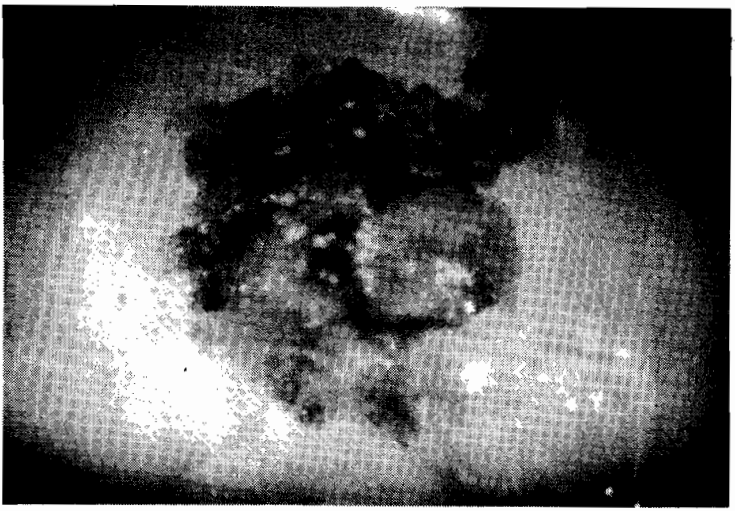

Fig.1. Callus formation on peanut (*Arachis hypogaea* L.) shoot apices when cultured on MS medium supplemented with BA (1.0 mg/l) and NAA 0.01 mg/l). The cultures were grown under 4000 lux fluorescent light of 16 hrs duration in each 24 hrs cycle. The temperature of growth chambers was regulated at 25±1°C.

mg/l each of BA and 2ip. Further increase in callus was noticed without regeneration even upto 5 months interval. The callus was then inoculated on MS medium supplemented with 0.5 mg/l each of BA, 2 ip and Z. Further increase in callus mass was noticed without any differentiation. When the concentrations of the cytokinins were altered as detailed in Table 1, mainly callogenesis was observed. No differentiation could be observed when the callus was cultured for two passages on MS medium supplemented with BA and NAA and for one passage on MS supplemented with 0.5 mg/l each of BA, 2ip and Z, again transferred to the MS containing the same concentrations of cytokinins and different concentrations of IAA.

In further experiments the apices were cultured on MS supplemtned with 1.0 mg/l of BA and 0.01 mg/l NAA for one month only. Then the calli were transferred to MS medium containing different concentrations of various growth hormones (Table 2). Efficient plantlet regeneration alongwith good callus growth was noticed on MS medium supplemented with 0.5 mg/l each of BA, 2 ip and Z but containing IAA (0.1 mg/l) as well (Fig.2). This regeneration occurred only when the MS medium containing BA (1.0 mg/l) and NAA (0.01 mg/l) induced calli were subcultured on fresh MS medium containing the above concentrations of phytohormones. It is interesting to note that only callus proliferation occurred after one passage of culture on MS supplemented with BA (1.0 mg/l) and NAA (0.01 mg/l). The plantlet, formation could be further increased by inoculating the regenerating callus on MS supplemented with 0.5 mg/l each of BA, 2 ip and Z alongwith 0.1 mg/l of IAA. The number of plantlets was about 10 per callus mass of about 1 cm diameter which yielded sufficient number of plantlets after 3 months. The plantlets were excised from the calli and inoculated on the rooting medium. Rootlets were induced after about 3 weeks. These plantlets were then transferred to MS medium containing 3% sugar only. The roots developed further and after they attainted a length of about 3-5 cm, the plantlets were transferred to pots containing perlite. The plants

Table 2. The effect of indoleacetic acid on *callo-* and *organo-genesis* when supplemented to MS medium alongwith 0.5 mg/l each of BA, 2 ip and Z. The other growth conditions were the same as in Table 1.

		IAA			
	mg/l	0	0.01	0.05	0.1
2 ip	0.1	-	-	-	-
	0.5	-	-	+	+
	1.0	+	-	+	-
	2.0	+	-	+	+ +
BA	0.1	-	-	-	-
	0.5	-	-	+	+
	1.0	+	-	+	+ +
	2.0	+	+	+ +	+ + +
Z	0.1	-	-	-	-
	0.5	+	-	+	+
	1.0	+	+	+	+ +
	2.0	+	+ +	+ +	+ + +
K	0.1	-	-	-	-
	0.5	+	+ +	+ +	+ +
	1.0	+	+	+	+ +
	2.0	+	+ +	+ +	+ + +
AS	0.1	-	-	-	-
	0.5	-	-	-	-
	1.0	-	-	+	+
	2.0	-	-	+ +	+ +

Legends: (no regeneration), + (2 plantlets per flask), + + (6 plantlets per flask) and + + + (10 plantlets per flask).

established themselves and exhibited a prolific growth after one month. The plants were ultimately transferred to the field where they exhibited vigorous growth and produced flowers. These flowers then formed pods containing normal seeds.

Discussion

Plantlet regeneration in legumes in general and peanuts in particular has been difficult. Although plantlet regeneration has been reported in peanuts, the number of plantlets formed per callus mass is not so prolific as with other species (Atreya *et al.*, 1984; Narasimhulu & Reddy, 1983; Pitman *et al.*, 1984). Similarly in our laboratory we have been facing the same problem with various cultivars of peanuts (Pervez, 1980; Akhtar, 1984) having a very limited number of plantlet regeneration. Paradoxically an excellent callus formation and proliferation took place on the various media tested with varying degrees of greening. However, the calli could not become autotrophic and died if the sugar was omitted from the culture medium. Moreover, no greening was noticed

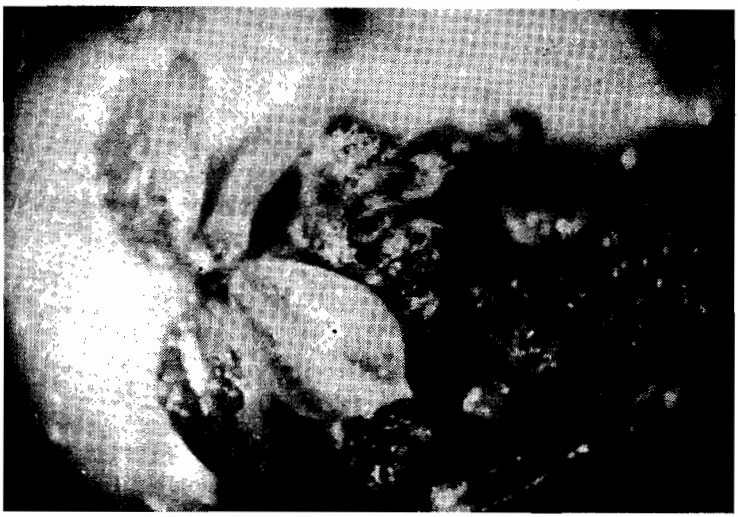

Fig.2. Plantlet formation from shoot apex derived callus induced on MS supplemented with BA and NAA. This time MS was supplemented with 0.5 mg/l each of BA, 2 ip, Z and IAA (0.1 mg/l). The callus was cultured under the same conditions as of Fig.1.

as reported for other species. Nonetheless, these findings are in contrast to those reported for *A. villosulicarpa,* a wild relative of peanuts because meristematic shoot primordia developed in calli when grown on BM supplemented with or without a carbon source (Pittman *et al.*, 1984). Although, similar meristematic shoot primordia were noticed in our cultures as well, these then produced callus when subcultured on the same BM with similar phytohormones or with variations in hormone concentration.

MS supplemented with 1.0 mg/l BA and 0.01 mg/l of NAA induced callus in peanut species. This callus exhibited a prolific growth when subcultured on the same combination of growth hormones. However, with change both in the concentration and kind of hormone, a reasonable differentiation was obtained. When calli induced on MS supplemented with BA (1.0 mg/l) and NAA (0.01 mg/l) were transferred to the MS containing 0.5 mg/l each of BA, 2 ip and Z alongwith 0.1 mg/l of IAA, differentiation could be achieved after the second passage each of one month duration. No regeneration occurred if the mother calli were not inoculated on MS containing BA and NAA. In our experiments with apices being cultured only on MS containing BA, 2 ip and Z (0.5 mg/l each) and IAA (0.1), right from callus induction, were without any success in inducing differentiation even after a prolonged culture period. These findings are not in agreement with others who reported plantlet formation on BM supplmented with the identical phytohormones (Atreya *et al.*, 1984; Kartha *et al.*, 1981; Mroginski *et al.*, 1981; Narasimhulu & Reddy, 1983) for one passage only. An alteration in concentration and nature of the phytohormones permitted a continuous and prolific regeneration not reported earlier. Moreover, this treatment was also able to check browning reported by various workers (Pervez, 1983; Oelck & Schieder, 1983; Hameed *et al.*, 1993).

In the present studies usually plantlet formation occurred on yellow callus rather than on lush green. Nonetheless, the regenerated shoots turned green and became

autotrophic to a large extent because plantlets have been excised, induced to root and then transferred to soil. Probably this protocol will enable us to obtain a large number of regenerants on calli stressed with salt for further studies.

Acknowledgements

This research was supported by the National Scientific Research Development Board of the University Grants Commission, Islamabad.

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(Received for Publication 6 March 1993)