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PLANTLET REGENERATION FROM MATURE EMBRYOS OF PEANUT (*ARACHIS HYPOGAEA* L.)

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Abstract

Mature embryos, excised from the soaked peanut seeds, when cultured on MS medium supplemented with BA and 2,4-D each @ 0.5 mg/l induced, callus. Regeneration occurred when this callus was subcultured on to MS containing BA and kin each @ 1.0 mg/l. Similarly, development of callus followed by regeneration was noticed in mature embryos in MS medium containing 0.5 mg/l kin and 1.5 mg/l NAA. Direct regeneration was also observed on MS containing 1.0 mg/l each of BA and kin. All the regenerated shoots when transferred to the rooting medium (1/2 MS) supplemented with 0.1 mg/l NAA showed development of root.

Introduction

Of the leguminous plants, peanuts contain 20-40% oil 25-30% protein besides a rich source of vitamins like thiamine, riboflavin and niacin. During the last decade there has been an enormous loss in its yield caused by various pathogens especially viruses. Of the various methods of plant improvement, the cell and tissue cultures are now established for *in vitro* regeneration of a large number of plant species. Studies were therefore, carried out to examine some of the factors affecting the establishment of callus cultures in peanut and its regenerating potentials. Information on callus initiation and plant regeneration in *A. hypogaea* is limited (Bajaj *et al.*, 1981; Mroginski *et al.*, 1981). Whereas plant regeneration has been obtained in different cultivars of peanut, however, the number of regenerants produced per callus mass or flask was less and can not be satisfactorily utilized for breeding and stress tolerance studies (Mroginski *et al.*, 1981; Kartha *et al.*, 1981; Bhattia *et al.*, 1985; Narasimhulu & Reddy, 1983). An efficient and quick method of *in vitro* peanut regeneration and to increase the number of regenerants per callus mass has also been studied (Ilahi, 1993). The present investigation describes the procedure employed to induce peanut plant regeneration either directly from the excised segments of mature embryos or plant regeneration from callus of mature embryos cultured under defined conditions.

Materials and Methods

Seeds of *Arachis hypogaea* L. cv. BARD (699) were obtained from the National Oil Seed Development Project of NARC, Islamabad. The seeds were washed in tap water and soaked for half an hour so that the testa could be easily removed. The seeds were surface sterilized with 1% mercuric chloride for 2 minutes followed by several washing with sterilized distilled water. The testa was then removed from the seeds with

Table 1. Callagenesis, organogenesis and regeneration on embryo explants of *Arachis hypogaea*.

Growth Hormones (mg/l) BA	Kin	2,4-D	NAA	MS Strength	Period in weeks	Callogenesis	Remarks
0.5	--	0.5	--	Full	4-6	+ + +	Few embryos developed single leafy shoots, while others formed withish-green callus.
1.0	1.0	--	--	Full	4-6	--	Regeneration of callus into multiple shoots
--	0.5	--	1.5	Full	4	+ + +	Most embryos formed callus while a few developed single leafy shoots which later devel-opped into multiple shoots.
1.0	1.0	--	--	Full	4	--	No callus formation occurred. All embryos pro-duced single leafy shoots.
1.0	1.0	--	--	Full	4	--	Multiple shoot formation in above step.
--	--	--	0.1	1/2	1-2	--	Rooting of shoots.

the help of scalpel. Mature embryos were excised from the seeds and cultured on Murashige & Skoog's (1962) basal medium, supplemented with 3% sucrose, 1% Difco-bacto agar containing growth hormones viz, 2,4-dichlorophenoxy acetic acid (2,4-D), 6-furfuryl amino purine (kin), Benzyladenine (BA) and Naphthalene acetic acid (NAA). The MS solution was used at full and half strength throughout this experimental work.

The pH of the medium was adjusted to 5.6 with 1.0 N NaOH or 1.0 N HCl prior to the addition of agar. The medium was then autoclaved at 15, psi for 15 minutes. Two hundred ml capacity flasks each containing 25ml of the medium were used. Growth hormones were added before sterilization. The cultures were kept in biotrons with 16/8 hours light/dark cycle and the temperature was regulated at 25 ± 1°C.

Results and Discussion

Full strength of MS medium is generally used for tree cultures and because of its toxicity to herbaceous plants some regeneration has been reported by Atreya *et al.*, (1984), Narasimhulu & Reddy (1983) and Pitman *et al.*, (1984). In the present study excised mature embryos of *Arachis hypogaea* L., were cultured on to the Murashige & Skoog's (1962) basal medium, supplemented with different concentrations of phytohormones.

Full MS was found conducive for the regeneration of peanut shoots, while 1/2 MS was effective for rooting of these shoots. When embryos were inoculated on to the BM (full MS) with 0.5 mg/l each of BA and 2,4-D, a whitish-green and compact callus with single-leafy shoots was produced after 4-6 weeks of culture (Table 1), indicating 2,4-D and BA as the best set of combinations used for callus induction as also reported by Ilahi & Jabeen (1987). When this callus was transferred to BM containing 1.0 mg/l each of BA and Kin, regeneration occurred and multiple buds were induced which after prolonged period of culture on the same medium, developed into multiple shoots (Table 1 and Fig.1). Similar effects of BA and Kin on callus regeneration has been reported by Ullah (1986). The calli of *A. hypogaea* were subcultured two times on 1.0 mg/l kin in combination with 1.0 mg/l of BA, and the resultant callus regenerated into multiple shoots after 8 weeks of subculture where rooting was observed in NAA @ 0.1 mg/l (Table 1).

Fig.1. Efficient plantlet formation on regenerating callus of mature embryo explants when cultured on Full MS medium supplemented with 1.0 mg/l each of BA and Kn after 4 to 6 weeks.

Fig.2. Rooting of the shoots on (1/2) MS medium containing 0.1 mg/l of NAA within a culture period of 1 to 2 weeks.

Most of the embryos formed callus with few single leafy shoots on BM, supplemented with 0.5 mg/l of Kin in combination with 1.5 mg/l of NAA. Regeneration of shoots occurred which, after 8-10 weeks, developed into multiple-shoots (Table 1). Kartha *et al.*, (1981) and Mroginski *et al.*, (1981) and Akram (1983) also found shoot regeneration in the callus cultures of *Rauwolfia serpentina* on a medium containing NAA in combination with a BA, without concomitant root differentiation. It is interesting to note that embryos cultured on BM supplemented with 1.0 mg/l each of BA and Kin gave direct regeneration of shoots without callus formation (Table 1), which later on developed into multiple shoots. These shoots when transferred to a rooting medium showed development (Table 1) of roots within a culture period of 1-2 weeks attaining a length of 3-5 cm (Fig.2). These *in vitro* raised rooted plantlets were transferred to pots to monitor their growth and development. Use of more than one cytokinin for organogenesis has been found beneficial in certain cases, especially, legumes (Akhtar, 1984). The results are similar to those obtained by Hanes *et al.*, (1985), who were able to induce new corms on slices of mother corms of saffron without formation of callus.

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