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STUDIES ON *IN VITRO* CULTURE OF *ARACHIS HYPOGAEA* HYPOCOTYL EXPLANTS

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Arachis hypogaea hypocotyl explants exhibited callus formation when inoculated on Murashige & Skoog (MS) medium supplemented with both an auxin and cytokinin. Although best callus resulted in 0.5 mg/l of 2, 4-D and 1.0 mg/l of K but it did not support further callus proliferation. 2, 4-D @ 1.0 mg/l and K @ 4.0 mg/l supported callus growth. Browning of callus tissues in subcultures for long duration was overcome by the addition of coconut milk (CM) and sodium diethyl dithio carbonate (SDDC).

Introduction

Plant tissue outltures have been used to study differentiation, morphogenesis and plant regeneration. White (1934) established the first callus oultures capable of potentially unlimited growth. Since then a variety of plants have been induced to form callus and regenerate plants *in vitro.* Callus induction and organogenesis has been reported for pollen of *Arachis hypogaea* and *A. villosa* (Bajaj *et al.*, 1981). Cotyledon explants have been induced to form callus and exhibit organogenesis (Ilahi & Ullah, 1983). Bajaj (1979) found shoot maristems of peanut to differentiate plantlets after freeze preservation. The regeneration of apical meristems of peanuts has been observed (Mrognski *et al.*, 1981). There is however, no report about the use of hypocotyl of peanut in culture. In the present communication, the behaviour of these explants *in vitro* is reported.

Materials and Methods

Arachis hypogaea seeds were surface sterilized with alcohol containing Tween 80 as the wetting agent and 1% mercuric chloride solution for 5 and 3 minutes respectively. The seeds were then rinsed three times with sterile distilled water and inoculated one seed/flask on 2% sucrose agar. After 10 days of germination, the hypocotyl was separated, cut into thin discs and inoculated on Murashige & Skoog (MS) medium as modified by Gamborg & Wetter (1975). Sucrose, growth hormones and other ingredients were used with 0.8% Difco-Bacto agar and adjusted to pH 5.8. The medium was sterilized at 15 Psi for 15 min. Erlenmeyer flasks containing 50 ml of the medium were inoculated with one piece of hypocotyl per flask. Experimental cultures were kept in a cooled incubator at 27 ± I°C having 16h light cycle every 24h.

Results

Excised hypocotyl pieces inoculated on modified MS medium supplemented with 2% sucrose did not exhibit any activity and died after a subculture of three weeks. The basal medium was then supplemented with various concentrations of auxins, cytokinins and organic supplements for callus induction and its further growth.

A. *Effect of 2, 4-D:*

When a disc or a piece of disc of peanut hupocotyl was inoculated on modified MS medium supplemented with 0.1, 0.5 or 1.0 mg/l of 2, 4-D no growth was observed at 0.1mg/1 of 2, 4-D. Slight swelling of the tissues was found in 0.5mg/1 of 2, 4-D, and after some-time the hypocotyl piece turned brown. At 1.0 mg/l of 2, 4-D, although no callus formation was observed but a single root primordium was induced after three weeks of culture.

B. *Effect of knietin:*

Hypocotyl pieces when inoculated on modified MS medium supplemented with 0.1, 0.5 or 1.0 mg/l of K (6-furfurylaminopurine) showed no callus formation. Whereas at 0.1mg/1 of K, there was no change in the explant, at 0.5mg/1 of K, a slight swelling of the excised hypocotyl tissue was observed. Hypocotyl explants when inoculated on 2.0 and 4.0 mg/l of K, showed some callus formation at 2.0 mg/l with none at 4.0 mg/l of K.

C. *Effect of 2, 4-D and K:*

Hypocotyl explants inoculated on the basal medium supplemented with a combination of 2, 4-D and K @ 0.01, 0.05, 0.1, 0.5 and 1.0 mg/l showed no callus formation in the medium supplemented with 0.01 or 0.05 mg/l of 2, 4-D in combination with K (Table 1). Slight callus formation occurred at 0.1 mg/l each of 2, 4-D and K. Increase in both auxin and cytokinin concentration enhanced callus formation. Good callus resulted on the medium supplemented with 0.5 mg/l of 2, 4-D and 1.0 mg/l of K (Fig. 1). When the callus induced on this medium was excised and transferred to the fresh medium of a similar constitution, no further growth in callus mass could be observed. The induced callus was then inoculated on a medium containing varying concentrations of 2, 4-D and K. The medium supplemented with 1.0 mg/l of 2, 4-D and 4.0 mg/l of K, was found to be the best combination for further callus proliferation since copious callus resulted. The callus surface was shiny, green and compact (Fig. 2).

Table 1. Callus induction in hypocotyl explants of peanut inoculated on modified MS medium under the influence of growth hormones.

Growth hormone mg/l 2. 4-D	K	Callus Formation	Remarks
0.01	0.01	–	No growth, explant died after 3 weeks.
0.01	0.05	–	" " " " " "
0.01	0.1	–	" " " " " "
0.05	0.01	–	" " " " " "
0.05	0.05	–	" " " " " "
0.05	0.01	–	Slight swelling of the tissue, died after 3 weeks.
0.1	0.1	+	Yellow-brown in colour.
0.1	0.5	++	Bright green callus, hard in texture.
0.1	1.0	++	Compact green callus, hard in texture.
0.5	0.1	++	Bright green callus.
0.5	0.5	++	Green callus.
0.5	1.0	+++	Compact whitish-green callus, hard in texture.
1.0	0.1	++	Brownish-green callus.
1.0	0.5	++	Brownish-green callus.
1.0	1.0	+	Slight callus formation. brown in colour.

– No callus; + Slight callus; ++ Moderate callus; +++ Good callus.

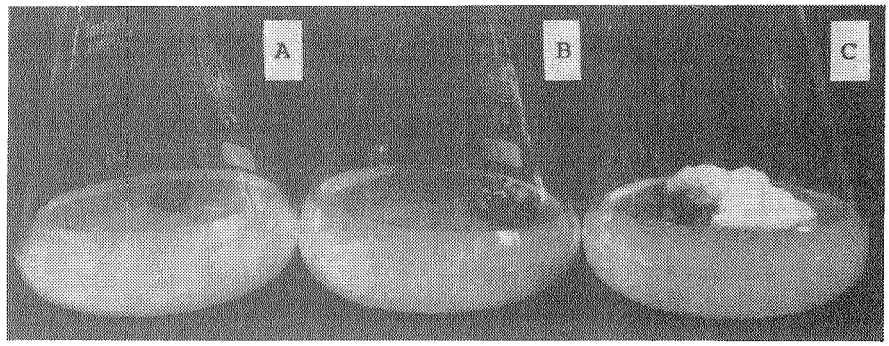

Fig. 1. Callus induction/growth on modified MS medium supplemented with A. 0.01 mg/l of 2, 4-D and 0.01 mg/l of K, B. 0.1 mg/l 2, 4-D and 0.1 of K and C. 0.5 mg/l of 2, 4-D and 1.0 mg/l of K after ten weeks of inoculation. (Note good callus formation in C).

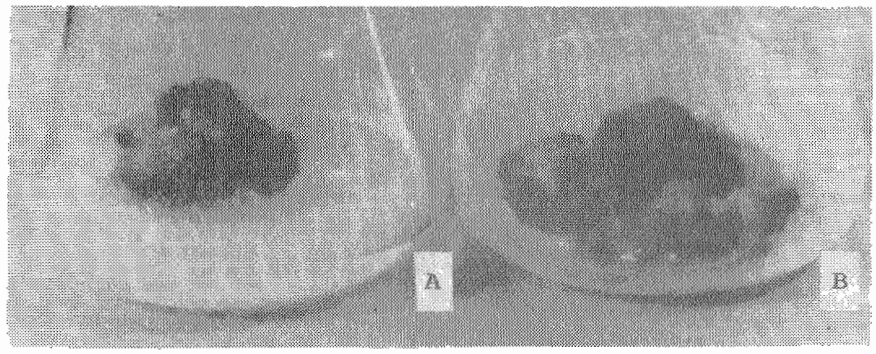

Fig. 2. Proliferation of callus cultured on modified MS medium supplemented with A. 1.0 mg/l of 2, 4-D and 2.0 mg/l of K and B. 1.0 mg/l of 2, 4-D and 4.0 of K after eight weeks of culture.

D. *Effect of sodium diethyl-dithio-carbamate and coconut milk:*

The calli induced on the basal medium supplemented with either 0.5mg/1 2, 4-D and 1.0 mg/l K or 1.0 mg/l 2, 4-D and 4.0 mg/l of K, exhibited browning after a period of 8 weeks with a decrease in callus growth rate. Browning and slow growth could not be overcome even on transfer to fresh modified MS medium containing same concentrations of the growth hormones. Callus browning was avoided by the supplementation of the medium with 250mg/1 of sodium diethyl-dithio carbamate (SDDC) and coconut milk (CM) at 10% v/v coupled with 2, 4-D and kinetin in different concentrations (Table 2) showed no callus browning, the callus was hard and compact which turned green with shiny and smooth surface.

Table 2. Proliferation of callus when inoculated on modified MS medium supplemented with CM (10% v/v) and sodium diethyl-dithio carbamate (250 m/g1) and the hormones.

Growth hormones mg/1		Callus	Remarks
2, 4-D	K	Formation	
0.5	1.0	–	Callus died after 3 weeks of inoculation.
0.5	2.0	+	White brown callus with abundant roots.
1.0	1.0	++	Brownish green callus with some roots.
1.0	2.0	+++	Bright whitish green, compact and hard callus with abundant roots.

– No callus; + Slight callus; ++ Moderate callus; +++ Good callus.

E. *Effect of casein hydrolysate and coconut milk:*

Modified MS medium supplemented with CH at 500mg/1 and CM at 10% (v/v), in addition to 2, 4-D and kinetin in various combinations showed very little callus development at 1.0 mg/l of 2, 4-D in combination with 1.0 mg/l of kinethin. Initiation of numerous roots from this callus after three weeks of culture was observed. At 0.5mg/1 of 2, 4-D a moderate callus formation took place in combination with 2. Omg/1 of kinetin. A better greensih white callus with compact and hard texture developed at 1.0 mg/l of 2, 4-D in combination with 2.0 mg/l of K (Table 3).

Table 3. Callus formation in hypocotyl explants inoculated on modified MS medium supplemented with CM (10 v/v), CH 500 mg/1 and growth hormones.

Growth hormones mg/1		Callus	Remarks
2, 4-D	K	formation	
0.5	1.0	+	Brownish callus
0.5	2.0	++	Brown callus, campact and hard in texture.
1.0	1.0	+	Greenish-brown callus with numerous roots.
1.0	2.0	+++	Compact, brightly greenish-white callus hard in texture.

+ Slight callus; ++ Moderate callus; +++ Good callus.

Discussion

Growth hormones viz., 2, 4-D and K were unable to induce callus on hypocotyl explants when used alone. No callus formation occurred in juniper shoot pieces when inoculated on MS supplemented with various concentrations of. 2, 4-D (Javeed *et al.*, 1980). However, Parveen (1978) reported callus formation of *Rauwolfia* leaf segments in presence of 2, 4-D. Some callus induction was reported on sugarbeet root explants on MS supplemented with K (Jabeen, 1978). Similarly, slight callus induction took place in peanut hypocotyl explants on MS medium supplemented with slightly higher levels of K.

Tobacco pith tissue exhibited rapid cell expansion forming giant cell when cultured on Indoleacetic acid (IAA). Tissues cultured on a medium containing both IAA and K exhibited marked cell division with a large number of relatively small cells, while K alone had little or no effect on cell division (Das *et al.*, 1956). Similarly, a marked increase in callus growth has been reported in tissues of Gerbera (Murashige *et al.*, 1974). In the present investigations with *A. hypogaea*, both callus induction and its growth increased in presence of an auxin and cytokinin.

According to Gresshoff (1981) legumes pose problems for culture and micropropagation. Bajaj *et al.* (1981) observed that the original meristem of epicotyle segments of *A. hypogaea* elongated into a shoot, while the mesocotyl segment formed a bud. The origin of this bud could have been from the dormant lateral buds. Excised cotyledons and petioles formed profused callus masses, but exhibited no differentiation. Similarly, Ilahi & Ullah (1983) have reported profuse callus formation on cotyledon explants of peanut. Although root formation has been induced, shoot buds formed are difficult to grow beyond a certain stage which needs investigation.

The stimulatory effects of casein hydrolysate (CH) have been reported in studies of cell division of carrot tissues (Steward & Shantz, 1954) and the tissue of tobacco (Skoog & Miller, 1957). Konar (1974) reported the requirement of CM and CH in addition to 2, 4-D in the medium for callus growth in *Pinus gerardiana*. In hypocotyl explants of peanut, addition of both CH and CM didnot exhibit callus formation. Since addition of these promoted root formation in calli cultured on lower levels of K, while at higher K levels no root formation occurred. Addition of 2, 4-D had no effect. Whether root formation is due to CM or CH needs investigation. An auxin and a cytokinin was necessary for both callus induction and its further proliferation. Addition of CM and SDDC further enhanced callus growth and checked tissue browning as also reported for other plant species (Mitra *et al.*, 1965; Konar, 1974; Krishnamurti, 1981).

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