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IN VITRO RESPONSE OF DIFFERENT EXPLANTS ON CALLUS DEVELOPMENT AND PLANT REGENERATION IN GROUNDNUT (*Arachis hypogeae* L.)

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ABSTRACT

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Different explants namely, cotyledon, epicotyl and hypocotyl were used to study the *in vitro* plant regeneration of groundnut (*Arachis hypogaea* L.). The explants of two different genotypes BARI groundnut 5 and BARI groundnut 6 were cultured in MS medium with different concentration of 2,4-D, BAP and NAA. In case of different explants, hypocotyls showed better performances than epicotyls and cotyledon. Among different concentrations, 2,4-D @ 2mg/l was found more suitable for good callus induction. When callus was sub cultured in different concentration of 2,4-D, good callus growth was also observed. MS medium supplemented with different concentrations of BAP produced small shoot bud at different subculture. Maximum number of shoot bud differentiation was observed from 2.5mg/l BAP concentration. Among the different growth hormones, 2,4-D found good for callus induction and BAP was found more suitable for organogenesis compare to NAA.

Key words: *Arachis hypogeae, in vitro, regeneration, tissue culture*

INTRODUCTION

Groundnut is a day neutral important oil seed crop. In Bangladesh, it occupies third place in respect of acreage. However, in terms of yield it ranks first among other major oil seed crops viz. mustard, sesame, sunflower and linseed. Groundnut seed contains 48-52% oil and 24-26% protein. Nutritionally it is superior to mustard oil. The content of essential amino acid e.g. linoleic acid is higher in groundnut than mustard.

The edaphic and the agro climatic conditions of Bangladesh are suitable for the cultivation of groundnut but it's per acre production is low due to proper management. However, the economic return obtained from this crop is more than any other oil seed crops (Reddy and Kaul, 1986; Khaleque and Mia, 1988) in Bangladesh. So, there is an ample scope for increasing its production. To improve upon the characters of agronomic importance in groundnut, conventional breeding method is not very successful. Besides, the sexual breeding techniques are tedious.

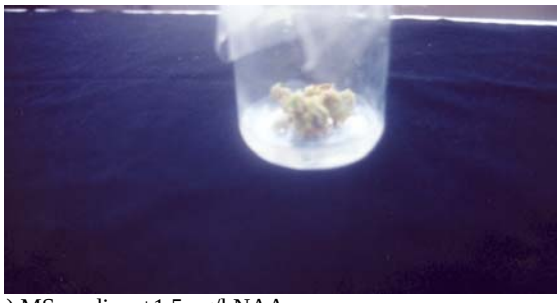
According to Scowcroft *et al*. (1987), tissue culture techniques can play a significant role for the enrichment of genetic variability giving rise to variations/mutations at an unexpectedly high rate and may be a novel source of genetic variability in many plant species. Tissue culture techniques may be utilized conveniently to overcome incompatibility barrier through fusion of vegetative cells of interspecific, intraspecific, intergeneric and interfamilial group (Nickell and Heinz, 1993; Engler and Grogan, 1982). Therefore, the present investigation was done to find out suitable growth regulator for callus induction and root-shoot development of groundnut plant lets.

MATERIALS AND METHODS

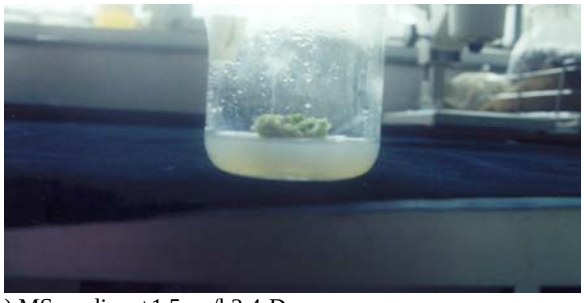
The experiment was conducted in the Tissue Culture Laboratory of Biotechnology Division, Bangladesh Agricultural Research Institute (BARI) Joydebpur, Gazipur. Two varieties of *Arachis hypogeae* L. namely BARI groundnut 5 and BARI groundnut 6 were collected from the Oil Seed Research Centre, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur as a test materials. The seeds of each material were washed under running tap water for 3 to 5 minutes to reduce the level of surface organisms. Then the seeds were washed 5 times with sterilized water and $HgCI_2$ (0.1**%)** solution for 20 minutes. After exposure to the sterilent, the seeds were washed 5 times with sterilized distilled water. Then the seeds were kept on a sterilized petridish containing sterile blotting paper to soak the water droplets. Seeds were then cultured in MS (Murashige and Skoog, 1962) medium with different concentrations of 2,4-D, BAP, NAA to initiate callus. Cultures were then transferred to fresh media containing various hormonal supplements, singlely or in combination at 30-days intervals and routinely examined for different morphological development. The subculture tubes or flasks were again incubated at 25 ± 2^0C with 12/12 hr light/dark cycle under fluorescent light. The sub cultured calli continued to proliferate and differentiated into shoots. When these shoots grew about 3 cm, they were excised aseptically from the callus mass and were separated from each other and cultured on freshly prepared medium containing different hormonal supplements for root differentiation. Data were recorded on callus induction, callus growth, callus type, number of multiple shoot, shoot and root length.

RESULTS AND DISCUSSION

The response of different explants, such as cotyledon, epicotyl and hypocotyl from two groundnut genotypes were investigated. Both genotypes and explants gave callus when cultured on MS medium supplemented with different concentrations of 2,4-D and NAA. The effect of different concentrations of 2,4-D and NAA on callus induction of peanut are presented in Table 1. The best callus growth was obtained when 2.0 mg/l 2, 4-D was used in the medium. In case of NAA the maximum callus growth was observed in 2 mg/l NAA. Friable, compact, light green, brownish and watery types of callus were observed irrespective of genotypes. They were maintained on the same medium by repeated sub culturing every 28-30 days to observe the passage of in their differentiation into shoots and roots. Callus obtained from epicotyl and hypocotyl explants were brown, watery and friable (Fig. 1a). Calli from cotyledon explants proliferated profusely and turned deep green and friable (Fig. 1b). Growth regulator NAA and 2,4-D were found to be equally good for callus induction. The best callus growth was obtained when 2 mg/l 2, 4-D and 0.5 mg/l kinetin were used in the medium (Hoque *et al*. 1992).



a) MS medium+1.5mg/l NAA



b) MS medium+1.5mg/l 2,4-D

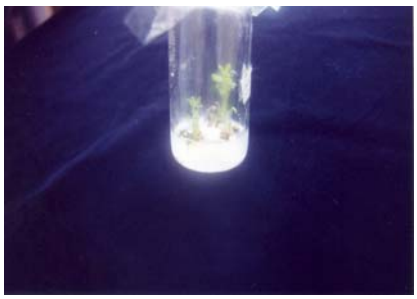
Fig.1. Callus induction derived from a) Hypocotyl b) Cotyledon on MS medium supplemented with hormone.

Table 1. Effect of 2,4-D and NAA in MS medium on callus induction of groundnut genotypes

Concentrations of 2,4-D mg/l	Concentrations of NAA (mg/l)	Nature of callusing*	Callus type
MS + 0	0	0	No. response
MS + 0.5	0	++	Light green, compact
MS + 1.0	0	++	Light green compact on middle and friable in side
MS + 1.5	0	+++	Light green, friable
MS + 2.0	0	+++	Brown, friable and loose
0	MS + 0	0	No. response
0	MS + 0.5	+	Compact brownish
0	MS + 1.0	+	Greenish
0	MS + 1.5	++	Brownish, friable and watery
0	MS + 2.0	++	Light green, friable

* '+' Poor Callus; '++' good Callus '+++' very good callus

The individual effect of 2,4-D, BAP and NAA in MS medium on organogenesis and plantlet differentiation are presented in Table 2. Among different concentrations 2,4-D good callus growth with bud primordia was observed in



2(a)

2(b)

2(c)

Fig. 2. Regeneration of large number of shoot from 2(a) Epicotyl 2(b) Hypocotyl 2(c) Cotyledon explants on MS medium supplemented with 2.0 mg/l BAP

MS medium with suplimented 1.5mg/l 2,4-D. In case of BAP good callus growth as well as shoot development was observed. Slow callus growth was found at different concentrations of NAA. So, from the above study we can assume that among the different growth hormone BAP was found more suitable for organogenesis compare to 2,4-D and NAA. Different levels of BAP ranging from 1.0 to 2.5 mg/l were used in epicotyl and hypocotyl as a explant, when cultured on only MS medium without hormone, none of the explants did not show any response (callusing or shoot bud differentiation). When higher concentrations of BAP (2.0 to 2.5 mg/l) were used, the explants directly developed shoot bud (Fig. 2.). No callusing was observed in higher concentration of BAP. In case of lower concentration of BAP epicotyl and hypocotyl explants remain unchanged for a longer period of time. Bhuiyan *et al.* (1992) observed similar performances when cotyledon used as explants. Epicotyl and hypocotyl explants were used because the cut surface of explants would favor an easy access of *Agrobacteriums tumefaciens* during transformation. Such as, proximity to the cut surface would favor an easy access of *Agrobacteriums tumefaciens* to the meristematic cells during co-cultivation. (Hendrix *et al.* 1987; Srivastava *et al.* 1988 and Gambly and Dodd, 1990).

Single shoot was produced by the 2.0 mg/l concentration of BAP in the cotyledon explant (Fig. 2c). Bhuiyan (1992) observed maximum number of shoot (20.3) at 3.0 mg/l concentration of BAP from cotyledon explants of variety DM-1. McKently *et al*. (1989) reported that the number of shoot was lowest in the de-embryonated cotyledon section of groundnut. The epicotyl and hypocotyl explants showed better performances than the cotyledon explants. This may be due to the presence a meristematic cell near the cut surface of the epicotyl and hypocotyl explants. The present results are consistent with the hypothesis that the shoot initiation is determined by the availability of the totipotent cells.

Table 2. Individual effect of 2,4-D, BAP and NAA in MS medium on organogenesis and plant let differentiation from callus

2,4-D mg/l	BAP mg/l	NAA mg/l	Morphogenic response
MS + 0.5	0	0	Slow callus growth
MS + 1.0	0	0	Good callus growth
MS +1.5	0	0	Good callus growth
MS +2.0	0	0	Good callus growth
0	1.0	0	Callus growth
0	1.5	0	Callus growth with shoot bud primordia
0	2.0	0	Only shoot developed
0	2.5	0	Only shoot developed
0	0	0.5	No. Response
0	0	1.0	Callus growth slow
0	0	1.5	Callus growth slow
0	0	2.0	Callus growth slow

CONCLUSION

From the results on regeneration system of two groundnut genotypes, BARI groundnut 6 was found more suitable for the regeneration. For callus induction MS medium supplemented with 2,4-D was found more suitable and also for callus induction. MS medium supplemented with different concentration of BAP was suitable for shoot development.

REFERENCES

Bhuiyan MSA, Haque MM, Hoque MI, Sarker RH, Islam AS (1992) Morphogenic responses of peanut leaflet explaints cultured *in vitro. Plant Tissue Cult.* 2(1), 49-53.

Engler DE, Grogan RG (1982) Protoplast regeneration. California Agriculture. August. 18-19.

Gambley RL, Dodd WA (1990) An *in vitro* technique for the production de novo of multiple shoots in cotyledon explants of cucumber (*Cucumis sativus* L.). *Plant Cell Tiss. Org. Cult.* 20, 177-183.

Hendrix RC, Litz RE, Kirchof BK (1987) *In vitro* organogenesis and plant regeneration from leaves of *Solanum candidum* Lindl., S. Quitoense Lam. (Naranjilla) and *S. Sessiliflorum* Dunal. *Plant Cell Tiss. Org. Cult.* 11, 67-73.

Hoque NI, Mahbub S, Sarker RH, Haque MM, Islam AS (1992) Callus induction and Plant regeneration from different explants of *Arachis hypogaea* L. *Plant Tissue Cult.* 1(1), 35-41.

Khaleque MA, Mia FU (1988) Status of groundnut in Bangladesh. Proceedings of the 2nd SAARC member countries counterpart scientists meeting on groundnut. March. Pp. 3-24.

Mckently AH, Moore GA, Gardner FP (1989) *In vitro* plant regeneration of peanut from seed explants. *Crop Sci.* 30, 192-196.

Nickell LG, Heinz DJ (1993) Potential of cell and tissue culture techniques as aids in economic plant improvement. In: Genes, Enzymes and populations. (Ed.: Srb, A.M.). New York. Plenum. 109-128.

Reddy LJ, Kaul AK (1986) Status and prospects of groundnut in Bangladesh. BARC, Dhaka, Bangladesh. p-1.

Scowcroft WR, Brettell RIS, Ryan SA, Davies PA, Pallotta MA (1987) Somaclonal variation and genomic flux. Plant Tissue and Cell Culture. Alan R. Liss. Inc. Pp. 275-286.

Srivastava V, Reddy AS, Mukherjee SG (1988) Transformation and regeneration of *Brassica oleraceae* mediated by an oncogenic *Agrobacterium tumefaciens.* Plant Cell Rep. 7, 504-507.