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Effect of different hormones in *in vitro* regeneration of groundnut (*Arachis hypogaea* L.)

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Abstract

Different combinations of BAP, IAA, NAA, Kn and Ads were used to observe the regeneration capacity of groundnut from calli in MS medium. Four groundnut genotypes were included in the study. The responses of shoot differentiation in different treatments were different. The medium supplemented with 1.0 mg/l BAP + 1.0 mg/l IAA showed the highest percentage of shoot regeneration (40 to 75%) while the lowest (0 to 60%) was observed in MS + 1.0 mg/l BAP + 2.0 mg/l NAA + 75.0 mg/l Ads. Half strength basal MS ($^1/_2$ MS) medium with IBA was used to investigate the rooting response of regenerated shoots. The highest percentage of root induction was observed by the genotypes Dhaka-1 and DG-2 in 0.5 mg/l IBA and the lowest by Acc.-12 in both the concentrations of IBA. Small plantlets with well develop root system were removed from the medium and transplanted in small pots. The pots were transferred to polythene shed for hardening. Survival rate of plantlets were about 75% for all the genotypes under study.

Keywords: *In vitro,* Callus, Regeneration, *Arachis hypogaea* L.

Introduction

The cultivated groundnut *Arachis hypogaea* L. is one of the most important oil and protein producing legume crop of the semi-arid tropics. Groundnut kernel contains 45-50 per cent high quality oil, more than 25 per cent highly assimable protein and vitamins B and E. Bangladesh is seriously deficit in edible oil production. The per capita consumption of edible oil is one of the lowest in the world (3 kg/head/year), is one fifth of recommended requirement for a balanced diet (Elias, 1998). Groundnut can be considered as one of the potential sources to meet up this deficiency. But its extremely recalcitrant seed often create problem in germination. *In vitro* regeneration technique can help to get available plants. During the last few decades, this technique of plant tissue culture has been a new and powerful tool in programmes of crop improvement, it received wide attention of modern scientists. Since the dawn of tissue culture, legumes have been identified as favourite materials. Akaska *et al.* (2000), Radhakrisnan *et al.* (2000) and Ihsan *et al.* (1995) carried out research on *in vitro* regeneration of groundnut. Therefore, the present study was under taken to observe the effect of different hormones in different combinations and concentrations on *in vitro* regeneration of groundnut, which will enrich the knowledge to get its higher number of plants through *in vitro* regeneration.

Materials and Methods

The experiment was conducted during the period from July 2000 to September 2001 in the tissue culture laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. The experiment was laid out in Completely Randomized Design. To induce shoots as well as roots from unorganized calli, 20 explants (hypocotyl, epicotyl, cotyledonary node, shoot tip and leaves) of each of the four genotypes (Dhaka-1, DG-2, DM-1, and Acc. No. 12) were cultured in MS medium (Murashige and Skoog, 1962)

with different combinations of auxin, cytokinin and also adenine sulphate (Ads.). The combinations such as 0.5 mg/l BAP + 0.5 mg/l IAA, 1.0 mg/l BAP + 0.5 mg/l IAA, 1.0 mg/l BAP + 1.0 mg/l IAA, 1.0 mg/l BAP + 1.5 mg/l IAA, 0.5 mg/l BAP + 0.5 mg/l 2,4-D, 0.5 mg/l BAP + 1.0 mg/l 2,4-D, 1.0 mg/l BAP + 1.0 mg/l NAA + 75 mg/l Ads, 1.0 mg/l BAP + 1.0 mg/l NAA + 75 mg/l Ads, 1.0 mg/l BAP + 1.5 mg/l NAA + 75 mg/l Ads and 1.0 mg/l BAP + 2.0 mg/l NAA + 75 mg/l Ads were used for this purpose.

Results and Discussion

For the induction of calli from different explants different combinations of 2,4-D and BAP were used, where MS+ 1.5 mg/l 2,4-D + 0.25 mg/l BAP was found to be the best for this purpose (Fig. 1).

i) Organogenesis via callus

In *in vitro* technique the primary objective is to establish the explants in culture, resulted in the formation of shoots and subsequently the development of roots for the production of free-living plantlets. The morphogenic response of explants of the genotypes to various combinations of auxin, cytokinin and also adenine sulphate are given in the Table 1.

Differential behaviour in shoot proliferation was observed in shoot regeneration media supplemented with different concentrations of hormones and additive. Among the genotypes in MS medium supplemented with 1.0 mg/l BAP + 1.5 mg/l IAA was found the best (80%) to induce shoot differentiation in DG-2 (Fig. 2) followed by Acc.-12 (75%) (Fig. 3) in 1.0 mg/l BAP + 1.0 mg/l IAA. Acc.-12 and DG-2 showed the poorest response (30%) in 0.5 mg/l BAP + 0.5 mg/l IAA combination.

Single shoot formation was also found at lower concentration of BAP combined with higher concentration of NAA (1.0 to 2.0 mg/l) or both the hormones in equal concentration (1.0 mg/l) with Ads.

BAP (0.5 mg/l) in combination with 2,4-D (0.5 to 1.0 mg/l) when used in MS medium were also found suitable for shoot/bud formation without intervention of callus. The best response was observed when MS medium was supplemented with 0.5 mg/l BAP + 0.5 mg/l 2,4-D (Table 1). Huang *et al.* (1986) obtained plant regeneration from NAA derived callus, Akaska *et al.* (2000) successfully produced shoots from callus and Baker and Wetzstein (1994) reported that the use of 2,4-D compared to NAA in the induction medium resulted in a higher rate of embryogenesis and mean number of plantlets, which partially supports the present investigations.

ii) Regeneration of root

Regenerated shoots from different explants need the roots to establish themselves in the field. In order to induce roots, the shoots were transferred to half strength MS medium supplemented with 0.5 mg/l and 1.0 mg/l IBA. In the ½ MS medium containing 0.5 mg/l IBA, root formation was found to be the best (Table 2, Fig. 4). Among the explants epicotyl and cotyledonary nodal segments of Dhaka-1 and cotyledonary nodal segments of DG-2 produced the highest percentage of roots (80%). The best root induction (60%) was found in epicotyl and cotyledonary nodal segment derived shoots of Dhaka-1 and DG-2 used in MS medium supplemented with 1.0 mg/l IBA. Root forming capacity of the genotype Acc.-12 was

comparatively poor in both the concentrations of IBA. Duffus (1985) and Venkatachalam *et al.* (1996,1997) used different concentrations of IBA for rooting in groundnut, where Akaska *et al.* (2000) and Ihsan *et al.* (1995) observed rooting in NAA.

Table 1. Effect of different combinations of auxin, cytokinin and adenine sulphate in MS medium on shoot and root differentiation from different explants of *Arachis hypogaea* L.

Supplements (mg/L)	Materials	No. of explants in which shoot initiated	% of shoot regeneration	Days to shoot regeneration	No. explants in which root induced	% of root induction	Days to root induction
0.5 BAP + 0.5 IAA	Dhaka-1	7	35.0	7-8	4	20.0	4-5
	DG-2	6	30.0	8-9	7	35.0	4-5
	DM-1	7	35.0	8-10	--	--	--
	Acc.-12	6	30.0	8-10	--	--	--
1.0 BAP + 0.5 IAA	Dhaka-1	14	70.0	7-10	5	25.0	5-6
	DG-2	12	60.0	6-7	5	25.0	5-7
	DM-1	11	55.0	7-8	--	--	--
	Acc.-12	12	60.0	7-8	--	--	--
1.0 BAP + 1.0 IAA	Dhaka-1	8	40.0	6-7	5	25.0	4-5
	DG-2	15	75.0	6-7	7	35.0	4-5
	DM-1	10	50.0	7-8	5	25.0	4-5
	Acc.-12	15	75.0	7-8	--	--	--
1.0 BAP + 1.5 IAA	Dhaka-1	7	35.0	5-6	--	--	--
	DG-2	16	80.0	6-8	--	--	--
	DM-1	10	50.0	7-8	--	--	--
	Acc.-12	10	50.0	6-8	--	--	--
0.5 BAP + 0.5 2,4-D	Dhaka-1	10	50.0	9-10	6	30.0	5-6
	DG-2	12	60.0	8-10	3	15.0	5-6
	DM-1	9	45.0	8-10	2	10.0	6-7
	Acc.-12	9	45.0	8-10	--	--	--
0.5 BAP + 1.0 2,4-D	Dhaka-1	10	50.0	8-9	--	--	--
	DG-2	11	55.0	8-10	3	15.0	9-10
	DM-1	8	40.0	8-12	2	10.0	9-10
	Acc.-12	8	40.0	8-12	--	--	--
1.0 BAP + 1.0 NAA + 75 Ads	Dhaka-1	14	70.0	9-10	--	--	--
	DG-2	13	65.0	7-9	--	--	--
	DM-1	12	60.0	9-10	--	--	--
	Acc.-12	12	60.0	7-9	--	--	--
1.5 BAP + 1.0 NAA + 75 Ads	Dhaka-1	10	50.0	7-8	10	50.0	7-8
	DG-2	12	60.0	7-9	4	20.0	8-10
	DM-1	9	45.0	8-10	3	15.0	8-9
	Acc.-12	9	45.0	8-10	--	--	--
1.0 BAP + 1.5 NAA + 75 Ads	Dhaka-1	10	50.0	6-7	6	30.0	8-10
	DG-2	12	60.0	7-8	--	--	--
	DM-1	11	55.0	7-8	--	--	--
	Acc.-12	11	55.0	6-7	--	--	--
1.0 BAP + 2.0 NAA + 75 Ads	Dhaka-1	12	60.0	8-10	5	25.0	9-10
	DG-2	7	35.0	8-10	--	--	--
	DM-1	--	--	--	--	--	--
	Acc.-12	--	--	--	--	--	--

Table 2. Effect of different concentrations of auxin in half strength of MS medium on root induction from regenerated shoots of *Arachis hypogaea* L.

Supplements (mg/l)	Materials	Name of explants from which shoot regenerated	No. of shoot sub-cultured	No. of shoot to which root induced	% of root regenerated	Days to root regeneration
0.5 IBA	Dhaka-1	Hypocotyl	5.0	3.0	60.0	4-5
		Epicotyl	5.0	4.0	80.0	6-9
		Cotyledonary node	5.0	4.0	80.0	5-8
		Shoot tip	5.0	2.0	40.0	7-10
	DG-2	Hypocotyl	5.0	2.0	40.0	5-7
		Epicotyl	5.0	3.0	60.0	6-8
		Cotyledonary node	5.0	4.0	80.0	7-8
		Shoot tip	5.0	3.0	60.0	9-10
	DM-1	Hypocotyl	5.0	-	-	-
		Epicotyl	5.0	-	-	-
		Cotyledonary node	5.0	2.0	40.0	9-11
		Shoot tip	5.0	3.0	60.0	9-11
	Acc.-12	Hypocotyl	5.0	-	-	-
		Epicotyl	5.0	-	-	-
		Cotyledonary node	5.0	1.0	20.0	9-10
		Shoot tip	5.0	2.0	40.0	9-10
1.0 IBA	Dhaka-1	Hypocotyl	5.0	2.0	40.0	5-9
		Epicotyl	5.0	3.0	60.0	5-7
		Cotyledonary node	5.0	2.0	40.0	9-11
		Shoot tip	5.0	2.0	40.0	8-11
	DG-2	Hypocotyl	5.0	2.0	40.0	5-7
		Epicotyl	5.0	2.0	40.0	5-8
		Cotyledonary node	5.0	3.0	60.0	8-10
		Shoot tip	5.0	2.0	40.0	7-8
	DM-1	Hypocotyl	5.0	-	-	-
		Epicotyl	5.0	-	-	-
		Cotyledonary node	5.0	2.0	40.0	8-10
		Shoot tip	5.0	1.0	20.0	8-10
	Acc.-12	Hypocotyl	5.0	-	-	-
		Epicotyl	5.0	-	-	-
		Cotyledonary node	5.0	1.0	20.0	7-9
		Shoot tip	5.0	1.0	20.0	8-9

iii) Establishment of plantlets

After sufficient development of roots, plantlets were successfully transplanted into pot (Fig. 5). The survival rate of the transplanted plantlets was 75%. The plantlets after their transplantation to the soil were subsequently watered with Hoagland's solution. As soon as new leaves started to initiate, the plants were watered with ordinary tap water. Gradually the plantlets were adapted to the soil. Radhakrisnan *et al.* (2000) reported that 60% of the rooted plants could be established in the field.

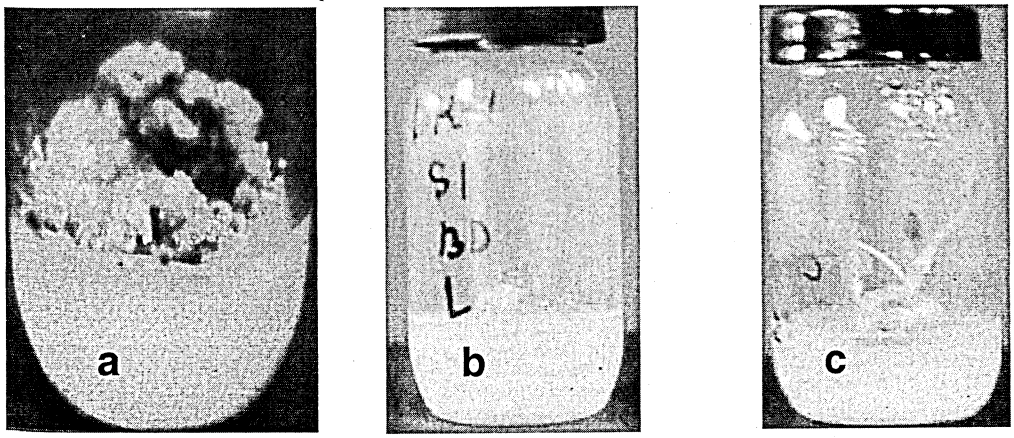

Fig. a) Development of callus. b) and c) Shoot initiation and regeneration from calli of DG-2 in MS+1.0mg/l BAP+1.5 mg/l IAA.

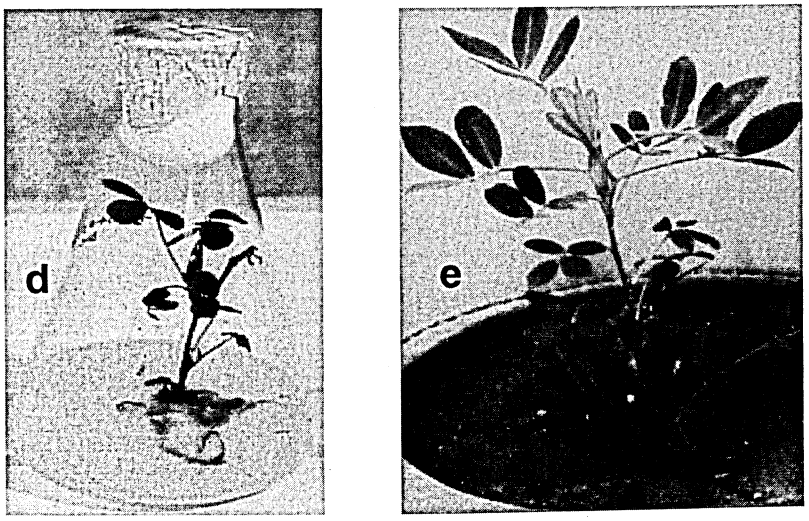

Fig. d) Root initiation and development from regenerated shoot of Dhaka-1 in ½ MS+0.5 mg/l IBA. e) Establishment of plantlet of DG-2 at early flowering stage.

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