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RESEARCH ARTICLE

EFFECT OF DIFFERENT GROWTH REGULATORS ON *IN VITRO* REGENERATION OF GROUNDNUT (*ARACHIS HYPOGAEA* L.)

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ABSTRACT

The present study was to carried out plant regeneration of *Arachis hypogaea* from in mature leaf explant was cultured on MS medium containing various concentration of IAA (2.5mg/l) and BAP (1mg/l) was maximum percentage of callus induction from shoot explants. KIN (2.5mg/l) and IAA (1mg/l) was maximum shoot regeneration of callus. BAP+ KIN (2.5mg /l) in combined effect of 1AA (1mg/l) results showed maximum yield of callus and shoot induction. IBA (3.0 mg/l) and KIN (0.5mg/l) was produced amount of maximum number of root induction. The present study to find out to the various concentrations of hormones was vital role of callus induction, shoot regeneration and root induction.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an economically important oil and protein rich crop, whose seeds contain about 43% oil and 25% protein that has a significant impact in tropical and sub-tropical regions of Asia, Africa, and North and South America. There are several constraints to the productivity of the peanut crop that result in great economic losses annually (Sharma *et al*., 2000). Groundnut (*Arachis hypogea* L.) or peanut is an oil, food and fodder crop which plays an important role in the agricultural economies of countries of the semi-arid tropics. It contributes significantly to food security and alleviates poverty (Reddy and Anbumozhi, V., 2003) and as a legume, improves soil fertility by fixing nitrogen and increases productivity for small holder farmers of the semi-arid cereal cropping systems (Giller *et al*., 2002). It is one of the edible oil seed and protein rich leguminous crop (Collino, *et al*., 2001), cultivated on over 20 million hectors in over 108 tropical and subtropical countries, with an annual yield of seeds estimated 28 million tons FAO, 2007. Although some of the wild relatives of *A*. *hypogaea* have been identified as resistance source to several diseases and pests Stalker, *et al.,* 1987 the success in transferring the desirable traits to cultivated varieties has been limited due to reproductive barriers, and frequent failures in the interspecific crosses.

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The application of biotechnological methods for the improvement of important crop plants of the semi-arid tropics have been shown to hold great potential (Sharma and Ortiz, 2000). Although several reports on efficient regeneration from diverse explants of peanut have been published (Cheng, *et al.,* 1992, Venkatachalam, *et al.,* 1992) not much success with genetic transformation of *Arachis* species has been achieved.

It has a high energy value (Cobb and Johnson, 1973) and suitable for wide variety of agroecological conditions (Norden *et al*., 1982). Tissue culture studies in groundnut have been well documented including some recent studies (Palanivel and Jeyabalan, 2000).

MATERIALS AND METHODS

Plant Materials

The certified seeds of groundnut (*Arachis hypogaea L.*) seeds were obtained from the Tamil Nadu Agriculture Research Station Extension, Bhavanisagar, Tamil Nadu. Seed moisture relatively (8.8%) was equilibrated under vaccum over a desicant.

Preparation of tubes for planting ground nut seeds

The test tubes were washed with soap water and rinsed water thoroughly with tap water and with distilled water. The tubes

were dried by placing them in a hot air oven at 60ºC, the MS basal medium (Murashige and Skoog, 1962) containing melted agar was poured into the test tubes at 10ml per tube. The tubes were plugged with non-absorbent cotton wrapped with one layer of gauze cloth. The test tubes were autoclaved at 121ºC for 15 minutes and stored for further investigation.

Preparation of Ground nut seeds for planting

Dry groundnut seeds were selected for the germination without any damage. The seeds were washed in tap water to remove impurities and contaminants. The seeds were imbibed for 16 hours for the removal of seed coat. After that, the following procedures were done in aseptic condition in laminar flow chamber. The seeds were placed in 5% teepol for10 min sand washed with sterile distilled water for 5 minutes. After it was placed in 70% ethanol for 3 minutes and rinsed with sterile distilled water for three times. Then the seeds were placed in 0.1% mercuric chloride for 10 minutes and rinsed with sterile distilled water for three times. Then the seeds were placed in 0.1% mercury chloride for 10 minutes and again rinsed with sterile distilled water for 3 to 5 times. The treated seeds were inoculated into the test tubes, which was contained MS medium. This process was carried out under aseptic conditions and the inoculated tubes were incubated for 48 h at 30ºC in the dark room.

Preparation of Explants

After germination the test tubes was removed and cotyledon from each germinated seeds was removed by excision nearby intercotyledonary region thereby producing a clear wound site adjacent to the immature leaf explants. All cultures were maintained at 25±2ºC under 16/8 light/dark conditions, provided by fluorescent lamps. Dried seeds for groundnut (*Arachis hypogaea*) gerotype morden were washed with tween 80%(V/V) for two minutes, followed by five minutes surface sterilization solution and rinsed five times in sterile distilled water. The seeds were placed in MS medium and seeds were germinated in darkness for 2 days and then exposed to light for 5 days at 30ºC. The 7 days old seedlings the cotyledons were removed and used as explants.

RESULTS AND DISCUSSION

The present study to develop a plant through *in vitro* techniques on plant regeneration of *Arachis hypogaea*. The immature leaf of *Arachis hypogaea* was cultured on MS medium containing various concentrations of auxin and combination with (IAA, IBA, 2, 4-D and BAP (0.5-3.0 mg/l). After 7 days best callusing was initiated from Explants cultured on MS medium supplemented with IAA (2.5mg/l) and BAP (1mg/l) was maximum percentage of callus induction for 36.0±3.1. IBA (2.5 mg/l) in combined effect of BAP (1mg/l) was maximum number of callus induction for 32.1± 0.9 from shoots explants. 2, 4-D (2.5 mg/l) in Combined effect of BAP (1mg/l) was effective for maximum of callus induction for 31.0±1.7 (Table 1 & Fig 1). BAP (2.5 mg/l) in combined effect of IAA (1mg/l) was lowest frequency of shoot regeneration from callus for 19.1 ±3.1. KIN (2.5mg/l) and IAA (1mg/l) was maximum shoot regeneration of callus for 24.1±2.0 (Table 2 & Fig 2).

Table 1. Effect of different concentration of hormone (IAA, IBA ,2,4- D and BAP) on callus induction from shoot tip explants of *Arachis hypogaea*

MS medium + Hormone concentration (mg/l)		Percentage of callus induction
IAA	BAP	
0.5	1.0	10.6±2.5
1.0	1.0	16.1±1.0
1.5	1.0	18.2±1.9
2.0	1.0	27.0±1.6
2.5	1.0	36.0±3.1
IBA	BAP	
0.5	1.0	--
1.0	1.0	16.1±1.0
1.5	1.0	22.0±1.2
2.0	1.0	32.1±0.1
2.5	1.0	32.1±0.9
2,4-D	BAP	
0.5	1.0	--
1.0	1.0	12.1±0.9
1.5	1.0	14.1±3.6
2.0	1.0	23.1±2.5
2.5	1.0	31.0±1.7

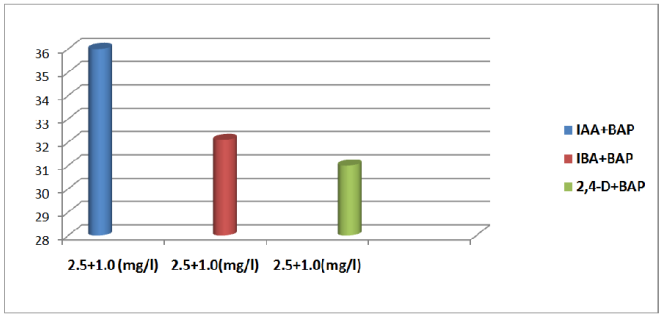

Fig. 1. IAA+BAP, IBA +BAP and 2,4- D +BAP on callus induction from shoot tip explants of *Arachis hypogaea*

Table 2. Effect of different concentration of (BPA, KIN and IAA) on shoot regeneration of callus induction of *Arachis hypogaea*

MS medium + Hormone concentration (mg/l)		Percentage of callus induction
BAP	IAA	
0.5	1.0	--
1.0	1.0	--
1.5	1.0	6.9±0.2
2.0	1.0	11.2±3.1
2.5	1.0	19.1±3.1
KIN	IAA	
0.5	1.0	--
1.0	1.0	--
1.5	1.0	17.0±0.6
2.0	1.0	15.7±1.8
2.5	1.0	24.1±2.0

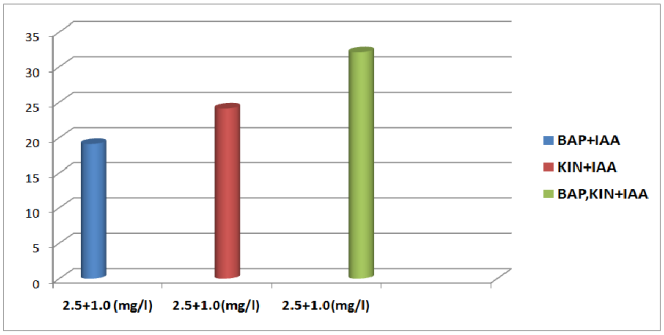

Fig 2. BPA+IAA, KIN+IAA and BAP+KIN,IAA shoot induction of *Arachis hypogaea*

Thus the combined effect of BAP+ KIN (2.5mg /l) and 1AA (1mg/l) results showed maximum yield of callus and shoot induction for 32.1±1.7 (Table 3 & Fig 3). Well elongated shoots was washed in sterile distilled water and cultured on root induction medium IAA (3.0 mg/l) and KIN 0.5 for 26.5 ±2.5. IBA (3.0 mg/l) and KIN 0.5 (1mg/l) produced maximum number of roots for 32.1±4.6 (Table 4 & Fig 3). Similar results had already been reported in strawberry (Bhatt and Dhar, 2000). Also the result was in consistent with the findings of in papaya (Cononer and Litz, 1978), as well as in *Eucalyptus grandis* (Teixetra and Silva, 1990). New shoot development from nodel explants was observed within three weeks of culture and more shoots were found to develop during subcultures (Zaman *et al*., 2008).

Table 3. Effect of BAP+KIN and IAA in multiple shoot induction of *Arachis hypogaea*

MS medium + Hormone concentration (mg/l)		Percentage of callus induction
BAP+KIN	IAA	
0.5	1.0	--
1.0	1.0	--
1.5	1.0	10.1±6.1
2.0	1.0	22.0±1.9
2.5	1.0	32.1±1.7

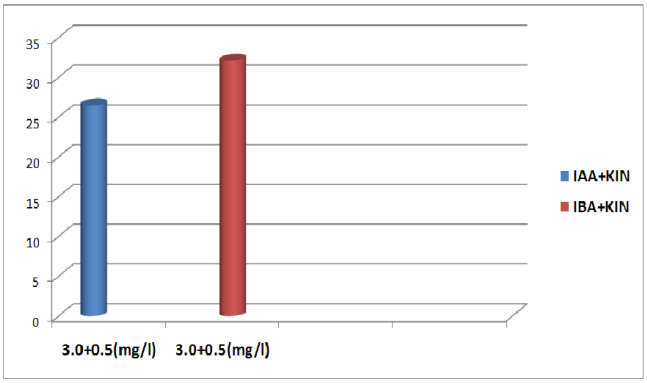

Fig 3. IAA+KIN and IBA+KIN root induction frequency of *Arachis hypogae*

Table 4. Effect of different concentration of IAA, IBA and KIN root induction frequency of *Arachis hypogaea*

MS medium + Hormone concentration (mg/l)		Percentage of callus induction
IAA	Kinetin	
1.0	0.5	--
1.5	0.5	--
2.0	0.5	8.5±1.2
2.5	0.5	13.6±2.4
3.0	0.5	26.5±2.5
IBA	Kinetin	
1.0	0.5	--
1.5	0.5	--
2.0	0.5	10.5±1.3
2.5	0.5	16.1±3.6
3.0	0.5	32.1±4.6

Arachis hypogea L. under *in vitro* conditions. The regenerated shoot lets were rooted on MS (Murashige and Skoog) basal medium with different concentrations of IBA (Indol butyric acid) and IAA (Indol acetic acid). The highest response of rooting was achieved with IBA at 0.05 + IAA at 0.05 $mg.L^{-1}$. The maximum frequency of rooting and highest number of roots were produced on medium containing IBA 0.05 $mg.L^{-1}$ and IAA 0.05 $mg.L^{-1}$ (Al-Joboury, 2012).

In seed germination, MS medium supplemented with concentration of 2, 4-D, (2 mg/l) with combination of different concentrations of KIN (0.2, 0.2,1 mg/l). The seed were responding concentrations of 2, 4-D (2 mg/l) and KIN (0.5 and 1 mg/l) (Kalpesh *et al*., 2012), *Solanum laciriatum* (Chandle *et al.,* 1982), *Nicotiana tabaccum* (Rathore *et al*., 1985) reported in the same species different combination of IAA, IBA and 2, 4-D and KIN combination gives more response in seeds. Recent advances in plant cell and tissue culture technology have opened up many new avenues for basic genetic research on higher plants at the cellular level and provided powerful tools in the hands of plant breeders for generating, selected and propagation of novel and economically important plant varieties. Functional genomics and biotechnological related approaches would play more and more important roles in the future for improvement of grountnut protein content/quality, oil content/quality as well as abiotic/biotic stress tolerance plants.

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