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Research Article

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Effect of Phytoregulators on *in vitro* micropropagation of *Arachis hypogea* (L.)

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ABSTRACT

A protocol has been developed for micropropagation of Arachis hypogea L. under in vitro conditions in south-east Rajasthan. Shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. The induction of multiple shoots from nodal segments was highest in MS medium supplemented with 1.0 BAP, 3.0Kn, 1.0NAA and 0.5 mg/l NAA+2.0 mg/l BAP. For rooting different concentration of IBA were used and maximum rooting was recorded on MS medium with 1.0 mg/l IBA. The rooted Plantlets were hardened initially in culture room conditions and then transferred to misthouse. Leaf petiole explants were used for the purpose of callus induction. Best growth was observed in MS medium supplemented with 3.0 mg/l 2,4-D.

Keywords: *- Arachis hypogea L., tissue culture, in vitro, plant regeneration.*

INTRODUCTION

Arachis hypogea L. or peanut (Groundnut) is an important oil; food and fodder yielding crop plant. It plays an important role in the agricultural economies of semi-arid tropical countries. It contributes significantly to food security and alleviates poverty [1], and as a legume it improves soil fertility by fixing molecular nitrogen of rhizosphere and increases productivity for smallholder farmers of the semi-arid cereal cropping systems .[2] Groundnut is a 30 to 50 cm (0.98 to 1.6 ft) tall annual herb. Synonymously it is also known as earthnuts, ground nuts, goober peas, monkey nuts, pygmy nuts and pig nuts. It is one of the major edible oil seed and protein rich leguminous crops in special reference to south-east Rajasthan .[3] It is cultivated on over 20 million hectors in over 108 tropical and subtropical countries, with an annual yield of seeds estimated 28 million tons.[4]It is an oil, food and fodder crop which plays an important role in the agricultural economies of countries of the semi-arid tropics. [5] Micropropagation can be achieved by using different parts of plant as explant. For this purpose various parts like apical meristem, nodal bud, shoot buds, axillary buds can be used as explant. Productions of somatic embryos by the well-known process of somatic embryogenesis this plant can also be micropropagated with the aims of production of large number of plantlets and propagation of the selected genotypes without inducing any genetic variation. A lot of research work has been carried out on nodal culture of several plants. [6, 7]

Effective explant sterilization is the first and must step for a successful tissue culture protocol. To conduct this exercise a comparatively simple and fast protocol using commercial bleach (sodium hypochlorite, NaOCl) was evaluated for explant sterilization was applied at the place of mercuric chloride ($HgCl_2$) which is mostly used in reported groundnut tissue culture studies [8]. In the other economic importance it is also used to make lactose-free milk-like beverage “Peanut milk”; their tops are used for hay [9]; the residual protein cake (oilcake meal) is used as an animal feed and as a soil fertilizer; low grade peanuts are sold as a garden bird feed .[10]

Present study is through focus on to micropropagation of *Arachis hypogea* L. It is necessary to device a rapid and efficient protocol for obtaining true type regenerants without

detriment to the survival of mother plant and saving its populations from getting rarer in nature.

MATERIAL AND METHODS

The branches (about 5-6 cm) of shoots of *Arachis hypogea* plants were collected from the Herbal Garden, Kota. The branches with node explants were washed in running tap water and then washed again thoroughly by adding a few drops of Tween-20 to remove the superficial dust particles as well as fungal and bacterial spores. They were surface sterilized with 0.1% $HgCl_2$ for 5 min followed by rinsing them five times with double distilled water inside the Laminar Air flow chamber. Nodal segments (with a single axillary bud) about 0.5-0.8 cm were prepared aseptically and were implanted vertically on MS medium prepared with specific concentrations of BAP, Kn (1.0-5.0 mg/l) singly or in combination were used for shoot proliferation. Same experiments were repeated for shoot multiplication.

The medium containing 3% sucrose was solidified with 0.8% agar (Qualigens). The *p*H of the media was adjusted to 5.9±0.02 with 1 N NaOH or 1 N HCl solutions prior to autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 121°C and 15 psi for 15-20 min. The cultures were incubated under controlled conditions of temperature (25±2°C), light (2000- 2500 lux for 16 h/d provided by fluorescent tubes) and 60-70% humidity. For each experiment a minimum of 7 replicates were taken and experiments were repeated thrice. Observations were recorded after an interval of 3 wk. Once culture conditions for shoot induction from explants were established, the shoots produced *in vitro* were subcultured on fresh medium every 3 wk. The nodal and shoot tip explants were inoculated in various concentrations and combination of BAP and Kn. Among these, the maximum number of shoots (3.42±0.39) was developed on MS media fortified with 0.5 BAP+3.0 Kn. Maximum shoot length was observed as 7.54±0.31cm.of a medium supplemented with 0.5 BAP+3.0 Kn. Rooting of elongated shoots was attempted under *in vitro* conditions. Auxins (IBA) alone in different concentrations (0.5-2.5 mg/l) were incorporated in the agar (0.8%) solidified medium containing 1/4 MS salts and 1.0% sucrose. The *in vitro*-rooted plantlets were transferred to culture bottles 1/4th filled with Soilrite composition (soil: sand: peat moss) and irrigated with 1/4 MS salt solution. These bottles were kept in controlled environmental conditions of culture room. After 3 wk of growth, the plantlets were transferred to misthouse for further growth.

RESULTS AND DISCUSSION

The nodal explants, when inoculated on MS medium containing BAP and Kn in the range 1.0-5.0 mg/l showed enhanced shoot proliferation. BAP at its 1.0 mg/l concentration evoked best response. Shoots after their initial proliferation on medium containing 1.0 mg/l BAP were sub-cultured on same fresh medium after every 21 days. Incorporation of BAP , Kn or NAA into MS medium supported multiplication of shoots in culture, NAA proved to be a better choice than BAP and Kn and the maximum number of shoots were obtained on its 1.0 mg/l concentration (Table 1& 3 , Fig. 1- A, B,D, Fig. 2, 4). When BAP was used in combination with NAA a variety of responses was observed (Table 2, Fig. 1-C, Fig.3). But best response was observed on medium containing 0.5 mg/l NAA + 2.0 mg/l BAP (Average number of shoots 3.52±0.39, shoot length 7.34±0.30 cm). The full or half strength of MS medium without any PGR was failed to induce rooting of regenerated shoots. However, shoots were capable to induce root when cultured on medium containing auxins.

Auxins in different concentration induced rooting when incorporated in the medium containing ¼ of MS salts. The best rooting response, however, was observed on medium containing 1.0mg/l IBA, where roots measuring 4.66±0.22 cm (average) were formed (Table 5, Fig. 1-E, Fig. 5). *In vitro* rooted plantlets were initially hardened in culture room conditions where leaves expanded. After 3 weeks, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly. Leaf petiole explants were used for the purpose of callus induction. Highest diameter of callus was observed on MS Medium fortified with 3.0 mg/l

2,4-D (callus diameter 4.60 cm), (Table 4, Fig. 1-f).

Table-1: Effect of Cytokinin (BAP and Kn) on shoot proliferation from Nodal shoot explant of *Arachis hypogea*

Hormone Con. (mg/ l) BAP	Hormone Con. (mg/ l) Kn	Response (%)	No. of Shoot/explant (mean±SE)	Shoot length (in cm) (mean±SE)
1.0	-	80	3.42±0.58	7.51±0.76
2.0	-	70	2.28±0.71	6.56±0.84
3.0	-	65	2.71±0.56	6.62±0.53
4.0	-	55	3.28±0.36	5.08±0.51
5.0	-	40	2.85±0.51	3.31±0.33
-	1.0	55	2.28±0.26	6.17±0.29
-	2.0	60	2.42±0.29	6.19±0.26
-	3.0	75	2.85±0.27	6.30±0.24
-	4.0	40	1.57±0.31	5.70±0.31
-	5.0	30	1.28±0.36	4.92±0.23

Medium: MS+ additives; mean± SE, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-2: Interactive effect of Cytokinin (NAA+BAP) on shoot multiplication by Subculture of shoot clumps of *Arachis hypogea*

Hormone Con. (mg/ l)	No. of Shoot/explant (mean±SE)	Shoot length (in cm) (mean±SE)	Shooting Response (%)
0.5 NAA + 0.5 BAP	1.71±0.38	3.70±0.28	70
0.5 NAA + 1.0 BAP	2.24±0.51	4.71±0.29	80
0.5 NAA + 2.0 BAP	3.52±0.39	7.34±0.30	90
0.5 NAA + 3.0 BAP	2.75±0.36	5.40±0.31	85
0.5 NAA + 4.0 BAP	2.67±0.40	6.30± 0.29	82

Medium: MS+ additives; mean± SD, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-3: Interactive effect of Cytokinin (NAA) on shoot multiplication by Subculture of shoot clumps of *Arachis hypogea*

Hormone Con. (mg/ l)	No. of Shoot/explant	Shoot length (in cm)	Shooting Response (%)
1.0 NAA	4.98±0.74	3.06±0.22	90
2.0 NAA	3.33±0.43	3.97±0.38	85
3.0 NAA	3.31±0.48	3.87±0.36	80
4.0 NAA	3.16±0.57	2.08±0.22	75
5.0 NAA	2.56±0.24	2.68±0.51	70

Medium: MS+ additives; mean± SD, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

Table-4: Effect of different Hormones on Callus proliferation and Morphology of *Arachis hypogea*

Hormone Con. (mg/ l)	Callus proliferation Scoring	Response (%)	Callus diameter after 4 weeks subculture (cm)
0.5 2,4-D	++	80	3.65
1.5 2,4-D	++	85	3.70
2.0 2,4-D	+++	86	3.71
2.5 2,4-D	+++	90	4.36
3.0 2,4-D	++++	95	4.60

'++++' Intense, '+++' Moderate, '++' Meager

Table-5: Effect of Auxin (IBA) on root induction from isolated shoot of *Arachis hypogea*

Hormone Con. (mg/ l)	No. of roots/explants (mean±SE)	Rooting Response (%)	Root length (in cm) (mean±SE)
0.5 IBA	3.56±0.34	83	4.31±0.28
1.0 IBA	3.98±0.24	85	4.66±0.22
1.5 IBA	3.71±0.38	80	3.37±0.34
2.0 IBA	2.88±0.17	75	3.06±0.25
2.5 IBA	2.76±0.26	73	2.73±0.53

Medium: MS+ additives; mean± SE, n= 7 replicates
Means having the same letter in each Colum, do not different significantly at P< 0.05 (Tukey's test)

CONCLUSION

The seedlings derived from explants, being juvenile, are frequently used for micropropagation, as they are easy to establish in culture. In *Arachis hypogea*, MS medium containing 1.0 mg/l NAA was the best for culture initiation. We have found that *Arachis hypogea* culture grew better on MS medium in comparison to other media. In *Arachis hypogea* 1.0 mg/l NAA was most suitable for shoot multiplication. We also observed improvement in shoot multiplication by different concentrations of BAP (0.5-4.0 mg/l) in medium along with NAA (0.5 mg/l). Best shooting response was observed on media containing 0.5 mg/l NAA+ 2.0 mg/l BAP (Average number of shoots 3.2±0.39, Average shoot length 7.34±0.30 cm). IBA (Auxin) has been widely used as root induction hormone under *in vitro* and *in vivo* condition. We also found positive role of IBA during *in vitro* rooting. In *Arachis hypogea*, 1.0mg/l IBA proved to be best for *in vitro* rooting. The *in vitro* rooted plants were hardened first under controlled conditions of culture room and then shifted to misthouse where they exhibited growth with 90% survival. Most responsive callus induction was observed on MS medium supplemented with 3.0 mg/l 2, 4-D.

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Figure 1 (A-G) Micropropagation of *Arachis hypogea* from nodal shoot explants

A. Shoot multiplication on MS medium supplemented with 1.0 mg/l BAP, B. Shoot multiplication on MS medium supplemented with 3.0 mg/l Kn, C. Shoot multiplication on MS medium supplemented with 0.5 mg/l NAA+2.0 mg/l BAP, D. Shoot multiplication on MS medium supplemented with 1.0mg/l NAA, E. Root Proliferation on Ms medium with 1.0mg/l IBA, F. Callus induction on Ms medium supplemented with 3.0mg/l 2,4-D, G. Hardening of plant

Figure-2: Effect of cytokine (BAP and Kn) on shoot proliferation from nodal shoot explants of *Arachis hypogea*

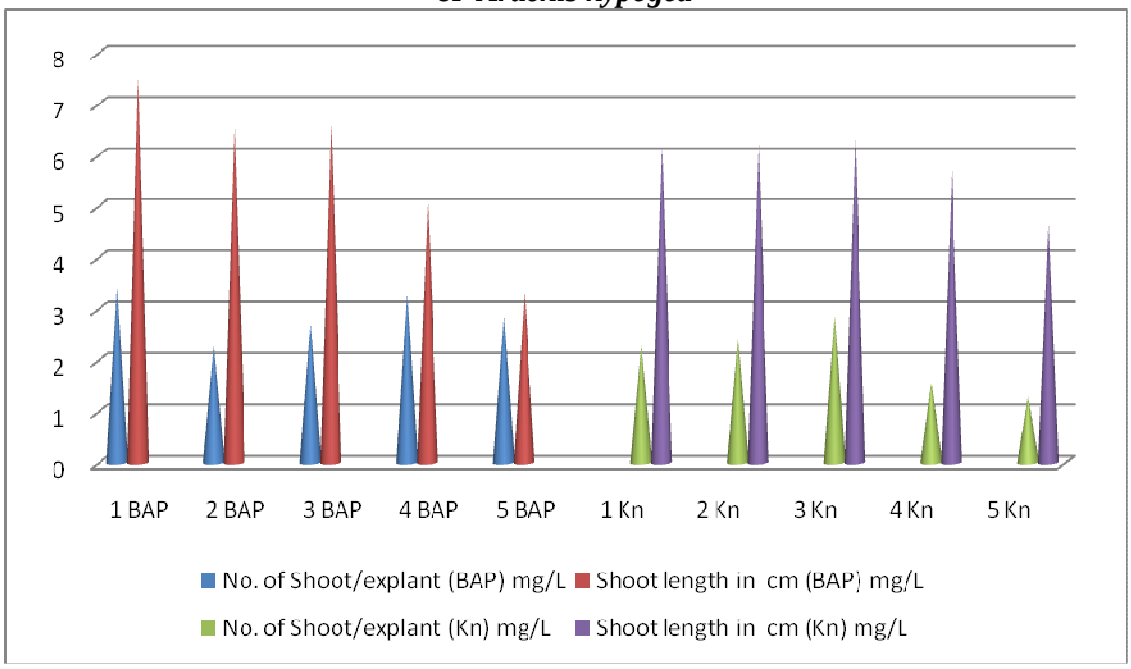


Figure-3: Effect of cytokine (NAA+BAP) on shoot proliferation from nodal shoot explants of *Arachis hypogea*

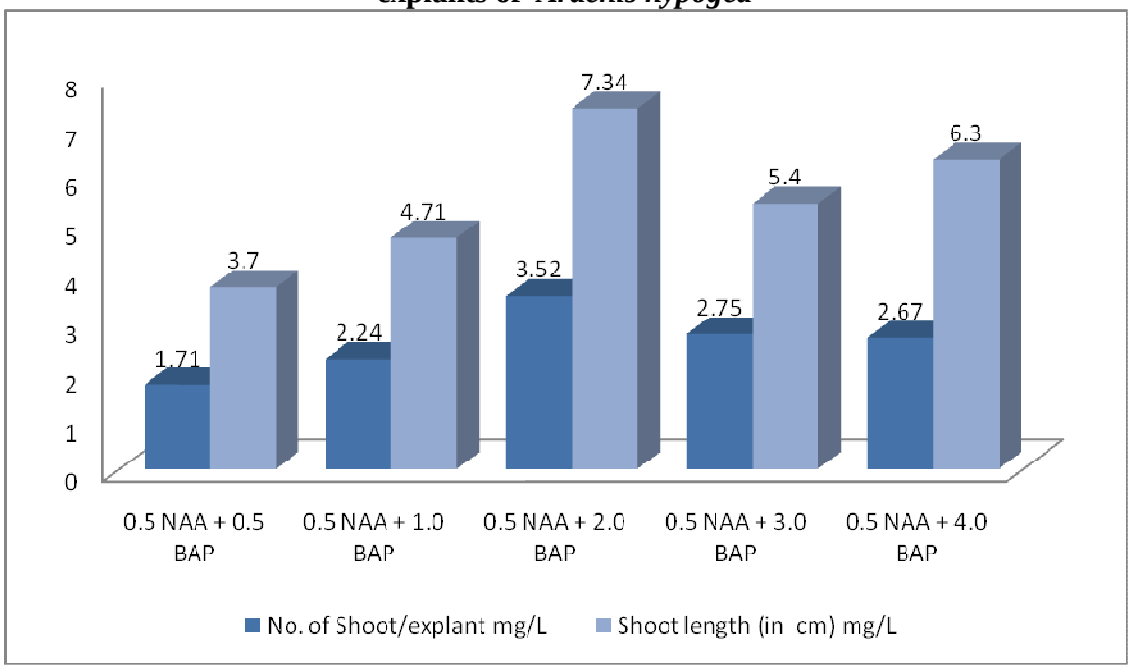


Figure-4: Interactive effect of cytokine (NAA) on shoot multiplication by subculture of shoot clumps of *Arachis hypogea*

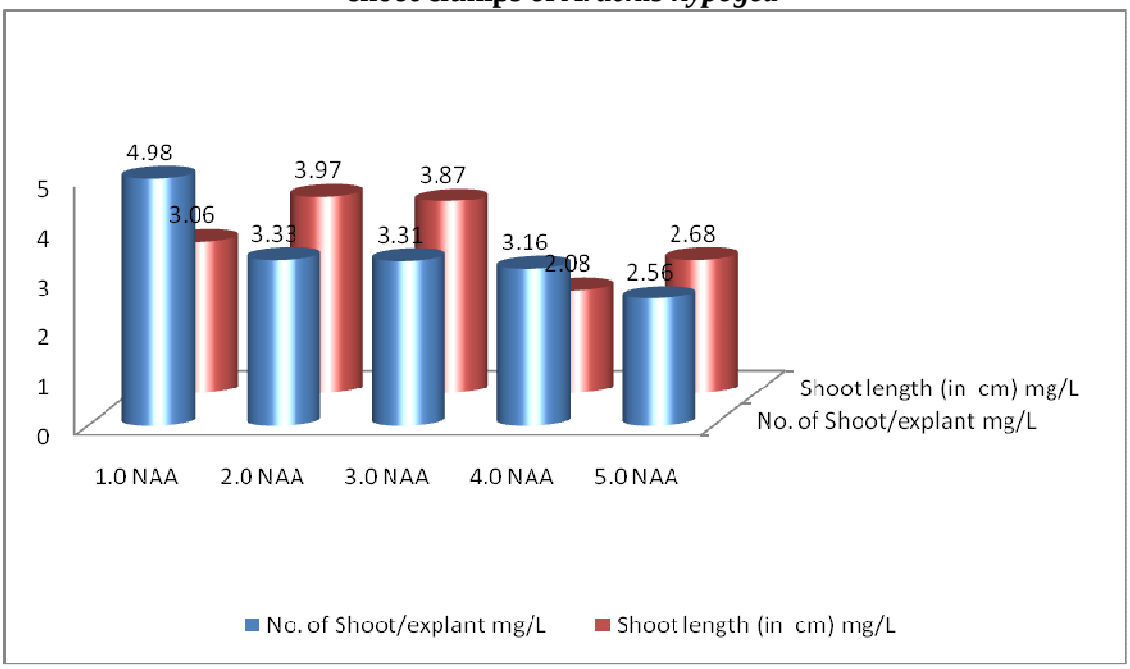
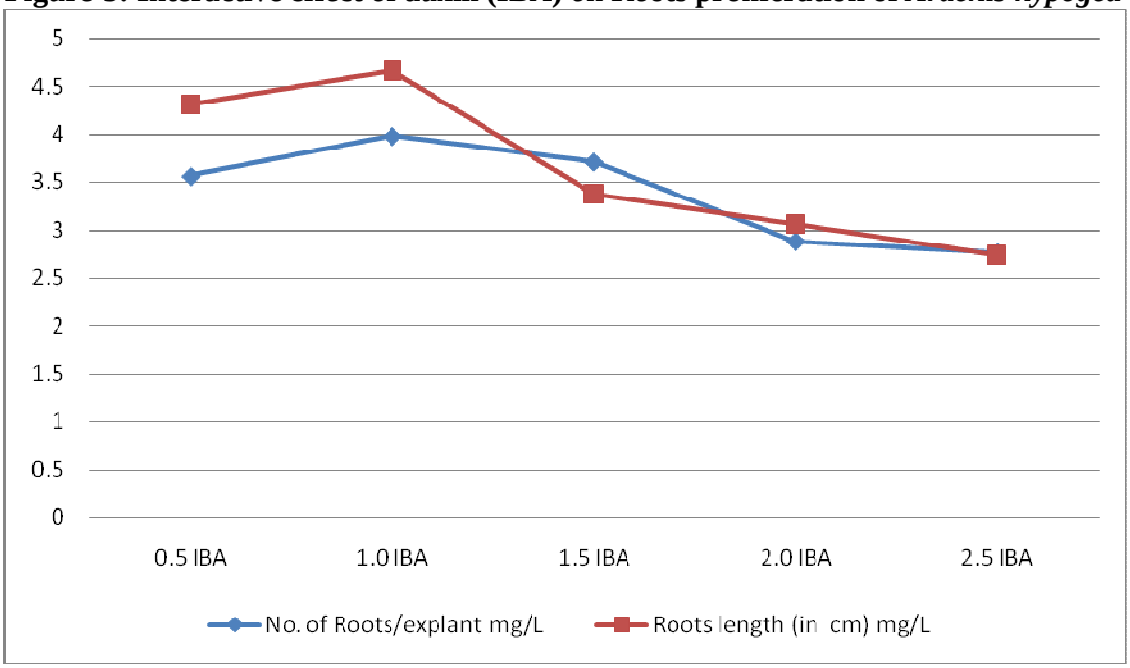


Figure-5: Interactive effect of auxin (IBA) on Roots proliferation of *Arachis hypogea*



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