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Research Article

RAPID *IN VITRO* PROPAGATION OF DIFFERENT VARIETIES OF *ARACHIS HYPOGAEA* L. THROUGH SHOOT APEX CULTURE

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ABSTRACT
Rapid clonal propagation through *in vitro* techniques using shoot apex as an explant has been attempted in the high yielding, tikka-susceptible (ICG 11337, AK 1224, ICGS 44, JL 24) and tikka-immune (ICG 6284) varieties of *Arachis hypogaea* L. Shoot apices were excised from the 12–15 days-old axenic seedlings and cultured in the presence of various concentrations of N6 –benzylaminopurine (BAP) (1, 5, 10, 15, 25 and 50 mg l^{-1}). BAP-free control showed very little or no sign of multiplication in terms of multiple shoot, axillary branch and shoot bud formation. In the presence of higher concentrations of BAP, cultured explants showed the development of multiple shoots, axillary branches and shoot buds. Thus, a tri-directional multiplication pathway (multiple shoot, axillary branch and shoot bud) in a single medium has been achieved in the tested varieties of *Arachis hypogaea* L. The regeneration of maximum number of shoot, axillary branches and shoot buds from the excised shoot apices and their corresponding requirements for BAP differed across varieties, which might be due to variation at their genotypic levels. The isolated shoots were quickly rooted in the presence of a-naphthalene acetic acid (NAA) (1 mg l^{-1}) and well-rooted plantlets were successfully transferred to the soil following a standard hardening protocol.

Keywords: *Genotype, Plant Growth Regulator, Shoot Apex, Arachis hypogaea, In Vitro Regeneration, Clonal Propagation*

Abbreviations: BAP– N6 –benzylaminopurine, NAA– *a*-naphthalene acetic acid, PGR – plant growth regulator

INTRODUCTION
The major breeding objectives of the groundnut programme are to develop varieties with high yield and quality, earliness, resistance to major pests, diseases, drought, salt and cold as well as higher protein and oil contents. Conventional methods for improving the groundnut crop have been inadequate in achieving such objectives (Murthy and Reddy, 1993).

Tools of genetic engineering are being exploited for the improvement of the crop plants. The most essential requirement for the production of transgenic plants is the availability of a reproducible protocol for the regeneration of complete plants. Regeneration can be effected either by primary organogenesis (Mroginsky *et al*., 1981; Banerjee *et al.*, 1988; Mc Kently *et al.*, 1991) or by indirect organogenesis (Bajaj *et al.*, 1981) through the development of shoots from the callus tissue. Regeneration can also occur through somatic embryogenesis (Ozias-Akins, 1989; Sellars *et al.*, 1990). Tissue culture response in the groundnut is strongly influenced by the plant genotype (Mroginsky *et al.*, 1981; Mc Kently, 1991; Mc Kently *et al.*, 1991; Banerjee *et al.*, 2007; Banerjee, 2013), the PGR levels of the culture medium (Mroginsky *et al.*, 1981; Mc Kently *et al.*, 1990, 1991), as well as the age of the explant source (Mroginsky *et al.*, 1981).

Although *in vitro* studies in this crop have been attempted by several workers at different times, detailed studies on the morphogenetic development patterns of shoot apex culture with reference to its multiplication potential and the role of genotype on the culture response are still inadequate.

Therefore, the present communication, deals with a tridirectional multiplication pathway (direct shoot formation, axillary branching and shoot bud formation) through the shoot apex culture of both high yielding (ICG 11337, ICGS 44, JL 24, AK 1224) and the tikka-immune (ICG 6284) varieties of *Arachis hypogaea*.

MATERIALS AND METHODS

Seeds of groundnut (*Arachis hypogaea* L.) varieties such as ICG 11337 (high yielding tikka-susceptible variety obtained from ICRISAT, Hyderabad), ICG 6284 (tikka-immune, obtained from ICRISAT, Hyderabad), AK 1224 (high yielding, tikka-susceptible, obtained from West Bengal State Seed Corporation, Midnapore), ICGS 44 (high yielding, tikka-susceptible obtained from BCKV, Jhargram) and JL 24 (high yielding, tikka-susceptible obtained from West Bengal State Seed Corporation, Burdwan) were selected as the experimental materials. Freshly collected healthy seeds were washed with few drops of liquid soap (Teepol) for 5 min, after which the surface was disinfected by 90% ethanol (v/v) for 2 minutes, followed by treatment with 0.1% (w/v) mercuric chloride solution for 5–6 min and finally washed repeatedly with sterile distilled water. The seeds were then aseptically germinated on a moistened cotton bed in a 250 ml conical flask and incubated in the culture room for germination and subsequent development into complete seedlings. From the 12–15 day- old seedlings cotyledonary nodes were excised and utilized for the initiation of cultures (Banerjee *et al.,* 2007). Shoot apices were inoculated into culture tubes each containing MS semisolid basal medium (Murashige and Skoog, 1962) solidified with 0.8% (w/v) agar (BDH, India) and supplemented with various concentrations of BAP (1,5, 10, 15, 25 and 50 mg l^{-1}).

The control set was devoid of any PGR. The pH of the medium was adjusted to 5.6–5.8 prior to autoclaving. The cultures were incubated at 25 ± 2 °C under a 10 hr photoperiod of 37.5 m mol m^{-2} s^{-1} light intensity. Direct shoots, axillary branches and shoot buds were counted after 45 days of incubation. The sum total of number of shoots, axillary branches and shoot buds was collectively considered as the multiplication potential of the particular explant. In vitro- grown shoots were rooted in MS medium containing NAA (1mg l^{-1}).

Regarding the transfer of in vitro plants to field conditions, around 40–50 well-rooted plantlets of each variety were taken out from the culture environment, hardened following a standard hardening protocol (Ghosh and Banerjee, 2003) and transferred to pots containing a sterile sand-soil mixture. After the establishment of these plantlets in the pots, which was visualized by emergence of new leaf, they were finally transferred to the experimental garden.

RESULTS AND DISCUSSION

Results

In all the treatments, including the BAP-free control, 100% shoot development was observed in all the varieties from shoot apex. All these varieties exhibited a general tendency to develop multiple shoots, axillary branches and shoot buds (Figure l A) with the increase in the BAP level up to a certain extent except the variety ICGS 44 where no axillary branching occurred. A higher level of BAP proved to be inhibitory to multiple shoot development, axillary branch formation and shoot bud development. In spite of these similarities of response in different varieties, they showed a striking difference in BAP requirement for optimum response in terms of direct shoot development, axillary branch formation and shoot bud development (Figure 1 B).

It was generally observed that both BAP-free control and lower concentration of BAP failed to generate axillary branching. Axillary branching was observed at relatively higher concentrations of BAP. As far as the production of shoot buds is concerned, the highest numbers were achieved at 25 mg l^{-1} BAP in the varieties ICG 11337, AK 1224, JL 24. However, ICGS 44 showed maximum shoot bud production (1.9 ± 1.07 per explants) at 15 mg l^{-1} BAP and ICG 6284 showed at 50 mg l^{-1} BAP. Multiplication potential of shoot apex explants in five varieties of *Arachis hypogaea* L. (Table 1) revealed evidence of intra-variety differences among the five varieties.

Isolated shoots of all these five varieties of *Arachis hypogaea* showed induction of roots when subcultured in the presence of NAA (1 mg l^{-1}) (Figure 1 C). The survival percentages of the plantlets of the varieties ICG 11337, AK 1224, ICGS 44, JL 24 and ICG 6284 in the field condition were recorded as 80.0, 83.33, 72.0, 73.33 and 84.0 % respectively (Figure 1 D). All the varieties grew normally and set viable seeds in the experimental garden.

Table- 1: Response of Shoot Apex of Different Varieties of *Arachis Hypogaea* L. Cultured on MS Agar Media Supplemented with Various Concentrations of BAP after 45 Days of Incubation

Variety	BAP (mg/l)	Mean No. of Shoots ± SE (A)	Mean No. of Axillary Branches ± SE (B)	Mean No. of Shoot Buds ± SE (C)	Multiplication Potential per Explant ± SE (A+B+C)
ICG 11337	0	1.0 ± 0	0	0	1.0 ± 0
	1	1.2 ± 0.13^{d}	0	0	1.20 ± 0.13^{d}
	5	2.3 ± 0.21^{c}	1.3 ± 0.21	5.30 ± 0.83	8.90 ± 0.83^{c}
	10	3.8 ± 0.41^{b}	2.6 ± 0.49	8.10 ± 1.05	14.5 ± 0.95^{b}
	15	5.4 ± 0.37^{a}	3.1 ± 0.48	8.60 ± 0.97	17.1 ± 1.19^{ab}
	25	4.0 ± 0.33^{b}	2.9 ± 0.43	11.5 ± 1.00	18.4 ± 1.27^{a}
	50	2.3 ± 0.26^{c}	0.6 ± 0.26	7.20 ± 1.35	10.1 ± 1.51^{c}
AK 1224	0	1 ± 0^{d}	0	0	1 ± 0^{d}
	1	1.3 ± 0.3^{d}	0	0	1.3 ± 0.3^{d}
	5	1.8 ± 0.24^{cd}	0.6 ± 0.4	0	2.4 ± 0.49^{cd}
	10	5.0 ± 0.81^{a}	0.5 ± 0.22	1.8 ± 1	7.3 ± 1.19^{a}
	15	3.8 ± 0.64^{ab}	0.8 ± 0.8	2.6 ± 0.97	7.2 ± 1.27^{ab}
	25	3.3 ± 0.85^{bc}	1.0 ± 0.63	3.3 ± 1.75	7.6 ± 1.6^{a}
	50	2.9 ± 0.34^{bc}	0	1.7 ± 0.63	4.6 ± 0.54^{bc}
ICGS 44	0	1.3 ± 0.21^{c}	0	0	1.3 ± 0.21^{c}
	1	1.6 ± 0.26^{c}	0	0	1.6 ± 0.26^{c}

	5	2.5 ± 0.37^{bc}	0	0	2.5 ± 0.37^{c}
	10	3.1 ± 0.58^{ab}	0	0	3.1 ± 0.58^{bc}
	15	4.0 ± 0.57^{a}	0	1.9 ± 1.07	5.9 ± 1.11^{a}
	25	3.8 ± 0.44^{ab}	0	1.2 ± 0.91	5.0 ± 0.94^{ab}
	50	2.5 ± 0.71^{bc}	0	0.7 ± 0.47	3.2 ± 0.47^{bc}
JL 24	0	1.0 ± 0^{c}	0	0	1.0 ± 0^{b}
	1	1.50 ± 0.34^{c}	0.50 ± 0.34	0	2.00 ± 0.36^{b}
	5	2.50 ± 0.75^{bc}	0	0.62 ± 0.37	3.12 ± 2.96^{b}
	10	3.60 ± 0.26^{ab}	1.00 ± 0.68	2.30 ± 1.3	6.90 ± 1.28^{a}
	15	4.50 ± 0.16^{a}	0.10 ± 0.1	2.20 ± 1.06	6.80 ± 1.04^{a}
	25	2.12 ± 0.71^{c}	1.50 ± 0.50	3.50 ± 1.19	7.12 ± 1.27^{a}
	50	2.40 ± 0.60^{bc}	0	0	2.40 ± 0.60^{b}
ICG 6284	0	1.0 ± 0^{f}	0	0	1.0 ± 0^{e}
	1	1.6 ± 0.22^{ef}	0	0	1.6 ± 0.22^{e}
	5	2.7 ± 0.36^{de}	0.7 ± 0.26	0	3.4 ± 0.30^{d}
	10	3.3 ± 0.30^{cd}	1.0 ± 0.29	1.2 ± 0.32	5.5 ± 0.40^{c}
	15	5.0 ± 0.53^{ab}	1.3 ± 0.33	1.9 ± 0.37	8.2 ± 0.46^{a}
	25	5.3 ± 0.61^{a}	1.2 ± 0.38	2.4 ± 0.49	8.9 ± 0.43^{a}
	50	3.9 ± 0.43^{bc}	0	3.0 ± 0.64	6.9 ± 0.70^{b}

Mean values followed by same letter are not significantly different at 0.05 level (DMRT)

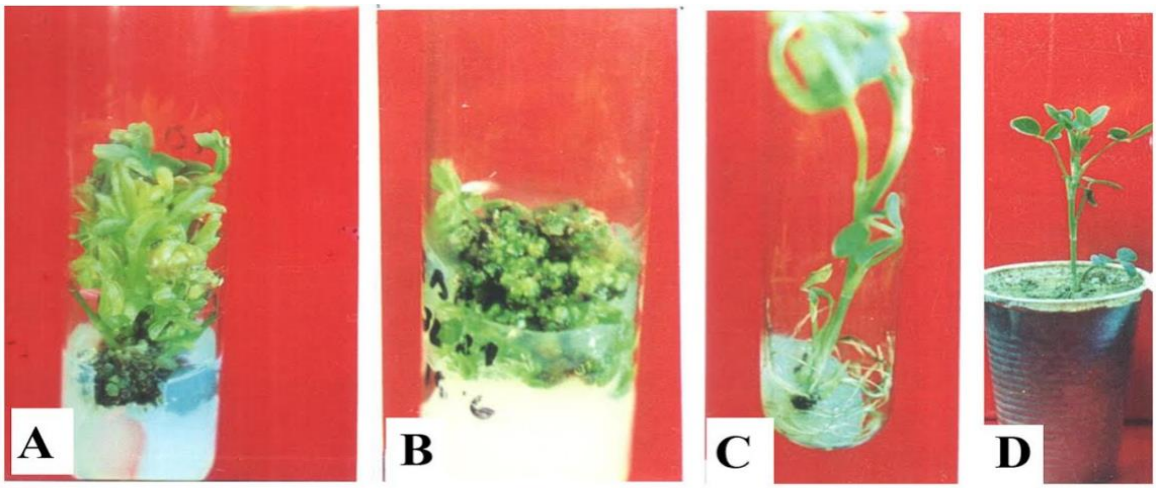

Figure- 1

Figure 1 A- D: *In Vitro* Propagation of *Arachis hypogaea* L. using Shoot Apex Explants

A. Multiple Shoots from Shoot Apex Explant
B. Shoot Buds Produced at Higher Concentrations of BAP
C. Induction of Roots
D. **Plantlet Established in Plastic Pot**

Discussion

BAP as cytokinin for in vitro multiplication and NAA as the auxin for induction of roots were used primarily due to the fact that BAP and NAA are very effective and less expensive plant growth regulators and can safely be autoclaved (Zaerr and Mapes, 1982; Thomas and Blakesley, 1987). Generally, a substantial amount of IAA, on the other hand, is degraded during the sterilisation of the culture medium in autoclave (Nissen and Sutter, 1988). 2,4-D, being a phenoxy auxin, promoted quick callus formation from the induced roots and also at the base of the shoots (Bonga and Aderkas, 1992), which is not desirable for successful transfer of in vitro-grown plants to the field. Therefore, 2, 4-D and IAA were purposely avoided for the induction of roots in the present study.

The response of the shoot apex explants of the varieties ICG 11337, AK 1224, ICGS 44, JL 24 and ICG 6284 revealed that the optimum number of shoots was generated (5.4 ± 0.37 shoots/explant) in ICG 1137 at 15 mg l^{-1} BAP followed by ICG 6284 (5.3 ± 0.61 shoots / explant at 25 mg l^{-1} BAP), AK 1224 (5.0 ± 0.81 shoots / explant at 10 mg l^{-1} BAP), JL 24 (4.50± 0.16 shoots / explants at 15 mg l^{-1} BAP) and ICGS 44 (4.0 ± 0.57 shoots / explant at 15 mg l^{-1} BAP). Further increase in BAP level in the medium did not reveal any significant enhancement in multiple shoot formation.

However, the effect of BAP concentration on shoot multiplication exhibited striking differences. It is clearly evident from the present study that such differential cytokinin requirements by varieties of *Arachis hypogaea* for shoot proliferation could be primarily due to their genotypic variation, which corroborated the findings of Radhakrishnan *et al.*, (1996). BAP alone could induce multiple shoot formation in *Gossypium hirsutum* (Banerjee *et al.*, 1999) and a low concentration of BAP was more effective in inducing multiple shoots in *Populus* (Agarwal and Gupta, 1999). So far as the development of axillary branches is concerned ICG 11337 was found to be the most efficient at 15 mg l^{-1} BAP followed by JL 24, requiring 25 mg l^{-1} BAP. Among the remaining varieties, AK1224 and ICG 6284 required 25 and 15 mg l^{-1} BAP respectively for the production of maximum axillary branching. However, ICGS 44 failed to generate any axillary branching.

So far as the production of the shoot bud is concerned, the number varied significantly among the varieties and the increase in the number of shoot bud generation could be directly related to the increasing

BAP levels in the media. These multiplications took place both by adventitious as well as by axillary shoot bud proliferation. In general, it is known that in an intact plant the apical bud exerts an inhibitory influence on axillary buds, preventing their development into leafy shoots (Street and Opik, 1986). Moreover, the adventitious production of multiple shoot buds could be directly controlled by the exogenous cytokinin concentration in *Rauvolfia tetraphylla* (Vishwanath and Jayanthi, 1997; Ghosh and Banerjee, 2003) *Vigna radiata* (Gulati and Jaiwal, 1994), *Canavalia virosa* (Kathiravan and Ignacimuthu, 1999) and *Lippia alba* (Gupta *et al.*, 2001).

A very high concentration of BAP in general showed an inhibitory effect on shoot bud proliferation in *Vigna radiata* (Badere *et al.*, 2002). The response in terms of shoot multiplication in the peanut variety GN 2 was not encouraging in the media fortified either with

NAA or BAP alone (Banerjee *et al.*, 1988). According to these authors the auxin: cytokinin ratio was crucial for the regeneration of multiple shoot buds in the groundnut. In the present study, on the contrary, BAP alone was capable of inducing proliferation of shoot buds in all the five varieties. However, the present findings supported the observations of Vishwanath and Jayanthi (1997) and Banerjee *et al.*, (1999 and 2007). The shoot multiplication potential of the five varieties of *Arachis hypogaea* revealed that the same differed at the varietal levels and the requirements of BAP for achieving optimum response differed markedly. Such variable response of different varieties in culture might be due to their differential genomic constitution (Radhakrishnan *et al.*, 1994; Banerjee *et al.*, 2007). This was also supported by the observations of Illingworth (1968), who succeeded in regenerating plantlets from cryopreserved tissues only in 2 out of 11 genotypes of groundnut.

The regenerative response of immature leaflet cultures of groundnut showed shoot multiplication only in six out of forty seven genotypes (Seitz *et al.*, 1987). Such differential response could also be due to different levels of endogenous PGRs within the explants. Levels of endogenous growth regulators in the explants are influenced by the duration of light, its quality and the intensity and also by the chemical environmental factors (Kefeli, 1978). Further, the effect of a particular PGR depended not only on the concentrations applied, but also on the presence of the other PGRs as well as its interaction with endogenous growth regulators (Roy and Banerjee, 2000).

In conclusion, the findings of the present study are of considerable significance, since it has described a tri-directional micro propagation technique in a single medium. Therefore, the results obtained here could be useful in improving this economically valuable crop.

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