Acta Bot. Croat. 66 (1), 15–23, 2007

CODEN: ABCRA 25
ISSN 0365–0588

Influence of genotype on *in vitro* multiplication potential of *Arachis hypogaea* L.

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Rapid clonal propagation through *in vitro* techniques using cotyledonary node 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. Cotyledonary nodes were excised from the 12–15 days-old axenic seedlings and cultured in the presence of various concentrations of N^6–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 shoots, axillary branches and shoot buds from the excised cotyledonary nodes 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 α-naphthalene acetic acid (NAA) (1 mg L^{-1}) and well-rooted plantlets were successfully transferred to the soil following a standard hardening protocol.

Key words: Genotype, plant growth regulator, cotyledonary node, *Arachis hypogaea*, *in vitro* regeneration, clonal propagation.

Abbreviations: BAP – N^6–benzylaminopurine, NAA – *a*-naphthalene acetic acid, PGR – plant growth regulator, ANOVA – analysis of variance

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

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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, MCKENTLY 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, MCKENTLY 1991, MCKENTLY et al. 1991), the PGR levels of the culture medium (MROGINSKY et al. 1981, MCKENTLY 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 cotyledonary node 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 cotyledonary node 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. Cotyledonary nodes 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 μmol 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. Two-way ANOVA for unequal observation per cell was performed to infer the significance of multiplication potential at varietal level (GOON et al. 1998). 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

In all the treatments, including the BAP-free control, 100% shoot development was observed in all the varieties from the lateral buds of the cotyledonary node. However, in the control only two shoots developed from the two lateral buds of the cotyledonary node in the varieties ICG 11337, JL 24 and ICG 6284 whereas the variety ICGS 44 showed a tendency to develop multiple shoots even in the BAP-free medium, 2.2 ± 0.13 shoots per explant. AK 1224 showed 1.4 ± 0.16 shoots per explant in the control medium. All these varieties exhibited a general tendency to develop multiple shoots, axillary branches and shoot buds (Fig. la) 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 except in the variety ICGS 44 where 50 mg L^{-1} BAP was necessary for regeneration of the maximum number of shoot buds. 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 (Fig. 2), axillary branch formation (Fig. 3) and shoot bud development (Fig. 4).

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

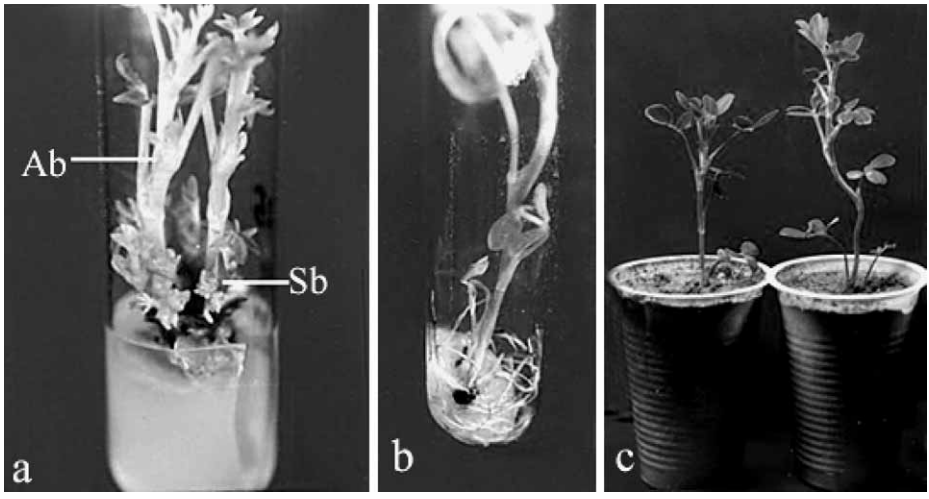

Fig. 1. Multiple shoot, axillary branch and shoot bud development from cotyledonary node explant of *Arachis hypogaea* (var. JL 24) (a). Regeneration of roots from single shoot (b). Transfer of plantlets to soil (c). Ab-axillary brach, Sb –shoot bud.

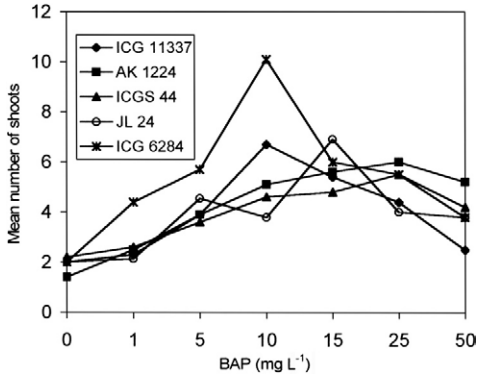

Fig. 2. Multiple shoot formation from cotyledonary node explant in five varieties of *Arachis hypogaea* L.

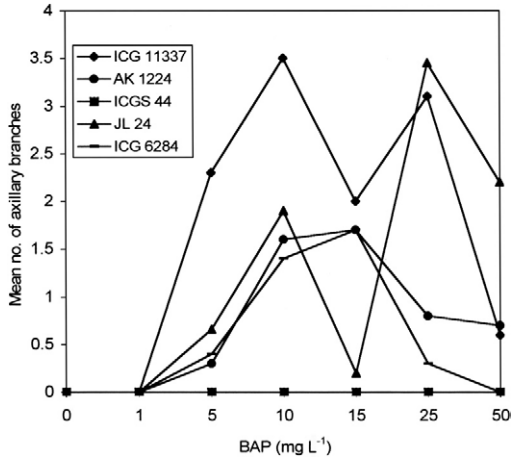

Fig. 3. Axillary branch formation from cotyledonary node explant in five varieties of *Arachis hypogaea* L.

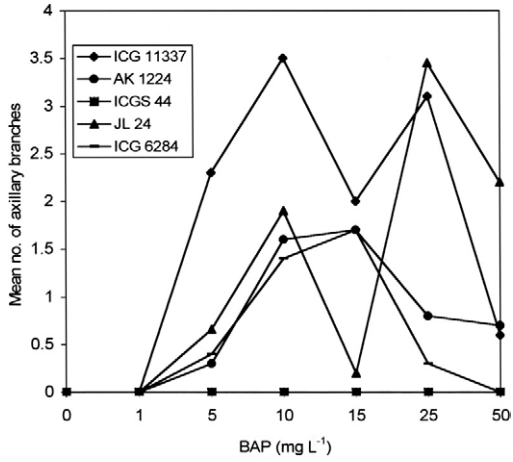

Fig. 4. Shoot bud development from cotyledonary node explant in five varieties of *Arachis hypogaea* L.

numbers were achieved at 25 mg L^{-1} BAP in the varieties ICG 11337, AK 1224, JL 24 and ICG 6284. However, ICGS 44 showed maximum shoot bud production (1.2 ± 0.62 per explant) at 50 mg L^{-1} BAP.

Multiplication potential of cotyledonary node explants in five varieties of *Arachis hypogaea* L. (Tab. 1) revealed evidence of intra-variety differences among the five varieties (Tab. 2, 3).

Tab. 1. Multiplication potential (multiple shoots + axillary branches + shoot buds) of cotyledonary node explant in five varieties of *Arachis hypogaea* L. after 45 days of incubation. Mean values followed by same letter are not significantly different at 0.05 level (Duncan's Multiple Range Test).

BAP (mg L^{-1})	Multiplication potential ± SE				
	ICG 11337	AK 1224	ICGS 44	JL 24	ICG 6284
0	2.0 ± 0.0^{c}	1.4 ± 0.16^{c}	2.2 ± 0.13^{e}	2.0 ± 0.00^{b}	2.0 ± 0.00^{e}
1	2.3 ± 0.2^{c}	2.5 ± 0.22^{c}	2.6 ± 0.22^{de}	2.1 ± 0.14^{b}	4.4 ± 0.49^{d}
5	8.9 ± 0.6^{b}	4.2 ± 0.35^{bc}	3.6 ± 0.45^{cd}	5.2 ± 1.03^{a}	6.5 ± 0.40^{bc}
10	16.3 ± 0.5^{a}	6.7 ± 1.05^{ab}	4.6 ± 0.47^{bc}	5.7 ± 1.10^{a}	12.2 ± 1.39^{a}
15	17.2 ± 0.8^{a}	7.8 ± 1.45^{a}	5.8 ± 0.29^{a}	7.5 ± 0.26^{a}	8.2 ± 0.48^{b}
25	18.6 ± 1.4^{a}	8.9 ± 1.51^{a}	6.5 ± 0.58^{a}	8.2 ± 0.94^{a}	7.6 ± 0.40^{b}
50	10.4 ± 1.6^{b}	6.7 ± 1.47^{ab}	5.4 ± 0.47^{ab}	6.0 ± 1.70^{a}	5.5 ± 0.63^{cd}

Tab. 2. ANOVA for multiplication potential of cotyledonary node explants of five varieties of *Arachis hypogaea*

Source of Variation	df	Sum Square	Sum Square	Source of Variation
Variety (Adjusted)	4	1859–2765	1757.0047	Variety (Unadjusted)
Dose (Unadjusted)	6	2846.5529	2948.8247	Dose (Adjusted)
Interaction (V x D)	24	1372.8797	1372.8797	Interaction (V x D)
Between Cells	34	6078.7091	6078.7091	Between Cells
Within Cells (Error)	300	1933.6389	1933.6389	Within Cells (Error)
Total	334	8012.348	8012.348	Total

Tab. 3. Table of significance

Source of Variation	$F_{obs.}$	$F_{tab.}$	
		5% level	1% level
Variety	45.54681667	2.41	3.40
Dose	48.15836626	1.14	2.88
Interaction (Variety x Dose)	8.8749747	1.52	1.79

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}) (Fig. 1b). 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 (Fig. 1c). All the varieties grew normally and set viable seeds in the experimental garden.

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 cotyledonary node explants of the varieties ICG 11337, AK 1224, ICGS 44, JL 24 and ICG 6284 revealed that the optimum number of shoots was generated (10.1 shoots/explant) in ICG 6284 at 10 mg L^{-1} BAP followed by JL 24 (6.9 shoots / explant at 15 mg L^{-1} BAP), ICG 11337 (6.7 shoots / explant at 10 mg L^{-1} BAP), AK 1224 (5.1 shoots / explant at 10 mg L^{-1} BAP) and ICGS 44 (4.6 shoots / explant at 10 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 10 mg L^{-1} BAP followed by JL 24, requiring 25 mg L^{-1} BAP. Among the remaining varieties, AK 1224 and ICG 6284 required 15 mg L^{-1} BAP 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).

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). 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 micropropagation technique in a single medium, which has not previously been reported. Therefore, the results obtained here could be useful in improving this economically valuable crop.

Acknowledgements

The financial assistance from the Department of Biotechnology, Government of India, New Delhi is gratefully acknowledged.

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