J. Indian bot Soc Vol 80 (2001) 15-18

IN VITRO CULTURE OF MATURE EMBRYO AXES OF GROUNDNUT (*ARACHIS HYPOGAEA L.*)

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(Received July, 2000; Accepted February 2001)

The mature embryo axes were inoculated on medium containing 2, 4-D with KIN or BAP for callus inducation. Maximum percentage of callus induction was noticed on medium supplemented with 3 mg/l of 2,4-D and 1 mg/l of BAP. The highly organogenic nodular calli were obtained in 2 mg/l of 2,4-D with 1 mg/l of BAP or KIN. Among the various concentrations of shoot induction medium BAP or KIN 3 mg/l in combination with 0.5 mg/l of 2,4-D produced higher percentage of shoot buds. The regenerated shoots were rooted with using IBA.

Key words: callus induction, organogenic calli, shoot induction, rooting.

Groundnut or peanut is an important crop in both developed and developing countries for the production of oil and protein from the seed (Mckently et al., 1989). The major breeding objectives of groundnut programme are to develop varieties with high yield and quality, earliness, resistance to major diseases, pests, drought, salt, cold, higher protein and oil contents.

Conventional methods for improving the groundnut crop have been inadequate in achieving all of the above objectives. Plant tissue culture have been alternatively proposed as a tool for propagation of useful genotypes or as a source of novel genetic variability to be exploited in selection program (Chaleff, 1981). The cultivated groundnut one of the grain legume species is recalcitrant in tissue culture, although some successful plantlet regeneration has been achieved either by organogenesis (Narasimhulu and Reddy, 1983; Cheng *et al.*, 1992; Vajranabhaiah et al., 1993; Venkatachalam *et al.*, 1994) or somatic embroyogenesis (Ozias-Akins, 1989; Mckently, 1990; Eapen *et al.*, 1993; Ramdev Reddy and Reddy, 1993; Venkatachalam *et al.*, 1999). In this present study, we have reported a successful plantlet regeneration of embryo axes derived from mature seeds.

MATERIALS AND METHODS

Experimental Material : Seeds of groundnut cultivars VRI-2 and VRI-3 were obtained from Tamil Nadu Agricultural University, Coimbatore used as an experimental material.

Surface Sterilization : Seeds of the groundnut cultivars Viz, VRI-2 and VRI-3 were thoroughly washed with tap water and immersed in detergent solution for 5 minutes. After washing with distilled water the seeds were again washed in 70% alcohol for few seconds and rinsed three to four times with distilled water. The seeds brought to the inoculation chamber, the seeds were surface sterilized with 0.1% $HgCl_2$ for 10 minutes and again washed with sterile distilled water for 4-7 times.

Collection of Embryo Axes : From the surface sterilized seeds, the cotyledons were removed and mature embryo axes were collected. In isolated embryo axes, the embryonal leaves were removed to avoid germination. Before inoculation, the embryo axes were injured all over the surface to facilitate callus inducation.

Media for Callus Induction and Plantlet Regeneration: The embryo axes were inoculated on MS basal medium (Murashige and Skoog, 1962) containing 30 g/l of sucrose, 8.0 g/l of agar and B_5 vitamins (Gamborg, 1968) supplemented with varying concentrations of auxin and cytokinins. For callus induction 2,4-D (1 to 3 mg/l) and KIN or BAP (1 mg/l) were used. For shoot bud regenetation (3 mg/l) of BAP or KIN along with 0.5 mg/l of 2,4-D were used.

Rooting : The regenerated shoots were rooted with using IBA ranged from 1-5 mg/l. The rooted plantlets were transferred to plastic cups containing sterilized soil.

Statistical Analysis : The data were analysed using standard deviation and mean separation was carried out using Duncans Multiple Range Test.

OBSERVATION AND RESULTS

Callus Induction and Plantlet Regeneration: From the

isolated mature embryos minor part of the radicle portion was also removed to facilitate callus induction. The whole axes were inoculated on the media containing 2,4-D and BAP or KIN for callus induction. Due to their embryogenic nature the explant showed higher efficiency of callus induction and callus growth (Table 1). The explants enlarged twice within 2 weeks and well developed callus formation ocurred within 4 weeks. When compared to radicle end, the plumular end showed vigorous callus growth. Maximum percentage of callus formation was noticed in 3 mg/l 2,4-D with 1 mg/l of BAP (91.8% in VRI-2 and 86.8% in VRI-3). The fresh and dry weight of the callus also influenced by hormonal concentrations. Highly organogenic, compact nodular calli were obtained in 2 mg/l of 2, 4-D with 1 mg/l of KIN or BAP.

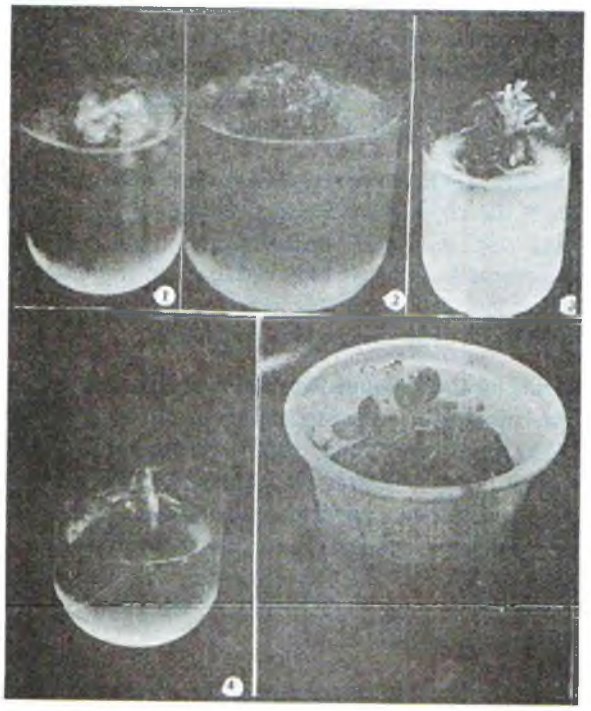

Figure 1-5 Callus induction and plantlet regeneraton from mature embryo axis of groundnut.

Fig 1. Compact calli derived from mature embryonal axis cultured on MS+2 mg/l2, 4-D+ 1mg/1BAP.

Fig 2. Regeneration of shoot buds from embryonal axis derived calli cultured on MS+BAP 3mg+0.5 mg/l of 2,4-D.

Fig 3. Development of multiple shoots in shoot elongation medium containing MS+5 mg/l of BAP.

Fig 4. Isolated shoot in root induction medium containing MS+3 mg/l of IBA.

Fig 5. Well developed plantlet established in plastic cup.

In both KIN and BAP in combination with 2,4-D the calli were pale white in colour.

Only organogenic calli were transferred to shoot induction medium containing KIN/2, 4-D or BAP-/2, 4-D. Before transferring the calli to shoot induction medium, they were subcultured in reduced auxin containing medium to favour shoot bud induction (Table -2). Among the various concentrations tested, 3mg/l of BAP with 0.5 mg/l of 2,4-D produced maximum shoot buds. The calli containing shoot buds were transferred to shoot elongation medium (5mg/l of BAP or KIN). Average number of shoots per calli varied from 4.6 to 20.0 in VRI-2 and 3.7 to 16.8 in VRI-3 in KIN/2,4-D containing medium and 6.0 to 23.6 in VRI-2 and 5.6 to 20.2 in VRI-3 using BAP and 2,4-D containing media.

Root Induction : Well developed shoots were transferred to root induction medium containing IBA ranged from 1 to 5 mg/l. Within 25 days of culture roots developed from cut ends. Among the five concentrations, 3 mg/l of IBA was found to be most suitable for root formation. Above this concentration rooting is associated with callus formation. Rooted plantlets were transferred to plastic cups cotaining sterilized sand, soil mixture.

DISCUSSION

In this regeneration system we have reported a successful plantlet regeneration from indirect organogenesis. Atreya *et al.*, (1984) cultured embryo axes in various media and obtained normal planlets without growth regulators. In our study 2,4-D with KIN or BAP produced callus in varying frequencies which later were differentiated into plantlets upon transfer to shoot induction medium. Mckently *et al.*, (1991) reported direct plantlet regeneration from mature embryo axes using BAP. Like our present study, Braverman (1975) produced pathogen free plantlets of groundnut by culturing mature embryo axes collected from seed that were infected with bacteria and fungi. The result of our study revealed that 2,4-D helped in growth and development of callus at higher level in combination with cytokinins. The similar results were supported by several workers. Ganesh, *et al.*, (1993) reported the formation of the seed callus in Sesame using 2,4-D with KIN or BAP. According to Pandey *et al.*, (1978) the leaf explants of *Vigna sinensis* proved to be more responsive to callusing when the basal medium was supplemented with 2,4-D than any other auxin tested. Like our present

Table 1: Effect of 2,4-D on callus induction and callus grwoth of mature embryonal axes derived callus cultures of groundnut cvs VRI-2 and VRI-3.

Hormones (mg/l)	Percentage of response		Callus fresh weight (g)		Callus dry weight (g)	
	VRI-2	VRI-3	VRI-2	VRI-3	VRI-2	VRI-3
2,4-D+KIN						
1.0+1.0	50.2±1.79^{e}	49.00±2.00^{d}	0.379±0.01^{d}	0.321±0.007^{d}	0.044±0.002^{bc}	0.034±0.0032^{e}
1.5+1.0	59.4±1.14^{d}	52.6±1.94^{cd}	0.872±0.01^{bc}	0.782±0.016^{bc}	0.052±0.0013^{bc}	0.044±0.0037^{de}
2.0+1.0	71.8±1.92^{c}	69.0±1.41^{bc}	0.877±0.13^{bc}	0.816±0.010^{bc}	0.076±0.0049^{b}	0.063±0.0021^{d}
2.5+1.0	82.8±1.48^{b}	75.6±2.07^{b}	0.945±0.32^{bc}	0.901±0.011^{bc}	0.136±0.018^{a}	0.109±0.015^{bc}
3.0+1.0	87.6±1.14^{ab}	80.0±1.58^{ab}	1.323±0.29^{b}	0.991±0.064^{b}	0.091±0.005^{ab}	0.900±0.0020^{a}
2,4-D+BA						
1.0+1.0	54.8±1.48^{de}	49.6±2.07^{d}	0.444±0.024^{d}	0.377±0.012^{d}	0.037±0.0043^{c}	0.034±0.0034^{e}
1.5+1.0	61.8±1.78^{cd}	56.2±1.48^{c}	0.717±0.02^{c}	0.636±0.021^{c}	0.070±0.0016^{b}	0.063±0.0024^{d}
2.0+1.0	73.6±2.07^{c}	70.0±1.58^{bc}	0.861±0.01^{bc}	0.749±0.025^{bc}	0.078±0.001^{b}	0.072±0.0019^{cd}
2.5+1.0	86.0±1.58^{ab}	82.0±1.87^{ab}	0.967±0.034^{bc}	0.914±0.016^{b}	0.022±0.0033^{d}	0.185±0.063^{b}
3.0+1.0	91.8±1.92^{a}	86.8±1.64^{a}	2.043±0.40^{a}	1.927±0.060^{a}	0.088±0.026^{ab}	0.090±0.0016^{c}

Values with the same superscript are not signigicantly different at the 0.5% probability level according to Duncan's multiple range test.

study several results were also reported in groundnut by several authors (Bajaj et al., 1981; Narasimhulu and Reddy 1983; Venkatachalam et al., 1998).

Shoot bud differentiation was observed using different concentration of cytokinins. The increasing concentration of KIN or BAP increased the percentage of shoot bud differentiation and mean number of shoots per callus. These results are in accordance with several previous reports of many legumes (Mehta and Mohanram 1980; Narasimhulu and Reddy 1983; Franklin *et al.*, 1991). They obtained shoots on medium containing KIN or BAP. In groundnut Venkatachalam *et al.*, (1994) reported that the highest frequency of shoot bud differentiation was observed using BAP. Generally BAP was essential for higher percentage of shoot multiplication in many plant species and was most effective than KIN (Murashige, 1974).

Table 2: Effect of KIN and BA on shoot induction from mature embryonal axes derived callus cultures of groundnut cvs VRI-2 and VRI-3 in combination with 0.5 mg/l of 2,4-D.

Hormones (mg/l)	Shoot bud regeneration (%)		Mean numbers of shoot/explant	
	VRI-2	VRI-3	VRI-2	VRI-3
KIN+2,4-D				
1.0+0.5	36.0±2.09^{d}	32.0±1.41^{d}	4.6±1.02^{d}	3.7±0.75^{d}
1.5+0.5	42.2±1.60^{cd}	37.4±1.02^{cd}	9.0±1.41^{c}	7.4±1.02^{c}
2.0+0.5	51.2±1.17^{bc}	43.6±1.62^{c}	11.6±1.01^{bc}	10.8±0.75^{bc}
2.5+0.5	61.0±1.41^{ab}	50.6±1.36^{b}	16.0±1.5^{b}	14.8±1.17^{ab}
3.0+0.5	68.2±1.33^{ab}	63.6±2.06^{ab}	20.0±0.63^{ab}	16.8±1.16^{ab}
BA+2,4-D				
1.0+0.5	40.0±1.41^{cd}	38.80±1.60^{cd}	6.0±0.64^{cd}	5.6±1.01^{cd}
1.5+0.5	48.8±1.60^{c}	43.20±1.79^{c}	12.07±1.41^{bc}	10.8±0.74^{bc}
2.0+0.5	59.2±1.47b	55.2±1.52ab	16.2±1.17^{b}	13.8±1.16^{b}
2.5+0.5	67.2±1.46^{ab}	64.4±2.96^{ab}	20.6±1.02^{ab}	17.6±1.02^{ab}
3.0+0.5	71.98±1.60^{a}	67.00±2.23^{a}	23.6±1.36^{a}	20.2±0.88^{a}

Values with the same superscript are not signigicantly different at the 0.5% probability level according to Duncan's multiple range test.

The regenerated shoots of both the varieties were rooted using different concentrations of IBA alone. It has been found that JBA was potent root auxin for *in vitro* produced shoots. The similar studies were already reported by several workers in groundnut (Mroginski *et al.*, 1981; Mckently *et al.*, 1991; Cheng *et al.*, 1992; Venkatachalam *et al.*, 1998).

In conclusion, we described a method for *in vitro* regeneration of groundnut from mature embryo axes. Among the two varieties of groundnut VRI-2 responded well than that of VRI-3. Moreover, based on available literature regarding this particular explant was not exposed much in terms of callus induction and plantlet regeneration.

The authors are grateful to Council of Scientific and Industrial Research (CSIR), Government of India for financial support.

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