J. Phytol. Res. **8** (1):31-34, 1995

AN EFFICIENT MULTIPLE SHOOT AND PLANTLET FORMATION SCHEDULE IN CHICKPEA *(CICER ARIETINUM L.)*

S.BANSAL and Y.K.BANSAL

Department of Biosciences, R.D.University, Jabalpur - 482 001, India.

Seedling explants of *Cicer arietinum* were cultured on MS medium enriched wih different concs. of auxins and/or cytokinin. Shoot buds emerged from the hypocotyl established on media containing IBA (0.1 - 1 mg/l), IAA (1-5 mg/l) and BAP (0.1-1mg/l) independently or in combination of IBA (0.5 mg/l)+ BAP (0.5 mg/l) and IAA (1 mgl)+ BAP (5 mg/l) had the maximum shoot regeneration potential from the hypocotyl explant. Proximal part of the hypocotyl proved more suitable than the distal. Shoot regenration occurred as direct shoots buds. Shoots developed roots when transferred to media having IBA (0.1 mg/l). The genotype JG 317 showed higher potential to regenerate shoots than JG 1265.

Keywords:BAP; chickpea; IAA; IBA.

Introduction

Cicer arietinum L. (Gram or chickpea) belonging to subfamily papiloinoideae of the family Leguminosae is a major nutritive pulse crop of dryland agriculture in India often used as a vegetable. Conventional breeding methods have yielded little genetic improvement due mainly to poor genetic base of the crop and the presence of strong sexual incompatibility barriers with its wild relatives[1]. Plant cell, tissue and organ culture techniques have been extensively used as alternative tools in the crop improvement programmes [2,3].

There are only a few reports available on callusing and plant regeneration in chickpea [4-7], and in majority of them, the plantlet regeneration frequencies have been rather low. The present report deals with the *in vitro* regernation, multiple shooting and plantlet formation from hypocotyl, cotyledon and leaflet from a cultivar of *C.arietinum.*

Materials and Methods

Seeds of *Cicer arietinum* cultivars JG-317 and JG-1265 obtained from Jawahar Lal Nehru Krishi Vishvavidyalaya (JNK VV), Jabalpur, were presoaked overnight and surface sterilized with 0.1% aqueous $HgCl_2$ for 6-8 min, rinsed thoroughly with sterile distilled water and germinated on moist cotton contained in sterile 250 ml flasks at room temperature in dark. Explants were prepared in the form of leaflet, root and hypocotyl (5-10mm each) from 4-6 day old seedlings and embryonal axis (5mm) and cotyledonds from decorticaled seeds and inoculated on MS medium[8] (pH 5.8) containing 0.8% agar (Qualigens), 3% (W/V) sucrose (BDH) and supplemented with various concentrations and combinations of cytokinin viz. BAP and/or auxins viz. 2,4-Dichlorophenoxy acetic acid (2,4-D), Indole-3 acetic acid (IAA) and Indole butyric acid (IBA) (Table 1). A minimum of 20 explants were cultured in each treatment and all treatments were replicated

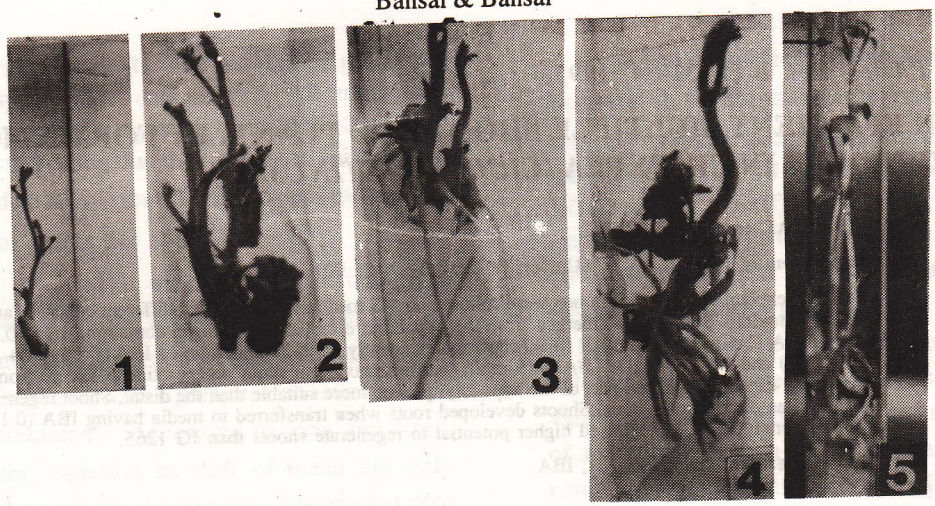

Fig. 1-5 1 Differentiation of shoot buds from hypocotyl in CV JG-1265 with IBA (0.5 mg/l) + BAP (0.5 mg/l) (3 weeks); 2. Multiple shooting from hypocotyl In CV JG - 317 with IAA (1 mg/l) + BAP (5mg/l) (4 weeks); 3. Shoot showing root formation in shooting medium (4 weeks); 4. Induction of rooting in shoots with IBA (0.1 mg/l) (4 weeks); 5. Complete plantlet showing floral bud formation (arrow) (6 weeks.)

three times. All cultures were kept under fluorescent light (1500 lux intensity)/dark cycles of 16hr/hr at 25±2°C and 70% RH.

The frequency of callus initiation and shoot regeneration was recorded four weeks after inoculation. Callus initiated from different explants was subcultured on media supplemented with different concs. of BAP (0.1 - 5.0 mg/l), IAA (0.1 to 1 mg/l) and IBA (0.1 to 1 mg/l) independently or incombination for plantlet regeneration.

Results and Discussion

Callus induction: All the chickpea explants responded well for callus initiation and growth on MS medium supplemented with different hormonal combinations (Table 1). The explants swelled considerably within 1-2 weeks of culture and showed profuse callus formation at the edges. The frequency of callusing, however, varied with the medium composition. Frequency of callus initiation from hypocotyl and cotyledonary explants was higher compared to leaf and embryonal axis. Callusing response was higher at 1-5 mg/l with IAA & 2,4-D. However, this response declined at low (0.1 mg/l) as well as high (10 mg/l) concs. Genotype had little effect on the induction of callus which was creamish and failed to show the occurrence of pigmentation as recorded earlier[9]. Calli did not show shoot organogenesis but often showed the occurrence of rhizogenesis.

Shoot morphogenesis: The regeneration of complete plantlet in chickpea was observed to be a two step process with shoot bud/shoot formation

Table 1. Efficiency of callusing and shoot budding from cotyledon (Co) and hypocotyl (H) with different hormones.

Hormone combination (mg/l)	Explant	% Explants callusing	No. of shoots per explant
1. MS + 0.1 IBA	Co	22.6	2-3
	H	28.8	3-4
2. MS + 1 IBA	Co	62.4	2-4
	H	50.2	4-6
3. MS + 1 IAA	Co	37.6	1-2
	H	36.2	2-4
4. MS + 5 IAA	Co	38.4	1-2
	H	30.2	1-2
5. MS + 5 2,4-D	Co	62.4	-
	H	57.6	-
6. MS + 10 2,4-D	Co	24.8	-
	H	26.6	-
7. MS + 0.1 BAP	Co	-	2-3
	H	-	2-4
8. MS + 1 BAP	Co	-	2-4
	H	-	3-5
9. MS + 0.5 IBA + 0.5 BAP	Co	27.4	1-2
	H	26.6	1-2
10. MS + 1 IBA + 0.5 BAP	Co	42.4	6-8
	H	30.6	8-10
11. MS + 0.1 IAA + 1 BAP	Co	32.8	1-2
	H	34.6	1-2
12. MS + 1 IAA + 5 BAP	Co	28.6	4-6
	H	26.8	5-7
13. MS + 0.1 2,4-D + 1 BAP	Co	29.4	-
	H	36.6	-
14. MS + 10 2,4-D + 1 BAP	Co	20.6	-
	H	24.8	-

Effieiency of shoot differentation and plantlet regeneration (%) from explants of two genotypes in *C. arietinum* cultured on MS medium.

	Genotype			
	JG — 317		JG — 1265	
	Shoot	Plantlet	Shoot	Plantlet
Cotyledon P	14.2	12.6	12.2	9.6
D	0	0	0	0
Hypocotyl	10.8	8.6	10.4	8.8
Leaflet	0	0	0	0
Embryonal axis	8.6	8.4	8.0	7.0
Root	0	0	0	0

Average of 20 explant cultures / treatment; P = Proximal, D = Distal.

followed by rooting. Different concentrations of IBA (0.1-1 mg/l), IAA (1-5 mg/l) and BAP (0.1-1 mg/l) either independently or in combination induced shoot morphogenesis and plantlet regeneration. Moderate concentrations of IBA (1 mg/l), IBA (0.5 mg/l) + BAP (0.5 mg/l) (Fig.1) and IAA (1 mg/l) + BAP (5 mg/l) (Fig.2) had the maximum shoot regeneration potential from the hypocotyl (Proximal) explants. Roots, leaflets and distal cotyledon segments failed to yield any shoot. Shoot regernation occurred in the form of the direct differentiation of shoot buds from the explants. The number of shoots per regenerating explant varied considerably ranging from 2-6 with

same explant regenerating as many as 8-10 shoots. Leaf senescence was the major problem observed in these cultures incubated for long periods on shoot induction media. Subsequent culturing of such shoots on hormone free MS medium rid the cultures of this problem. The shoots grew well on this medium and attained a height of 10-12 cms within a month.

Induction of rooting: Sometimes shoot induction media yielded whole plants with both shoots and roots (Fig.3). However, for achieving consistent rooting individual shoots had to be subcultured on IBA (0.1 mg/l) enriched medium (Fig.4). Frequency of root differentiation declined when IBA was deleted from the medium or was used at a high (5-10 mg/l) concs. The plantlets thus established sometimes showed occurrence of floral bud formation (Fig.5). Of the three auxins (2,4-D, IAA, IBA) tested in combination with BAP in the present case, the best shoot regenerated occured in the presence of IBA + BAP contrary to regeneration of shoot bud in chickpea reported in the past with NAA + BAP[6], BAP + IAA or BAP + 2,4-D[10]. In the present study 2,4-D despite inducing callus had an inhibitory effect on shoot differentiation as was reported earlier[9]. This is in sharp contrast to high plant regeneration frequencies induced by 2,4-D in legumes.[11-13]

The genotype has been established in the past to have an effect on plant regeneration[11,14]. In the present study too genotype has been found to have good effect on shoot organogenesis. Of the two genotypes used viz. CV JG-317 and JG-1265 the former showed higher potential to regenerate shoots then the latter.

Acknowledgements

Authors wish to thank Prof. S. K. Hasija, Head, Department of Biosciences, R.D. University, Jabalpur for the facilities provided and to U.G.C. for the award of Associateship to one of the authors (SB).

References

1. Lal J and Chandra S 1987, *Legume Res.* **10** 53
2. Mroginski LA and Kartha KK 1984, In : *Plant Breeding Reviews Vol.* 2 J. Janick (ed) Avi Publishing Co. Inc. West Port Ct. USA, p. 215
3. Cocking EC 1986, In : *Plant tissue culture and its agricultural* application L.A. Withers and P.G. Anderson (eds) Butterworths, London, p. 3.
4. Neelam A, Reddy CS and Reddy GM 1986, *Int Chickpea News Lett* **14** 9
5. Divakar Rao J and Reddy GM 1987, In : *Plant Cell and tissue culture of economically important plants* G.M. Reddy (ed) Hyderabad, p 355.
6. Rao GR and Chopra VL 1987, In : *Plant Cell and tissue culture of economically important plants* Hyderabad, p 343
7. Bansal YK and Shrivastava R 1992, *Ind J Appl Pure Biol* **7** 41
8. Murashige T and Skoog F 1962, *Physiol Plant* **15** 473
9. Parkash S, Chowdhury JB, Jain RK and Chowdhury VK 1992, *Ind. Jour. Exptl. Biol.* **30** 1149
10. Sharma DR, Kumari R and Chowhdury JB 1979, *Ind. Jour. Exptl. Biol.* **17** 607
11. Jain RK, Chowdhury JB, Sharma DR and Fredt W 1988 *Plant Cell Tiss Org. Cult.* **14** 197
12. Pickardt T, Huancaruna PE and Schieder O 1989, *Protoplasma* **149** 5
13. Eapen S and George L 1990, *Ann. Bot.* **66** 219
14. Pierik RLM 1987, In : *In vitro culture of higher plants* R. L. M. Pierik (ed) Martinus Nijhoff Publishers, The Netherlands.