SHORT COMMUNICATION

SHOOT REGENERATION IN CALLUS CULTURES OF CHICKPEA CULTIVARS

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Plant regeneration protocol from callus cultures of chickpea cultivars was developed using cotyledonary node, shoot tip, leaflet, internode and hypocotyl as explant source. Except leaflet explant, all the explants used showed high degree of callus induction on B5 basal medium supplemented with BAP (1.5 mg/l) and NAA (1.0 mg/l). MS medium supplemented with TDZ (1 mg/l) was found suitable for callus induction in leaflet explants. For callus induction as well as for shoot regeneration, cultivar HC-I showed higher response than cultivars C-235 and H86-18. Regenerated shoots were rooted on half strength MS medium. The hardened regenerated plantlets were finally transferred to soil. However, survival percentage of the plantlets was low (11%) in soil.

Key words: ***Cicer arietinum*** **L., shoot morphogenesis, thiadiazuron (TDZ)**

Chickpea (*Cicer arietinum* L.) is a major pulse crop of India, Middle-East and North-Africa (Singh and Reddy 1991) and is an important source of dietary protein in the Third World countries. Chickpea yields are low due to various factors such as diseases (*Fusarium* wilt, *Aschochyta* blight) and insects/pests (pod borer etc.) as well as abiotic stresses (Salinity, alkalinity, frost etc.). Genetic improvement of traits has been very slow through conventional plant breeding methods because of poor sexual compatibility with its wild relatives and its narrow genetic base. Like other legumes, chickpea has been a recalcitrant crop for tissue culture response. However, shoot regeneration from immature cotyledons (Batra *et al.* 2002), leaflets (Barna and Wakhlu 1994), shoot tips (Surya Parkash *et al.* 1992), cotyledonary nodes (Subhadra *et al.* 1998) and embryo axis (Krishnamurthy *et al.* 2001) has been reported. In this communication, we report a regeneration protocol for three chickpea, cultivars, viz. HC-I, C-235 and H86-18.

Seeds of three chickpea cultivars, viz. HC-I, C-235 and H86-18 were washed with teepole and were pre-soaked surface sterilized with 0.1% mercuric chloride for 10 minutes and were soaked over night in sterilized distilled water. Seeds were cultured aseptically on germination medium having sucrose (2.0%) and agar (0.8%). Ten-day old *in vitro* grown seedling were used for excision of explants i.e. shoot tip, cotyledonary node, internode and hypocotyls for callus initiation. Leaflet explant obtained from one-month old field grown plants, surface sterilized with 0.1% $HgCl_2$ for 10 minutes washed with sterilized distilled water 4-5 times were cultured on various callus induction media. A number of media having MS salts (Murashige and Skoog 1962) and B_5 (Gamborg *et al.* 1968) vitamins with varying concentrations and combinations of cytokinins auxins and growth regulators were tested for callus initiation from different explants (Table 1). A total of around 150 explants were cultured per treatment. Observations were recorded for per cent explants showing callus induction and shoot regeneration. Regenerated shoots (aseptically separated) were inoculated on various root induction media (Table 1) and the cultures were observed for root formation after thirty days of inoculation. Regenerated plantlets were transferred to potted soil.

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Table 1. Media tested for callus induction, shoot regeneration and root induction in chickpea

S.No.	Basal medium	Vit.	Cytokinin (mg/l)	Auxin (mg/l)	Sucrose (mg/l)	Any other growth regulator (mg/l)
Callus induction media						
A1	B5	B5	BAP 1.0	NAA 1.0	20	-
A2	B5	B5	BAP 1.5	NAA 1.0	20	-
A3	MS	B5	BAP 1.0	IBA 1.0	30	Picloram 0.1
A4	MS	MS	BAP 0.5	IBA 1.0	30	Picloram 0.05
A5	MS	MS	-	-	30	TDZ 1.0
A6	MS	MS	-	-	30	TDZ 0.05
Shoot regeneration media						
A7	MS	B5	BAP 1.5 Kinetin 1.0	-	30	-
A8	MS	B5	BAP 2.0	-	30	-
A9	MS	MS	BAP 3.0	NAA 0.04	30	-
A10	MS	B5	BAP 1.0	-	30	-
Root induction media						
A11	MS	MS	-	-	30	-
A12	½ MS	MS	-	-	30	-
A13	MS	MS	-	NAA 1.0	30	-
A14	½ MS	MS	-	-	15	-
A15	MS	MS	-	IBA 1.0	30	-

Chickpea cultivar HC-1, showed highest callus initiation (84.3%) followed by C-235 and H86-18 in shoot tip explants (Table 2). This feature of cultivar variations, has already been well documented in several other crop species (Jain *et al.* 1988, Surya Parkash *et al.* 1992), including large seeded legumes (Bajaj and Dhanju 1979). After 10-15 days of incubation, explants swelled and callus initiation took place only at the cut surface. Among the media used, medium A2 (B_5 + BAP 1.5 mg/l + NAA 1.0 mg/l) was found to be most suitable for callus initiation in all the cultivars and explants used (cotyledonary node, shoot tip, hypocotyl and internode) except leaflet explant (Table 2). However, per cent explants producing shoots was maximum (34.4%) in shoot tip explants of the cultivar HC-I followed by cotyledonary node, hypocotyl and internodal explants (Table 2). These observations lend support to the findings of Surya Parkash *et al.* (1992) in chickpea where they stressed effect of different explants

Table 2. Per cent callus induction, callus proliferation and shoot regeneration from different explants in chickpea cultivars on respective media

Explants	Callus induction (%)			Calli producing shoots (%)			No. of shoots per callus		
	C-235	HC-1	H86-18	C-235	HC-1	H86-18	C-235	HC-1	H86-18
Cotyledonary node	73.6	82.6	68.4	31.8	26.7	24.2	3.2	3.0	2.4
Shoot tip	72.4	84.3	63.9	27.0	34.4	22.3	3.0	3.4	3.0
Hypocotyl	68.6	75.7	51.4	14.6	11.3	9.2	1.5	1.6	1.5
Internode	42.5	63.3	40.2	11.7	9.2	7.4	0.8	1.0	0.2
C.D. at 5%	4.2	4.1	4.6	2.7	2.7	2.8	0.6	0.6	0.6

for callus initiation (Table 2). The leaflet explants of HC-1 cultivar showed high degree (85%) of callus formation on MS medium supplemented with 1 mg/l TDZ (Table 3). Callus obtained from leaflet explants, was creamish yellow soft and friable. This confirms the finding of Gill and Saxena (1992), who reported that TDZ is able

Table 3. Effect of TDZ on per cent callus induction in leaf explants of chickpea cultivars C-235, HC-I and H86-18

TDZ (mg/l)	C-235	HC-1	H86-18
0.1	15.0	10.0	6.0
0.2	35.0	30.0	18.0
0.5	38.0	25.0	12.0
1.0	8.0	85.0	45.0

Media listed – MS medium supplemented with following concentrations of TDZ

Medium	AC_1	AC_2	AC_3	AC_4
TDZ mg/l	0.1	0.2	0.5	1.0

to convert non-embryogenic cells into an embryogenic state. In all the three cultivars and explants used (cotyledonary node, shoot tip, hypocotyls and internode), shoot morphogenesis was maximum in HC-I cultivar on shoot regeneration medium (A8) supplemented with B5 vit. and BAP (2 mg/l) from shoot tip derived calli (Table 2). In general, shoot tip and cotyledonary node explants showed better response for shoot regeneration as compared to hypocotyls and internode explants which is in agreement with previous studies (Rao and Chopra, 1989; Subhadra *et al.* 1998). However, calli derived from leaflet explants failed to regenerate shoots. Root differentiation from regenerated shoots was observed on half-strength MS medium (A 12) in all the three cultivars. Regenerated plantlets (8-10 cm) were transferred to soil. However, the survival of plantlets in the soil was poor (11%). Krishnamurthy *et al.* (2001) resorted to grafting of regenerated shoots on five-day old dark grown seedlings to recover the mature plants. However, hardening of regenerated plantlets in chickpea is still a major constraint in successful transfer of regenerated plants to potted soil. Kumar *et al.* (1995) have also suggested that the factors governing the survival of chickpea plantlets in potted soil, are yet to be worked out.

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