DIRECT *IN VITRO* SHOOTS PROLIFERATION OF CHICK PEA (*CICER ARIETINUM* L.) FROM SHOOT TIP EXPLANTS INDUCED BY THIDIAZURON

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ABSTRACT

A rapid, simple and efficient protocol for direct *in vitro* multiple shoot induction and plantlet regeneration was achieved from shoot tip explants of *Cicer arietinum*. The shoot tips were cultured on MS medium fortified with Thidiazuron (TDZ) (1.0-7.0 mg/L) for multiple shoot induction. Multiple shoots proliferation was best observed at 3.0 mg/L TDZ from the shoot tip explants within three weeks of culture. Shoot number per explant ranged between 2 and 10. Individual shoots were aseptically excised and sub cultured in the same media for shoot elongation. The elongated shoots were transferred to Indole Butyric Acid (IBA) (1.0mg/L–5.0mg/L) for root induction. Rooting was observed within two weeks of culture. Rooted plantlets were successfully hardened under culture conditions and subsequently established in the field conditions. The recorded survival rate of the plants was 86%. Plants looked healthy with no visually detectable phenotypic variations.

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KEYWORDS: shoot tip; multiple shoots; rooting; hardening
Abbreviation: TDZ (1-Phenyl -3 - (1, 2, 3-Thiadiazo l-5-yl) Urea, IBA-Indole-3-butyric acid

INTRODUCTION

Legumes, broadly defined by their unusual flower structure, podded fruit, and the ability of 88% of the species examined to date to form nodules with rhizobia (De Faria *et al.,* 1989), are second only to the Poaceae in their importance to humans. The 670 to 750 genera and 18,000 to 19,000 species of legumes include important grain, pasture. Legumes should be planted in light soils, not so much for their own crops as for the good they do to subsequent crops. Leguminous plants are quite different to regenerate *in vitro.* Grain legumes represent one of the most valuable sources of proteins for human and animal nutrition and are also responsible for nitrogen enrichment of soil through symbiosis with rhizobium. Legumes are largely cultivated in the Mediterranean basin, Middle East, Asia and South America and to date, they have been qualitatively and quantitatively improved by conventional breeding. However, the lack of resistance to several pest diseases still remains the major cause of significant loss of edible product. Recent advances in genetic engineering have clearly demonstrated the possibility of incorporating foreign genes for desired agronomic traits while preserving the existing characteristics of improved genotypes.

Chickpea (*Cicer arietinum* L.) is an important grain legume of the Indian subcontinent, West Asia, Mediterranean region, North and East Africa, Southern Europe and Central America and Australia. Various attributes of chickpea made it the most cultivated pulse crop and the most appreciated protein source among vegetarians all over the world. Chickpea straw has forage value comparable to other straws commonly used for livestock feed. It is able to drive more than 70% of nitrogen from symbiotic dinitrogen fixation, which makes it a promising crop for "alternative agriculture" that is now attracting a considerable attention in the industrialized world. The heavy demand created by the pressure of increasing population in the developing world requires a tremendous scientific effort to meet the requirements of food, fiber, fuel and other necessities of life. Since the conventional techniques employed in crop improvement may not keep pace with the demands of the increasing population (3 person/s) and decreasing land resources, the importance of *in vitro* technologies in crop improvement has great relevance. Recent advances made in the field of tissue culture have brought about new emerging technologies for crop improvement.

Plant tissue culture offers new ways for the improvement of this crop after many years of recalcitrance. Several researchers have reported on the regeneration of *Cicer arietinum* via direct organogenesis (Kartha, *et al.,* 1981; Islam, *et al.,* 1995; Barna Wakhlu, 1995; Anju Chawla, 2005).

Thus the objective of the present study was to induce maximum number of shoots and regenerate. Whole plants from shoot tips explants of *Cicer arietinum* cv (ICCC-34) (kranthi).

MATERIALS AND METODS

The seeds of *Cicer arietinum* L.cv (ICCC-34) (kranthi) cultivar were obtained from ICRISAT Hyderabad A.P. The seeds were washed thoroughly in tap water 3–5 times and placed in 1% (v/v) Teepol solution (Reckitt Benckiser, India) which was kept under running tap water for 15 min. Then the seeds were disinfected with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 min. Finally the seeds were rinsed 3–4 times in sterile distilled water and inoculated on moist cotton in sterile test tubes. To assure uniform and rapid germination of seeds, test tubes were placed in dark at 28°C for 24–48 h. Then the germinated seeds were transferred to light intensity (15 µmol/s2/s), 16 h light per day photoperiod for another 4–7 days and maintained at 25 ± 2°C and 55–60% relative humidity.

Selection of explants

Shoot tips with one or two leaf primordia, of 15-d old *in vitro* raised seedlings were selected as explants for direct shoot multiplication. The shoot tips, segments of 5–8 mm in length were excised aseptically.

Culture media and culture conditions

MS media containing 3.0% sucrose and supplemented with various concentrations cytokinin such as TDZ (1.0 – 7.0 mg/L) were used. The initial pH of the culture media was adjusted to 5.8 before addition of 0.8% (w/v) agar- agar. The medium was dispensed into culture tubes (25 + 150 mm) each containing 15 ml of the culture medium capable with non-absorbent cotton and was autoclaved at 121^{o} C for 15 minutes. In each cultures tube one shoot tip explants was implanted. The cultures were maintained under 16h light provided with white fluorescent tubes (40 µ mol m-2s-2) at 25 ± 2 ^{o}C.

RESULTS AND DISCUSSION

Data on multiple shoot induction from shoot tip explants cultured on MS medium fortified with different concentrations of TDZ alone is presented in (Table-1). The important part of the present study was the preparation of contamination free explants. This was achieved by using *in vitro* germinated seedlings as an explant source. Sterilization of seeds required 0.1% (w/v) $HgCl_2$ 5 min treatment for maximum germination (98%) and minimum contamination (Narashimhulu and Reddy, 1983). A similar observation was also reported in *Vigna aconitifolia*, confirming the view that the pretreatment of seeds with specific surface sterilizing agents would predetermine the regenerating behavior of explant tissues (Godbole, *et al.,* 1984). The use of direct and large sized explants had higher survival and growth rates than the smaller ones (Hu Wang, 1983).

Table 1: Effect of different concentration of TDZ on multiple shoot induction from shoot tip explants of *Cicer arietinum* (ICCC-34) (kranthi).

Growth regulators (mg/L)	% of explants showing response	No. of shoots per explant SE*	Average length of shoots SE*
TDZ			
1.0	60	4.0 ± 0.4	2.0 ± 0.4
2.0	65	6.0 ± 0.3	3.2 ± 0.3
3.0	85	10.0 ± 0.6	4.3 ± 0.2
4.0	80	8.0 ± 0.3	6.5 ± 0.5
5.0	70	5.0 ± 0.2	3.3 ± 0.5
6.0	68	3.0 ± 0.3	2.3 ± 0.5
7.0	53	2.0 ± 0.5	1.3 ± 0.5

* Mean ± Standard Error.

Table-2: Rooting ability of regenerated shoots from shoot tip explants culture of *Cicer arietinum.* cv (ICCC-34)) (kranthi).cultured on MS medium supplemented with IBA.

Growth Hormones (mg/L) IBA	Percentage of response	Average no of roots (S.E)*
1.0	62	6.2 ± 0.13
2.0	72	3.4 ± 0.37
3.0	95	9.8 ± 0.38
4.0	60	6.9 ± 0.38
5.0	50	5.4 ± 0.36

*** Mean ± Standard Error.**

Fig 1: Direct *in vitro* shoots proliferation of *Cicer arietinum* L. (a) *In vitro* raised seedlings (30-d old), (b) Formation of multiple shoots on MS+TDZ (3.0) mg/L from shoot tip, (c) Proliferation of multiple shoots on MS+TDZ (4.0mg/L) from shoot tip, (d) rooting of individual shoots on MS+IBA (3.0mg/L) (e) hardening of plantlet

Effect of TDZ:

The meristem containing explants shoot tip were excised from the surface sterilized, *in vitro* grown, 30-d old seedlings and cultured on MS medium augmented with TDZ (1.0–7.0 mg/L) for multiple shoot induction of all the different concentrations of TDZ tested, (2.0 mg/L) TDZ was found to be more effective in inducing (10.0 ± 0.3 shoots/explants) (Fig b). But at high concentration of TDZ (4.0 mg/L) considerably the number of shoot induction was found to be reduced. As the concentration of TDZ was increased up to 2.0mg/L the multiple shoots number was increased but as the concentration of TDZ (4.0mg/L) to (7.0 mg/L) TDZ resulted the number of shoots were reduced. (Fig c).

Rooting of Micro-shoots:

The shoots raised *in vitro* (2-3 cm long) were cultured on MS medium supplemented with various concentration of IBA (1.0 – 5.0 mg/L) for the induction of roots (Table-2). High percentage of rooting ability with several roots was observed at 2.0 and 3.0 mg/L IBA, longer roots were also observed at 3.0 mg/L IBA. The percentage of response was increased gradually from 1.0 to 3.0 mg/L IBA. Maximum numbers of roots were induced at 3.0 mg/L IBA. The number of roots increased from 1.0 – 3.0 mg/L IBA roots number was decreased from 3.0 to 5.0 mg/L IBA. (fig d).

Hardening of well-rooted plantlets:

The elongated shoots were transferred to MS medium augmented with IBA (1.0–5.0) for root induction. Rooting was observed within two weeks of culture. Well-rooted plantlets were isolated and washed in running tap water. Later they were transplanted into plastic cups containing sterile sand and soil mixture (3:1) for hardening purposes. The well-grown plants were transferred to larger pots containing soil mixture and maintained in the field conditions. Plants grown in the field were further observed for growth and survival.

The result of present investigation show that the shoot tip explants from mature plants of *Cicer arietinum* L. (ICCC-34) (kranthi) could be induced to produce multiple shoots *in vitro* maximum number of shoots was induced on MS medium fortified with various concentrations of TDZ. In recent years, shoot tip explants have been preferred to produce large number of genetically identical clones (Bajaj Dhanju, 1979). Multiple shoot formation from shoot apices was obtained on MS medium supplemented with 20µM BA, 0.1µM NAA in pea (Griga *et al.,* 1986). MS- solid medium fortified with TDZ and Kn alone and in combination increased the regeneration potential of shoot apical meristems of soybean, cowpea, peanut, chickpea and bean (Kartha *et al.,* 1981). It was reported that BAP was proved to be an ideal hormone for shoot multiplication of shoot tip culture in grain legumes (Sounder Raj *et al.,* 1989).These results are also in agreement with those on *Tectona grandis* (Gupta *et al.,* :1980) *Abizzia lebbeck* (Gharyl and Maheshwari 1982) multiple shoot induction was also observed in *Ziziphus manritiana* (Sudharshan *et al.,* 2000) and *Vanilla plantifolia* (Geetha *et al.,* 2000) shoot tips cultured on MS + cytokinin alone as it was observed in the present studies.

Nasir *et al*., (1997) has studied the shoot meristem culture in 16 cultures of cotton using several media formation. They observed the best shoot developmentation MS media containing TDZ alone compared to other media with NAA / IAA in combination with TDZ. These results are to the present observation in *Cicer arietinum* L. (ICCC) (kranthi) which contain with cytokinins showed the increased number of shoots/ explants have also observed the similar results when they have cultured the shoot tips of F1 hybrids of *Paulownia*.

The capacity of shoot bud differentiation and shoot proliferation from shoot tip explants of *Cicer arietinum* (ICCC) (kranthi) depended on hormonal variation. There was good shoot bud induction and proliferation response only in the presence of cytokinin and no response in the basal medium. Similar results are well documented in several medicinal plants (Pattnaik and Chand 1996), *Emblila officinale* (Verma and Kant :1996) and *Withania somnifera* (Deka *et al.,* 1999). From our study it was clear that 2.0 mg/L BAP and Kn were significantly more effective for inducing shoot organogenesis. Well-developed shoot lets from our experimental data, it is evident that TDZ are the best suited for inducing multiple shoots In conclusion, this communication describes an efficient rapid propagation system of *Cicer arietinum* L. (ICCC-34) (kranthi).

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