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REGENERATION FROM IMMATURE COTYLEDONS OF CHICKPEA *(CICER ARIETINUM* L.)

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In vitro shoot regeneration was achieved from different explants viz. immature cotyledons, hypocotyls, cotyledonary nodes, shoot apex and embryo axis of chick pea cv. ICC 4918 (Annigiri) cultured on B_5 medium supplemented with BA as sole growth hormone or in combination with IBA, IAA or NAA. BA at 2 mg/l was optimal for high frequency shoot induction.

Keywords: Chick pea; Cotyledonary nodes; Hypocotyls; Immature cotyledons; Multiple shoots.

Plant regeneration has been quite difficult in legumes. Most grain legumes have a higher propensity for root formation than for shoot formation. The frequency of root initiation is quite high despite auxins and cytokinins. Root initiation was observed in attempts to obtain plant regeneration for *Psophocarpus tetragonolobus*[1], *Glycine max*[2] and *Phaseolus vulgaris*[3].

In generation plant regeneration needs reduced auxin concentration or increased concentration of cytokinins. In some cases addition of some substances were necessary. Bean seed extract was necessary for *Phaseolus vulgaris* regeneration[4] and 5KR gamma radiation was needed for *Cajanus cajan*[5].

Plant regeneration can be initiated from non-meristematic tissue of a number of species. However, explant source can greatly affect the frequency of plant regeneration[6]. Plant regeneration has been reported from different explants using cotyledonary nodes of soyabean[7,8], *Phaseolus vulgaris*[9,10] and *Pisum sativum*[11]. The influence of genotype on regeneration efficiency has been shown in a number of grain legumes[12]. In chickpea shoot regeneration was observed from immature cotyledons[13] and from shoot meristerms[14].

In the present study, different explant of Annigiri of chick pea (Cicer arietinum cv. ICC 4918 were tested for regeneration capability.

The seeds of the *Cicer arietinum* cv. Annigiri were obtained from National Seeds Corporation, Anatapur, Andhra Pradesh. Seeds were surface sterilized with 0.2% $HgCl_2$ for 5 min followed by five rinses with sterile distilled water. The seed were germinated on B_5 basal medium[15] with 3% sucrose and solidified with 0.8% agar. The cultures were grown under fluorescent light with 16h photoperiod at 25±2°C for one week.

Different explants such as hypocotyls, shoot apex, cotyledonary nodes from *in vitro* grown plants were used for regeneration studies. The embryo axis and cotyledons from immature pods were collected from field after 21 days of pollination. Different explants were cultured on B_5 medium supplemented with BA (0.5, 2.0 mg/l) as a sole growth hormone or in combination of IBA, IAA or NAA. Thirty cultures were maintained for each treatment.

The percentage of explants forming shoot and the number of shoots per explant varied with the concentration of BA (Table 1), when B_5 medium was supplemented with 0.5 mg/l BA, the explants produced were green and compact callus which later turned brown due to production of phenolic compounds. Increase in the concentration of BA simultaneously enhanced the frequency of regeneration and the number of shoots per explant. The number of shoots per culture was highest on B_5+ 2 mg/l BA (8.2± 0.6 shoots/explant) in immature

Table 1. The response of different explants of chickpea (Annigiri) on MS medium.

Explant	Frequency of shoot production	Number of shoots explant
Immature Cotyledons	76	8.2±0.6
Hypocotyl	75	6.3±0.4
Embryo axis	68	6.2±0.3
Cotyledonary node	62	3.4±0.3
Shoot Apex	65	3.6±0.2

Minimum number of explants used were 30

cotyledons, embryo, hypocotyls etc.

From immature cotyledons with embryo axis shoot regeneration occurred from the proximal end within 15-21 days. Cotyledons without embryo axis showed green compact callus when cultured at 0.5 and 1.0 mg/l of BA. The response of different explants of ICC 4918 is given in Table 1. Highest number of shoots (8.2 ± 0.6) was recorded from immature cotyledons followed by hypocotyls, embryo axis, shoot apex and cotyledonary nodes. The shoots produced from different explants were excised and rooted on B_5 medium with 1 mg NAA or IBA.

The embryo axis was removed from soaked seeds and cultured on B5 medium with 2 mg/l BA. Within two weeks multiple shoots were observed.

In the above study BA at 2 mg/l found to be most suitable for high frequency shoot induction (Fig. 1 & 2). Similar results were obtained by Barna & Wakhlu[16]. Sudhavai and Reddy[17] obsserved that root formation occurred after 4 weeks of culture on medium supplemented with 1 mg IBA or 1 mg NAA from the shoots. Root formation was not observed. When shoots were cultured on medium lacking growth regulators. They observed direct regeneration from epicotyl explants of cv. JG-62. The response was 100% with 5-6 shoots/explant on B5 medium with 1 mg/l BA + 1 mg/l Kn + 1 mg/l IAA. When used with BA in combination with IAA or IBA the explants failed to form shoots. Gulati and Jaiswal[18] reported plant regeneraton from cotyledonary explants and produced an average of 2 shoots on basal medium. The variation in plant regeneration ability may be attributed to altered levels of endogenous hormones, variation in degree of differentiation, finally their response to exogenous hormones present in the regeneration medium.

It is clear from the results that the axillary buds must be present together with the cotyledon in the explants in order to obtain shoots. Multiple shoot production increase as a function of BA from 0.5 mg/l to 2 mg/of the cultivar Annigiri.

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