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MICROPROPAGATION OF CHICKPEA FROM SHOOT TIP AND NODE SEGMENT

J. D. BARSHILE

Department of Botany,

Shri Anand College, PATHARDI,

District- Ahmednagar-414 102 (M.S.)

E-mail:jagannath_barshile@rediffmail.com

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ABSTRACT

Present investigation was undertaken to standardize technique for *in vitro* micro-propagation of chickpea (*Cicer arietinum*) cultivar Vishwas (Phule G 12). Micropropagation method for chickpea was established and this method enabled much more efficient propagation of plants. The present work was aimed at evolving a protocol for rapid multiplication of chickpea using micropropagation technique. Explants from shoot tip and node segment were cultured on MS medium supplemented with different concentrations of BAP and Kinetin (1.0 to 2.5 mg/l) and their growth responses like shooting were elucidated. The maximum multiple response was observed with 2 mg/l concentration of BAP from both types of explant. The highest number of shoots (12.5 ± 0.3) was achieved on MS medium with 2 mg/l BAP using node segments. The medium supplemented with 2 mg/l of BAP was found better than all other concentrations. Individual shoots were transferred to IBA and IAA (1.0-1.5 mg/l) for root induction. MS medium supplemented with 2 mg/l of IBA proved better for rooting. Rooted plantlets were successfully hardened in greenhouse and established in the pot.

Figure : 01 References : 16 Tables : 03

KEY WORDS : *Cicer arietinum*, Kinetin, Micropropagation, Shoot tips.

Introduction

Chickpea (*Cicer arietinum*) is a widely cultivated important food grain legume in the Indian sub-continent. It is a major source of protein in human diet. It is also used for livestock feed. It is able to fix atmospheric nitrogen symbiotically, which makes it promising in crop rotation in agriculture. It is cultivated on about 10 million ha worldwide with a yield of 8.28 million tons annually[2]. Unfortunately in spite of its importance, yield of chickpea did not witness much appreciation during past decade[3], due to several biotic and abiotic factors. Chickpea is self pollinated grain legume, hence shows limited genetic variability. Hybridization, spontaneous and induced mutations are sources of variation for chickpea breeding. It has been argued that one of the reasons for failure to achieve a breakthrough in productivity of chickpea is the lack of genetic variability[15]. The improvement of chickpea using conventional breeding approaches has been hampered due to lack of sufficient genetic variability. To overcome production constraints through traditional breeding, tools are insufficient and must be assisted by modern biotechnology means. Recent advances in cell and tissue culture allowed the occurrence of new plants with interesting characters[13]. Plant tissue culture, somaclonal variation or genetic variability is source of new useful characters for plant improvement[12]. Although it seems to be an interesting way, its use requires a prerequisite and reliable standardized protocol. For leguminous plants like chickpea, this is rather difficult because of their recalcitrant nature[16]. The conventional techniques deployed in crop improvement may not keep pace with the increasing demands of the chickpea; hence 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. On this background in the present investigation, an attempt has been made for development of effective protocol for tissue culture in chickpea cultivar Vishwas.

Materials and Methods

The seeds of chickpea cultivar Vishwas (Phule G 12) were procured from Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.). Seeds were washed under running tap water. Then seeds were disinfected with 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 5 min. followed by rinsing with sterile distilled water 4-5 times. Seeds were then germinated on moist filter paper in Petri-dishes. The germinated seeds were incubated for 7 days at 25±1°C temperature.

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TABLE-1: Effect of different concentrations of BAP and Kn on shoot induction from shoot tips of chickpea cultivar Vishwas

Growth hormone	Concentration (mg/l)	% of explant showing response	Number of shoots Mean ± SE	Average length of shoot Mean ± SE
BAP	1.0	54.5	3.01 ± 0.28	7.62 ± 0.32
	1.5	70.8	3.15 ± 0.30	8.70 ± 0.41
	2.0	83.3	5.21 ± 0.26	10.87 ± 0.27
	2.5	66.6	4.14 ± 0.33	9.85 ± 0.38
Kn	1.0	61.9	3.16 ± 0.21	4.56 ± 0.19
	1.5	75.0	3.33 ± 0.18	6.21 ± 0.36
	2.0	91.3	5.77 ± 0.35	7.12 ± 0.51
	2.5	80.9	4.25 ± 0.29	5.46 ± 0.37

Selection of explants: Shoot tips and node segments of 7 days old *in vitro* raised seedlings were selected as explants for shoot multiplication. Shoot tips and node segments of 4-5 cm in length excised aseptically.

Establishment of culture and shoot induction: This research work was carried out in the plant tissue culture laboratory of Shri Anand College, Pathardi. Shoot tips and node segments of explant were surface sterilized using 70% ethanol and 1% sodium hypochloride solution for 5 min. Explants were rinsed three times with sterile distilled water and cultured on medium[9] supplemented with different concentrations of cytokinins like BAP (1.0 - 2.5 mg/l) and Kn (1.0 - 2.5 mg/l) were used. The basic nutrient medium contained mineral salts, vitamins, 3% sucrose and 0.7% agar. The pH of media was adjusted to 5.8 before addition of agar. The media were sterilized by autoclaving at 121°C for 30 min. The surface sterilized shoot tips and node segments were inoculated in 25 culture bottles for each treatment. The Cultures were incubated under 16 h fluorescent light (light 3000 lux, temperature 25 ± 1 °C and humidity 60–70%). The numbers of shoots were determined after 30 days of culture. The healthy grown shoots were transferred to the culture media containing different concentrations of auxins such as IBA (1.0 -2.5 mg/l) and IAA (1.0 -2.5 mg/l) for rooting.

Root induction and hardening: The elongated microshoots were transferred to MS medium supplemented with IBA (1.0 -2.5 mg/l) and IAA (1.0 -2.5 mg/l) for root induction. Rooting was observed within two weeks of culture. Rooted plantlets were isolated and washed in running tap water. Later they were transplanted into plastic pot containing sterile FYM and soil mixture (3:1) for hardening. The plantlets were covered with polythene bags to maintain high humidity. These were acclimatized at 25±3°C under 14h photoperiod and watered regularly. 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.

Results and Discussion

Data on shoot induction from shoot tips and node segments explants cultured on MS medium supplemented with different concentrations of BAP and Kn are presented in Table 1 and 2. It was observed that the different concentrations of BAP and Kn were found effective in induction of multiple shoots in both the types of explants in chickpea cultivar Vishwas. The explants type cultured on MS medium supplemented with different concentrations of BAP and Kn showed varied response for shoot induction (Table1 and 2). Among different concentrations of BAP and Kn, 2.0 mg/l concentration of BAP was found significantly effective for inducing multiple shoots in chickpea cultivar Vishwas. 2 mg/l concentration of kn produced the highest number of shoots per node segments as explants (Table 2 and Fig. 1b). However the shoot induction was found to be more on BAP as compared to Kn for explant like shoot tip and node segment might have triggered by the action of BAP in proper way for inducing more number shoots. Our result

TABLE-2: Effect of different concentrations of BAP and Kn on shoot induction from node segments of chickpea cultivar Vishwas

Growth hormone	Concentration (mg/l)	% of explant showing response	Number of shoots Mean ± SE	Average length of shoot Mean ± SE
BAP	1.0	45.0	7.14 ± 0.52	4.21 ± 0.35
	1.5	61.9	8.34 ± 0.43	6.73 ± 0.38
	2.0	75.0	12.53 ± 0.27	7.87 ± 0.51
	2.5	54.1	10.68 ± 0.65	5.95 ± 0.64
Kn	1.0	52.3	6.13 ± 0.49	3.22 ± 0.17
	1.5	61.9	7.24 ± 0.29	3.54 ± 0.24
	2.0	71.4	9.84 ± 0.54	5.28 ± 0.39
	2.5	61.9	7.13 ± 0.47	3.95 ± 0.52

for multiple shoot induction is in agreement with those obtained in *Ocimum* species[1,11].

The node segment explants were found to initiate more multiple shoots than the shoot tips as explant. Average increase in length of shoot is directly proportional to the increase in the concentration of BAP and Kn in both types of explants up to the 2.0 mg/l concentration. As the concentration of BAP and Kn was increased up to 2.0 mg/l there was increase in shoot length gradually, whereas shoot length was found to be decreased when BAP and Kn concentrations were increased above 2.0 mg/l. The shoot tips explants showed better increase in length of shoots as compared to node segment using the BAP and Kn as growth hormones. Nodal explants were also used to get higher rates of shoot multiplication of several plants[13]. More experimental results indicate that the addition of a low concentration of cytokinin in callus culture medium often enhances callus regeneration[4].

Healthy shoots were transferred to half strength

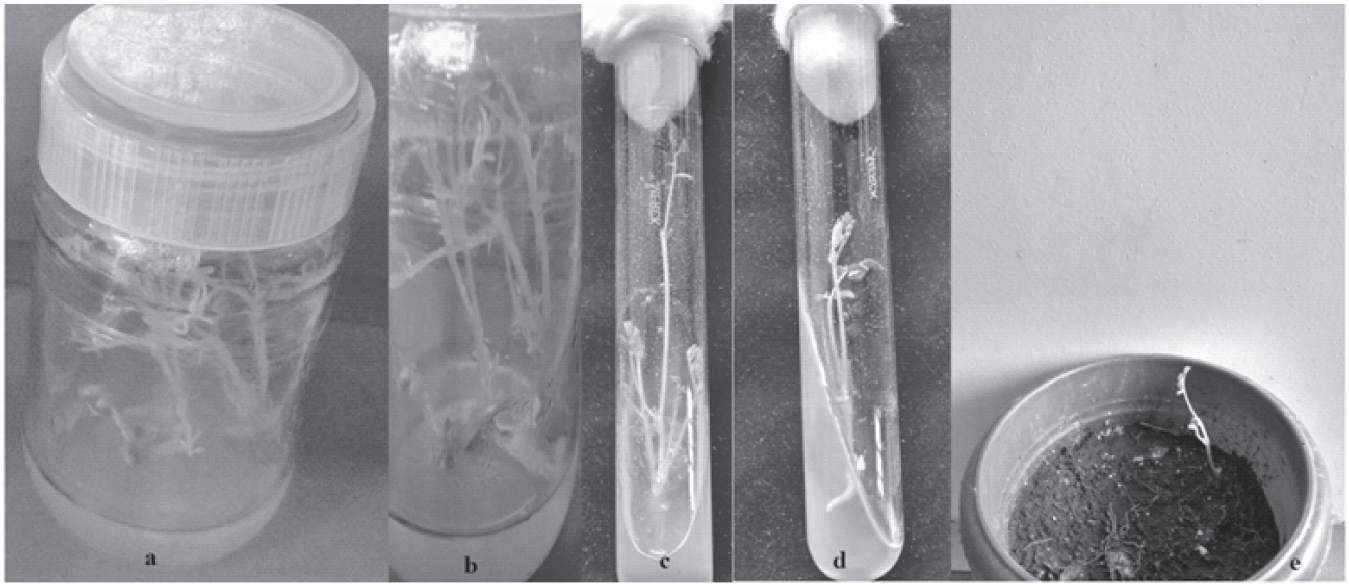

Fig. 1 : *In vitro* culture of chickpea (*Cicer arietinum*) cultivar Vishwas a. induction of multiple shoot from shoot tip b & c. multiple shoots from node segment d. rooting of individual shoot and e. hardening

TABLE-3: Effect of different concentrations of IBA and IAA on rooting of microshoot in chickpea cultivar Vishwas

Growth hormones		% of explant	Number of roots
IBA mg/L	IAA mg/L	showing response	Mean ± SE
00	-	18	1.6 ± 0.12
1.0	-	60	4.3 ± 0.31
1.5	-	74	7.1 ± 0.62
2.0	-	65	6.3 ± 0.42
2.5	-	61	5.2 ± 0.31
-	00	12	1.2 ± 0.19
-	1.0	54	2.3 ± 0.22
-	1.5	68	3.2 ± 0.37
-	2.0	62	5.6 ± 0.45
-	2.5	58	4.8 ± 0.36

MS medium for root induction with different concentration of IBA (1 - 2.5 mg/l) and IAA (1 - 2.5 mg/l). Profuse root development was observed on 1.5 mg/l IBA, compared to 1 - 2.5 mg/l IAA on MS medium. In the present investigation, it was observed that the root induction was maximum (74%) in MS medium supplemented with 1.5% IBA (Table 3). MS medium containing 1.5 mg/l of IBA showed 74% of response regarding root induction with 7.1 ± 0.62 roots per plant. Rooted plantlets were initially hardened under culture conditions (Fig. 1e) and subsequently established in the field conditions. Half strength MS media supplemented with IBA was found to be better for root regeneration and this was in accordance with previous report in *Clitoria*[8]. For rooting of *in vitro* raised shoots, 0.1% IBA was used in soybean[5] and IAA in common bean[6]. It was reported earlier that half strength MS medium induced maximum rooting in cowpea[7]. Our observations for *in vitro* culture of chickpea cultivar Vishwas are in agreement with previous reports of legume.

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