*Pak. J. Bot.*, 39(6): 2067-2073, 2007.

# MULTIPLE SHOOT FORMATION FROM DIFFERENT EXPLANTS OF CHICK PEA (*CICER ARIETINUM* L.)

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**Abstract**

The direct regeneration ability in different explants i.e., shoot and nodal explants of *Cicer* arietinum was investigated on M.S. basal medium supplemented with different concentrations of *Benzyl amino purine* (BAP*).* Rapid and early shoot initiation was observed in lower concentration of BAP. Higher concentrations delayed shoot initiation in both explants. The number of shoots per explant increased with BAP concentration while shoot length decreased with increasing BAP concentration. The overall response of nodal explant was better than shoot apex. The regeneration shoots were further multiplied by sub culturing on fresh medium. The multiple shoots were shifted to full and half strength M.S. medium supplemented with different concentrations of NAA and IBA for induction of roots while the half strength media with 1.0 uM NAA gave excellent response of root induction.

## Introduction

World population has been growing explosively from 1.7 billion people at the beginning of 20$^{th}$ Century to now more than 5 billion and by beginning of new millennium close to 6.5 billion people is to populate our planet. Situation is getting worst in Asia particularly in countries like India and Pakistan where growth rate of population is much higher (Abbas, 1989; Anon., 1998).

The unprecedented picture compel that an intensive environmentally compatible agricultural programme is more important than ever before in the history of mankind. More recently, plant biotechnology has emerged as spectacular discipline of life sciences. It has offered unprecedented opportunities and promises for the development of human resources and economic benefits (Sukapinda, 1993; Gamborg & Philip, 2005). Outstanding findings in plants cell and Tissue Culture- an important discipline of biotechnology have generated great optimism both in developed and developing countries (Mehra, 1993; Popelka *et al*., 2004).

Although many of the economically important plants have been improved regarding yield and productivity through genetic transformation and other cultural techniques, however certain plant species including legumes have generally proved notoriously recalcitrant due to the lack of reliable *In vitro* regeneration system (Barna & Wakhlu, 1993; Odutayo *et al*., 2005; Khawar *et al*., 2004). Efficient regeneration system is prerequisite for cellular and genetic manipulation. Generally, efforts have been concentrated for the potential improvement of legumes through Tissue Culture techniques, because legumes are the main and at time the only source of plant protein and essential amino acids for the inhabitants of developing countries where protein deficiency is common. Legumes are considered as second most important source of food after cereals for humans and animals consumptions (Smith & Jimmerson, 2005).

In legumes, chickpea is one of the most important grain legumes used for human food and animal feed in developing countries and is the rich source of dietary protein 20-22% (Singh, 1990; Mc. New *et al*., 2001). But it's per hectare yield is very low i.e., 500

kg. Many factors are responsible for low yield which include excessive vegetation growth, narrow genetic basis, old cultivated genotypes and poor harvest index due to wilt and blight (Hassan & Khan, 1991). Despite the intensive conventional research efforts and vast agricultural practices, desired production targets in chick pea improvement have not been achieved so far. Looking at the potential and promises of the tissue culture technology, efforts have been directed for implementing this technology to improve productivity by developing the disease resistance and high yield varieties in chick pea. There are very few reports on efficient regeneration system which is prerequisite of cellular and genetic manipulation. Development of highly reproducible and efficient regeneration protocol in chick pea is still awaited.

Keeping in view all these problems, this study was conducted to establish a reproducible protocol for multiple shoot formation from nodal and shoot apices explants.

## Materials and Methods

The seeds of *Cicer arietinum* L.CV, CM72 were thoroughly washed with household detergent for about five minutes. This was followed by second washing with tap $H_2O$ to make the material free from soap. These seeds were treated with 10% of commercial Sodium hypochlorite for 20 min. The seeds were washed with distilled $H_2O$ at least three times or more till the smell of Sodium hypochlorite was completely removed. These sterilized seeds were grown on M.S. basal medium (Murashige & Skoog, 1962) in complete aseptic conditions. These *In vitro* grown two weeks old seedlings were used for the preparation of node and shoot apices explants.

The freshly prepared cultures were grown under carefully regulated temperature and light conditions. The temperature of culture room was $24 \pm 2^{o}C$ and the light intensity varied from 2000-3000 lux with photoperiod of 16 hrs light and 8 hrs dark.

To standardize the medium for induction of multiple shoots, different concentrations of Benzyl amino Purine BAP ranging from 0.1-2.0 mg/l were used in M.S. medium. Observations on frequency of shoot formation, number of shoots/ explants and shoot height in cm were recorded at 30 days after establishing the culture.

The shoots proliferated from different explants were utilized for adventitious root formation to get plantlets *In vitro*. For induction of roots half or full strength of M.S. media with different concentrations of auxins were tried.

## Results and Discussions

Experiments were conducted to investigate the direct regeneration ability of shoot apices and nodal explants. Different concentrations of BAP supplemented to M.S. basal media were used.

Initiation of multiple shoots in both explants in most of the treatments ranged within 7-11 days of inoculation (Table 1). Rapid and early shoots initiation was observed in lower concentrations of BAP i.e., 0.1 mg/l. higher concentration of BAP delayed initiation response. The best shoot proliferation was observed in M.S. + 2.0 mg/l. In this treatment, 98% of nodal (Fig. a) and 89% of shoot apices (Fig. d) explants produced shoots, where the number of shoots were 25.4 per explant in nodal region and 15.2 in shoot apices. The regeneration frequency increased with increase in concentration of cytokinins and 2.0 mg/l was found to be optimal for maximum frequency of shoot bud formation.

**Table 1. Effect of different BAP concentrations on multiple shoot formation from nodal explants.**

| Conc. of BAP (mg/1) | Days to shoot initiation | Frequency of shoot formation (% age) | *No. of shoot/explant | Shoot length (cm) |
|---|---|---|---|---|
| 0.0 | - | - | - | - |
| 0.1 | $7.8 \pm 0.334^{a}$ | 90 | $5 \pm 0.282^{f}$ | $5.6 \pm 0.219^{a}$ |
| 0.25 | $9 \pm 0.4^{cd}$ | 95 | $7.6 \pm 0.219^{e}$ | $4.8 \pm 0334^{ab}$ |
| 0.50 | $8.4 \pm 0.219^{bc}$ | 98 | $11.6 \pm 0.357^{d}$ | $4.6 \pm 0.357^{b}$ |
| 1.0 | $8.8 \pm 0.334^{bcd}$ | 96 | $17 \pm 0.4^{c}$ | $3.4 \pm 0.334^{c}$ |
| 1.5 | $9.8 \pm 0.334^{cd}$ | 90 | $21 \pm 0.282^{d}$ | $3 \pm 0.282^{c}$ |
| 2.0 | $10.4 \pm 0.357^{d}$ | 98 | $25.4 \pm 0.219^{a}$ | $2 \pm 0.447^{d}$ |
| | **1.004** | | **0.903** | **0.916** |

*: Date was collected after 28 days of culture Each value is a mean of three replicate with standard error (Mean ± S.E) a,b,c: Mean with same superscript are not significantly different from each other at 5% level by Duncan' new multiple range test.

**Table 2. Effect of different BAP concentrations on multiple shoot formation from explants of shoot apices.**

| Conc. of BAP (mg/1) | Days to shoot initiation | Frequency of shoot formation (% age) | *No. of shoot/explant | Shoot length (cm) |
|---|---|---|---|---|
| 0.0 | - | - | - | - |
| 0.1 | $8.8 \pm 0.334^{ab}$ | 75 | $4 \pm 0.282^{e}$ | $4.2 \pm 0.334^{a}$ |
| 0.25 | $10.2 \pm 0.334^{bc}$ | 80 | $5.4 \pm 0.219^{d}$ | $3.6 \pm 0.606^{ab}$ |
| 0.50 | $10.2 \pm 0.593^{bc}$ | 82 | $6.2 \pm 0.282^{d}$ | $3.2 \pm 0.178^{b}$ |
| 1.0 | $9.4 \pm 0.537^{b}$ | 85 | $7.6 \pm 0.219^{c}$ | $2.6 \pm 0.219^{bc}$ |
| 1.5 | $9.4 \pm 0.219^{b}$ | 87 | $10 \pm 0.632^{d}$ | $1.8 \pm 0.521^{cd}$ |
| 2.0 | $8.6 \pm 0.357^{a}$ | 89 | $15.2 \pm 0.521^{a}$ | $1 \pm 0.282^{de}$ |
| | **1.25** | | **1.19** | **0.916** |

*: Date was collected after 28 days of culture Each value is a mean of three replicate with standard error (Mean ± S.E) a,b,c: Mean with same superscript are not significantly different from each other at 5% level by Duncan' new multiple range test.

Franklin *et al*., (1998) obtained maximum of 49 shoots on 3.0 mg/l BAP with seedling explants which is a combined cotyledonary node and shoot tip and only five shoots with cotyledonary node. Kumar *et al*., (1983) reported 20% of shoot bud regeneration from cotyledonary callus on Blaydes medium with 2.25 mg/l BAP and 14% from callus leaf tissue. Similar results were obtained by Polisetty *et al*., (1997) in chick pea.

Concentration of BAP also influenced the shoot length. Lower dosages of BAP resulted in increased length, while higher reduced the shoot length. Maximum shoot length (5.6 cm) was observed in BAP 0.1 mg/l (Fig. b, c) while the minimum length was observed (2.0 cm) at higher dosage i.e., BAP 2.0 mg/l in both explants (Table 1). It is inferred that with increase in BAP concentration the number of shoots per explants increased but shoot length decreased. Similar results were obtained in chick pea by Altaf & Ahmed (1986) and Rao & Chopra (1989).

The importance of BAP in multiple shoot formation in chickpea and other legumes is widely reported (Barik *et al*., 2004; Odutayo *et al*., 2005). The results of the presents study revealed that among the two explants used, nodal segments induced higher number of shoots/explants as compared with shoot apex. Singh *et al*., (1982) also reported better efficiency of nodal segments as compared to shoot apices in chick pea. In this study, the response of shoot length was negatively correlated with the increase in BAP concentration. Reduction in the number of shoots with increasing concentration of BAP was reported by Rao & Chopra (1989); Franklin *et al*., (1991) and Rahman *et al*., (2004).

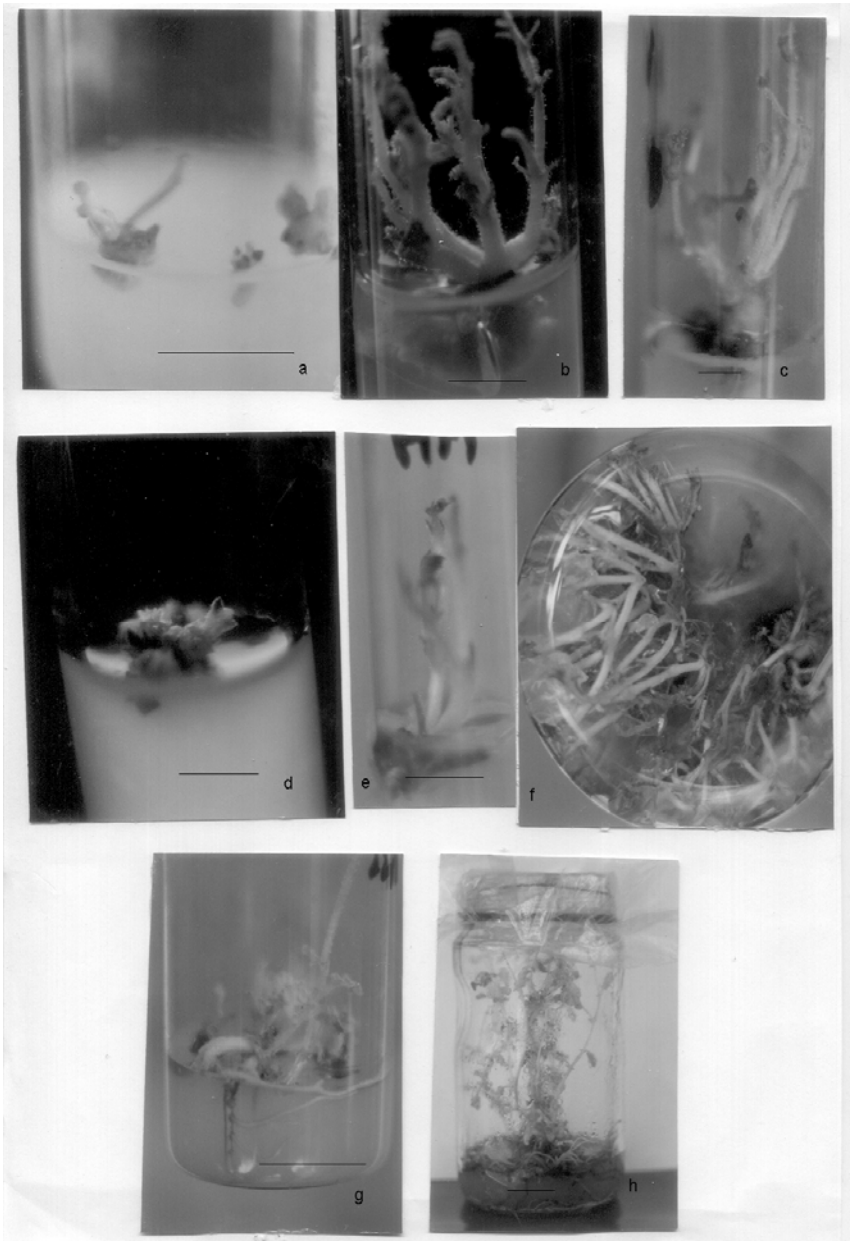


Fig. 1. Multiple shoot formation from different explants of chick pea on M.S medium. **a)** Initiation of shoot formation on BAP 2.0 mg/l after 7 days of incubation. **b-c)** Shoot proliferation after 25 days. **d)** Initiation of shoots on M.S +BAP 2.0mg/l from shoot apices explant. **e)** Shoot proliferation after 28 days. **f)** Multiplication of regenerated shoots. **g)** Emergence of roots from multiple shoots on root induction medium M.S ½+NAA 1uM after 4 weeks. **h)** Full grown plantlets after 6 weeks of incubation.

**Table 3. Effect of auxins on days to root initiation, frequency of root formation from regenerated shoots of chick pea.**

| Treatments (μM) | Days to root initiation | No. of shoots cultured | *No. of cultures showed root induction | Frequency of root formation |
|---|---|---|---|---|
| 0.0 | - | - | - | - |
| MS + NAA 0.5 | $11.6 \pm 0.456^{bc}$ | 10 | $1 \pm 0.4^{c}$ | 10 |
| MS + NAA 1.0 | $12.6 \pm 0.456^{ab}$ | 10 | $2 \pm 0.282^{c}$ | 20 |
| MS + NAA 2.0 | - | 10 | - | - |
| MS + IBA 0.5 | - | 10 | - | - |
| MS + IBA 1.0 | $13.4 \pm 0.219^{a}$ | 10 | $1 \pm 0.282^{c}$ | 10 |
| MS + IBA 2.0 | - | 10 | - | - |
| MS ½ + NAA 1.0 | $12.4 \pm 0.536^{ab}$ | 10 | $9 \pm 0.4^{a}$ | 90 |
| MS ½ + IBA 1.0 | $10.8 \pm 0.521^{c}$ | 10 | $1 \pm 0.4^{c}$ | 10 |
| | **1.08** | | **0.855** | |

Each value is a mean of three replicate with standard error (Mean ± S.E) a, b, c: Mean with same superscript are not significantly different from each other at 5% level by Duncan' new multiple range test.

**Multiplication of regenerated shoots:** To fulfil the increasing requirement of nutrients and space the directly regenerated shoots from both explants were transferred in fresh medium in culture flasks. Proliferation of shoots started and during this secondary proliferation stage, lateral shoots developed from axils of lower leaves of newly initiated shoots. As a result a dense mass of shoots (15-50) was formed in each culture flask (Fig. f). These proliferated shoots were cut into segments containing 2-4 shoots and transferred to fresh medium in jars. These segments gave rise to fresh clumps of 4-9 shoots within 4-5 weeks of transfer. In this way shoot proliferation was maintained for several passages by monthly transfer to fresh medium. (Fig. h) So by reculturing the clumps about 120-150 shoots were produced from single explants with 4-5 passages and maintained for one year.

Kartha *et al*., (1981) also achieved profuse axillary branching from a single shoot of chick pea. These propagated axillary shoots stock were maintained for several months. Maintaince of shoots for several months was also reported by Amin & Jaiswal(1988) Rehman *et al*., (2004)

**Rooting of regenerated shoots:** The proliferated shoots obtained from both explants from initial culture or from subsequent cultures were used for induction of roots by culturing them in full and half strength media with different concentrations of NAA and IBA. Clumps of regenerated shoots were transferred to these media. The rooting response was poor in full strength. M.S. medium containing 1uM NAA improved rooting frequency and continued to grow up to the end of $4^{th}$ week. NAA promoted better root formation as compared to IBA. In full strength MS media the maximum root frequency (20%) was observed in NAA 1u M medium (Table 3), while IBA with same concentration showed 10% frequency.

Keeping in view, the low frequency of root formation in full strength. M.S. media, MS lower salts i.e., ½ MS was tried for unrooted shoots. Like the full strength media, the same two auxins were utilized in half strength media. Of the two auxins tested, NAA at 1.0 uM gave an excellent response. Frequency of root formation was 90% while in IBA supplemented medium was only 10% (Table 3).

Same results were reported by Singh *et al*., (1982) and Phillips & Collins (1979). Among the two auxins used, NAA was found to be better than IBA for root induction. Singh & Singh (1989) and Singh *et al*., (1982) also preferred NAA over IBA for root

induction in chick pea. In pigeon pea, Kumar *et al*., (1983) had observed rooting with IAA 1.0 mg/l or NAA 0.01 mg/l and George & Eapen (1994) obtained 90% of rooting with 0.2 mg/l NAA.

The results obtained in the present study could be highly significant. This efficient and reliable plant regeneration system *via* direct regeneration system can be exploited for improvement in yield and productivity through genetic transformation and other cellular techniques.

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(Received for publication 7 February 2007)