African Journal of Biotechnology Vol. 10(40), pp. 7825-7830, 1 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2378
ISSN 1684–5315 © 2011 Academic Journals

*Full Length Research Paper*

# Callus induction via different growth regulators from cotyledon explants of indigenous chick pea (*Cicer arietinum L.*) cultivars KK-1 and Hassan-2K

**Saleem Khan[1]*, Farhad Ahmad[2], Farhad Ali[1], Hakim Khan[3], Ayub Khan[3] and Zahoor Ahmad Swati[1]**

[1]Institute of Biotechnology and Genetic Engineering, KPK, Agricultural University Peshawar, Pakistan.
[2]Agricultural Research Station, Baffa, Mansehra, Pakistan.
[3]Department of Agriculture, Haripur Campus, Hazara University Mansehra, Pakistan.

Accepted 17 June, 2011

**Callus induction from cotyledon explants was studied in indigenous chick pea (*Cicer arietinum* L.) cultivars KK-1 and Hassan-2K on MS and $B_5$ media containing different combinations and concentrations of growth regulators. Different MS and $B_5$ callusing media containing varying level of 2, 4-D (2 and 4 mg/l), NAA (0.50 and 1 mg/l), BAP (5 and 10 µM) and their combinations were tested for callus induction response. Percent callus and callus fresh weight (g) were recorded after two and four weeks of culture for both genotypes. For KK-1 cultivar, the maximum callus frequency (71 and 97%) followed by (65 and 96%) were observed on 4 mg/l 2,4-D+5 µM BAP in MS and 4 mg/l 2,4-D in $B_5$ media, respectively after two and four weeks of culture. Similarly, the highest callus fresh weight (0.411 and 0.787 g) were also recorded for MS+4 mg/l 2,4-D+5 µM BAP in contrast to $B_5$ where the highest callus weight (0.401 and 0.693 g) was achieved on 4 mg/l 2,4-D only. In Hassan -2K, the highest callus % (68 and 96) and fresh weight (0.572 and 0.821 g) were recorded on MS+4 mg/l 2,4-D+0.50 mg/l NAA after two and four weeks of culture, respectively. In $B_5$ medium, 2,4-D+BAP combination produced average callus induction response for both cultivars.**

**Key words:** Callus induction, chick pea cotyledons, growth regulators.

## INTRODUCTION

Chick pea (*Cicer arietinum* L.) commonly known as Gram, is a vital grain legume of Pakistan. In Asia, India is the largest producer of chick pea. Pakistan ranks second in term of acreages under its cultivation (Hassan and Khan, 1991).

Chick pea is an important source of protein, phosphorous, iron and certain water soluble vitamins. The protein is mainly located in the cotyledons and the embryonic axis, with small amounts present in the testa. Chickpeas are a good source of zinc, folate and are very high in dietary fiber and thus, are a healthy food source of carbohydrates for persons with insulin sensitivity or diabetes (Hulse, 1991).

Chick pea is a temperate crop; self pollinated, annual species with an erect or spreading habit become well adapted to sub-tropical conditions (Schnepf, 1965). Chick pea is highly sensitive to excess moisture; it cannot tolerate heavy rains and hence, is unsuited to wet conditions. It is susceptible to foliar diseases under humid and wet conditions (Muehlbauer and Singh, 1987; Malhotra et al., 1987).

Although, many of the economically important plants have been improved regarding yield and productivity through genetic transformation and other cellular techniques, however, legumes including chick pea have generally proved notoriously recalcitrant due to the lack of reliable *in vitro* regeneration system (Flick et al., 1983;

*Corresponding author. E-mail: khan.pesh@yahoo.com. Tel: +92 334 8431740. Fax: +92 997 511724.

**Abbreviations: MS,** Murashige and Skoog basal medium; **$B_5$**, gamborg $B_5$ vitamins, **2,4-D,** (2,4-dichlorophenoxy acetic acid); **NAA,** naphthylacetic acid α; **BAP,** benzyl aminopurine; **µM,** micro mole.

Barna and Wakhlu, 1993). The potential benefits of using advanced agricultural biotechnology in chick pea genetic improvement have not yet been realized in Pakistan. Establishment of an efficient callus induction protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement. For the successful application of the tissue culture technique in crop breeding, callus growth and plant regeneration potential of each crop must be determined (Khaleda and Al-Forkan, 2006). Production of callus and its subsequent regeneration are the prime in crop plant to be manipulated by biotechnological means and to exploit somaclonal variation. A plant regeneration system is necessary for gene transfer studies and selection for disease resistance (Geber-Medhin et al., 1988).

Much works has been carried out on chick pea to enhance callus induction, improve the frequency of plant regeneration from the callus and investigate the factors affecting plant regeneration. Both callus induction and plant regeneration from explants require the presence of appropriate concentrations and combinations of plant growth regulators in the culture media. Many researchers work to standardize the optimum concentrations of growth regulators for regeneration of chick pea and consequently, great progress has been made in callus induction and plant regeneration. Gosal and Bajaj (1979) reported that cotyledons and immature embryos of *C. arietinum* L. formed calli when incubated on MS (Murashige and Skoog, 1962) medium supplemented with 2 mg $l^{-1}$ of 2,4-dichlorophenoxy acetic acid (2,4-D). Induction of callus and plant regeneration from immature cotyledon explants of chickpea has been reported by Islam and Rizauddin (1994). Organogenic regeneration via callus induction has also been attempted using cotyledons, mature and immature embryo axes, leaflets, distal and proximal cotyledons and hypocotyl explants (Anju and Chawla, 2005). The effect of zeatin, GA3 on regeneration from immature cotyledons of chickpea has been studied by Hita and Gaerra, (1997). This study was undertaken to test the effect of different concentrations and combinations of growth regulators on chick pea callus induction and standardize the protocol for callus induction for two indigenous cultivars of chick pea widely grown in Pakistan, which would be efficient and suitable for the investigation of induced somaclonal variation and successful application of gene transfer technique.

## MATERIALS AND METHODS

### Plant material

Seeds of two indigenous chick pea cultivars, KK-1 and Hassan-2K (Kabuli), collected from Ahmad wala research station, Karak and Nuclear Institute of Food and Agriculture (NIFA), Peshawar, Pakistan respectively, were used as explants.

### Disinfection method

The fresh seed explants of chick pea were washed with clean water for 30 min and then put in a sterilized flask or universal bottle. Surface sterilization was carried out with 0.06 to 0.2% mercuric chloride ($Hg Cl_2$) for 5 to 6 min followed by gentle shaking. After surface sterilization, the seeds were thoroughly washed for several times with sterile distilled water in a laminar flow cabinet for the removal of any traces of $Hg Cl_2$. The seed coat and the embryo itself were removed and each of the two cotyledons was used as an explants. The media were sterilized by autoclaving at a pressure of 15 p.s.i and temperature of 121°C for 20 min. Then, explants were transferred in to sterilized media flask with 20 to 30 ml MS or B5 media supplemented with different phytohormones (2,4-D, NAA and BAP) for callus induction.

### Callus induction

Completely sterilized cotyledon seeds were used for culturing. For callus maintenance, MS (Murashige and Skoog, 1962) media with B5 vitamins (Gamborg et al., 1968) including thiamine (HCl) 10.00 $Mg^{-1}$, pyridoxine (HCl) 1.00 $Mg^{-1}$ and nicotinic acid 1.00 $Mg^{-1}$ were used with different concentrations and combinations of plant growth regulators. The medium contained 30 g/l sucrose (Sigma Chemical Co.) solidified with 8 g/l agar (Sigma Chemical Co.). The pH of the media was adjusted to 5.8 with 1 N HCL and 1 N NaOH before autoclaving. A varied range of phytohormones for NAA and 2,4-D as an auxin and BAP as cytokinins, prepared from Sigma Company, were applied for callus induction. The medium was supplemented with 2 mg and 4 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) alone or with in combination of 0.50 and 1 mg/l of 1-naphthylacetic acid α (NAA) and 5 and 10 µM benzyl amino purine (BAP). Similarly, NAA alone and in different concentrations and combinations with 5 µM (1 ml $L^{-1}$) and 10 µM (2 ml $L^{-1}$) of BAP in MS or $B_5$ media were also used for callus induction. Cultures were incubated at 25 ± 1°C under fluorescent light (2500 to 3000 lux) and 70% humidity in culture room. Data were taken when callus initiates and then their characteristics were recorded after 2 and 4 weeks of culture, respectively.

## RESULTS AND DISCUSSION

This study was conducted to observe the effect of different growth regulators on callus induction from cotyledon explants of two indigenous chick pea (*C. arietinum* L.) cultivars KK-1 and Hassan-2K. Callus induction was recorded onto MS and $B_5$ media containing different concentrations and combinations of 2,4-D (2 and 4 mg/l), NAA (0.50 and 1 mg/l) and BAP (5 and 10 µM) within 2 and 4 weeks of incubation of cotyledon explants depending upon the concentration and combination of hormones (Figure 1). Data were analyzed after 2 and 4 weeks of culture and the result showed that there was a wide range of variations in days to callus initiation, percentage of explants developed callus, callus texture, callus color and degree of callus formation depending on culture media formulations.

Response in tissue culture is highly genotype dependent. Significant genotypic differences in callus initiation response were observed between the two indigenous genotypes investigated. In both of the examined genotypes, callus induction was recorded in all media formulations with in average range of 8 to 12 days of callus initiation. Generally, specifics of the produced calluses on different media were light brown to whitish

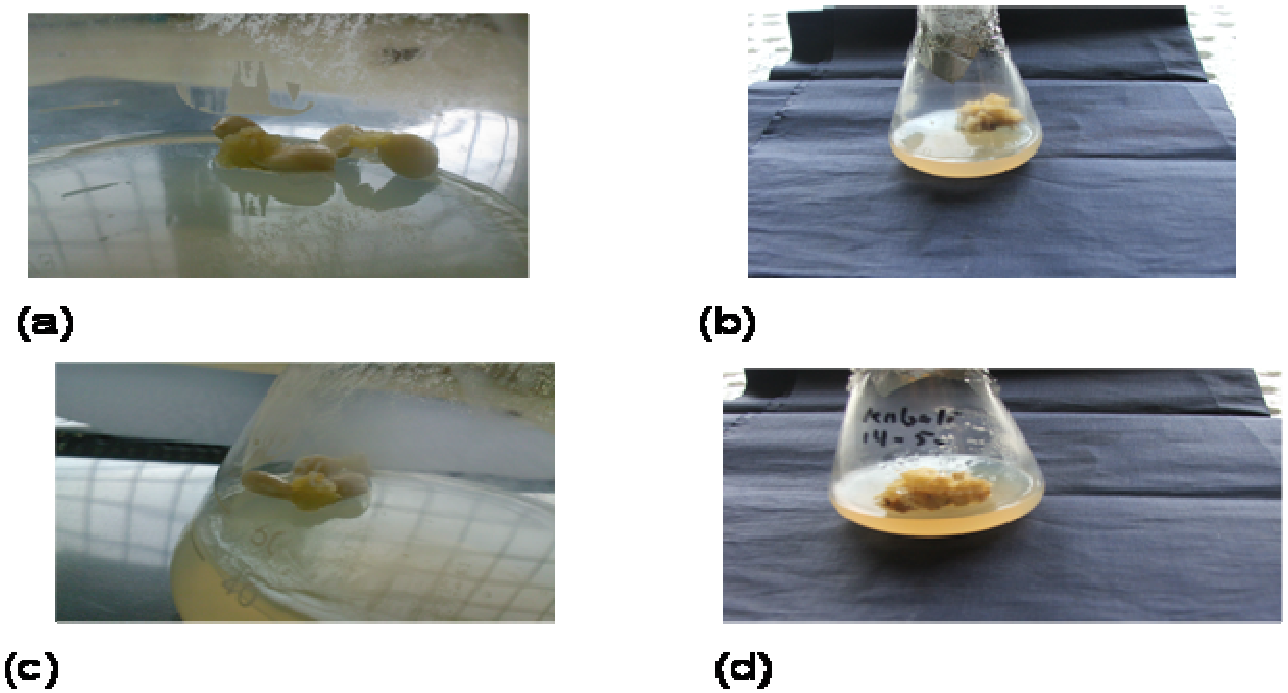


**Figure 1.** Callus induction from cotyledon explants of chick pea in KK-1 cultured on M.S medium containing 4 mg/l 2,4-D+5 µM BAP after 2 weeks of culture (a); 4 weeks of culture (b); in Hassan-2K (Kabuli) cultured on M.S medium containing MS+4 mg/l 2,4-D+0.50 mg/l NAA after 2 weeks of culture (c) and 4 weeks of culture (d).

green and light green with a hard surface on both B5 and MS media along with different concentration and combinations of phytohormones used for induction.

Significant difference between the media for growth of calluses at 5% level was observed. We realized that the selected ranges of phytohormones had significant effects on callus observation. Variance segregation of recorded data on percent callus and callus fresh weight showed that there was significant difference between genotypes in the 5% level. The percentage of callus formed is positively correlated with callus dry weight. In KK-1 cultivar, the highest frequency of callus induction (71%) and (97%) was recorded on MS containing 4 mg/l 2,4-D+5 µM BAP, while the minimum callus percent (30 and 53%) was observed in 2 mg/l 2,4-D alone in MS media after 2 and 4 weeks of culture, respectively (Table 1). It seemed that by increasing the 2,4-D in combination of NAA as an auxin, the callus inductions rates increased and induction time decreased significantly. Lower concentration of NAA (0.50 mg/l NAA) and high concentration of 2,4-D (4 mg/l 2,4-D) favored callus formation in both of MS and B5 media. Similar observations were recorded by Abdellatef et al. (2008) and Vesna et al. (1991) for cotton and pumpkin species, respectively. Similarly, the highest callus growth in term of fresh weight (0.411 and 0.787 g) was noticed in MS medium fortified with 4 mg/l 2,4-D+5 µM BAP and (0.401 and 0.693 g) on 4 mg/l 2,4-D when used in $B_5$ medium. The results showed that BAP as a cytokinin was more suitable in lower concentration (5 µM), whereas high concentration of this hormone is sub optimal in term of callus induction. Callus fresh weight increased significantly with lowering BAP and increasing 2,4-D concentration in both formulated media. This kind of auxin (2,4-D) alone or in combination with cytokinin (BAP) 100% callus induction has been reported by Panday and Ganopathy (1984). In over all auxin and cytokinin, combination in MS media showed better results for callus induction when compared with $B_5$ medium where callus growth average were recorded (Table 1).

Hassan-2K cultivar also showed a wide range of variations in the percentage of callus formation and average fresh weight. There was significant difference between calli formed among media (Table 2). MS medium showed the highest percentage and dry weight callus followed by B5 medium. Maximum callus % ( 68 and 96) and (65 and 64) callus fresh weight (0.572 and 0.821 g) and (0.520 and 0.800 g) were achieved on combination of higher auxin (4 mg/l 2,4-D) and lower cytokinin (0.50 mg/l NAA) concentration when used in MS and $B_5$ medium, respectively for both incubation periods (Table 2). In this study, it was observed that 2,4-D without cytokinin could induce callus but for better proliferation, cytokinin such as BAP and NAA were required. Castillo et al. (1998) reported that auxin 2,4-D by itself or in combination with cytokinins has been widely used to enhance callus induction and maintenance. Moreover, many researchers observed 2,4-D as the best auxin for callus induction as common as in monocot and dicot

**Table 1.** Effect of different concentrations and combinations of phytohormones on callus induction of KK-1 variety of chick pea when used in MS or $B_5$ media.

| Treatment | Day to callus | Color | Texture | After 2 week of culture | | After 4 week of culture | |
|---|---|---|---|---|---|---|---|
| | | | | % callus | callus fresh weight (g) | % callus | callus fresh weight (g) |
| MS+2 mg/l 2,4-D | 10-12 | LG | C | 30 | 0.211 | 53 | 0.537 |
| MS+4 mg/l 2,4-D | 10-12 | WG | C | 48 | 0.244 | 81 | 0.482 |
| MS+0.50 mg/l NAA | 8-12 | WG | C | 39 | 0.323 | 88 | 0.628 |
| MS+1 mg/l NAA | 10-12 | LB | F | 37 | 0.278 | 71 | 0.398 |
| MS+2 mg/l 2,4-D+0.50 mg/l NAA | 8-10 | LB | C | 42 | 0.214 | 78 | 0.582 |
| MS+4 mg/l 2,4-D+0.50 mg/l NAA | 8-12 | LG | C | 68 | 0.314 | 96 | 0.721 |
| MS+2 mg/l 2,4-D+1 mg/l NAA | 10-12 | WG | F | 43 | 0.124 | 83 | 0.321 |
| MS+4 mg/l 2,4-D+1 mg/l NAA | 10-12 | LG | F | 38 | 0.299 | 88 | 0.453 |
| MS+0.50 mg/l NAA+5 µM BAP | 8-10 | WG | C | 66 | 0.345 | 78 | 0.637 |
| MS+0.50 mg/l NAA+10 µM BAP | 10-12 | WG | C | 59 | 0.243 | 79 | 0.567 |
| MS+1 mg/l NAA+5 µM BAP | 10-12 | LG | C | 54 | 0.345 | 81 | 0.732 |
| MS+1 mg/l NAA+10 µM BAP | 10-12 | LB | C | 42 | 0.287 | 78 | 0.499 |
| MS+2 mg/l 2,4-D+5 µM BAP | 8-10 | LG | C | 44 | 0.251 | 85 | 0.387 |
| MS+2 mg/l 2,4-D+10 µM BAP | 8-10 | LG | C | 38 | 0.351 | 83 | 0.563 |
| MS+4 mg/l 2,4-D+5 µM BAP | 10-12 | LG | F | 71 | 0.411 | 97 | 0.787 |
| MS+4 mg/l 2,4-D+10 µM BAP | 10-12 | WG | F | 43 | O.267 | 91 | 0.569 |
| **Mean** | | | | **47.62**[a] | **0.281**[a] | **81.87**[b] | **0.553**[b] |
| $B_5$+2 mg/l 2,4-D | 10-12 | LG | C | 35 | 0.278 | 53 | 0.429 |
| $B_5$+4 mg/l 2,4-D | 10-12 | LG | C | 65 | 0.401 | 96 | 0.693 |
| $B_5$+0.50 mg/l NAA | 8-12 | WG | C | 35 | 0.315 | 64 | 0.683 |
| $B_5$+1 mg/l NAA | 10-12 | LB | F | 27 | 0.239 | 69 | 0.348 |
| $B_5$+2 mg/l 2,4-D+0.50 mg/l NAA | 8-10 | LB | C | 44 | 0.234 | 73 | 0.595 |
| $B_5$+4 mg/l 2,4-D+0.50 mg/l NAA | 8-12 | LG | C | 58 | 0.454 | 89 | 0.663 |
| $B_5$+2 mg/l 2,4-D+1 mg/l NAA | 10-12 | WG | F | 35 | 0.112 | 74 | 0.341 |
| $B_5$+4 mg/l 2,4-D+1 mg/l NAA | 10-12 | LG | F | 41 | 0.336 | 90 | 0.498 |
| $B_5$+0.50 mg/l NAA+5 µM BAP | 10-12 | LG | C | 43 | 0.223 | 61 | 0.400 |
| $B_5$+0.50 mg/l NAA+10 µM BAP | 10-12 | WG | C | 37 | 0.221 | 56 | 0.383 |
| $B_5$+1 mg/l NAA+5 µM BAP | 10-12 | LG | C | 47 | 0.268 | 68 | 0.456 |
| $B_5$+1 mg/l NAA+10 µM BAP | 10-12 | LB | C | 42 | 0.233 | 60 | 0.357 |
| $B_5$+2 mg/l 2,4-D+5 µM BAP | 10-12 | LG | C | 33 | 0.156 | 68 | 0.258 |
| $B_5$+2 mg/l 2,4-D+10 µM BAP | 10-12 | LG | C | 36 | 0.187 | 65 | 0.287 |
| $B_5$+4 mg/l 2,4-D+5 µM BAP | 10-12 | LG | F | 51 | 0.345 | 73 | 0.568 |
| $B_5$+4 mg/l 2,4-D+10 µM BAP | 10-12 | LG | F | 42 | O.234 | 69 | 0.462 |
| **Mean** | | | | **41.93**[a] | **0.264**[a] | **70.50**[c] | **0.463**[d] |
| LSD at 5% between treatment means | | | | 12 | 1.21 | 16.5 | 2.3 |

MS and $B_5$ means with same letters are not significantly different. F = Friable; C = compact; LB = light brown; LG = light green; WG = white green.

(Evans et al., 1984; Ho and Vasil, 1983; Jaiswal and Naryan, 1985; Chee, 1990; Mamun et al., 1996). The induction percentage of callus formation and dry weight of callus formed were increased with increasing incubition time. These results are similar to those reported by Huda et al. (2003).

Similarly, in Hassan-2K high concentration of BAP (10 µM) stimulated more callus proliferation when compared with lower concentration of BAP (5 µM), whereas high combination of 2,4-D (4 mg/l) and lower NAA (0.50 mg/l) also showed better results in term of callus percentage and fresh weight when used in MS medium in contrast to $B_5$, where it showed average callus results with a BAP (Table 2). Rao and Chopra (1987) have reported that initiation and development of calli were influenced by the medium and chick pea genotypes. These genotypic

**Table 2.** Effect of different concentrations and combinations of phytohormones on callus induction of Hassan-2K variety of chick pea when used in MS or $B_5$ media.

| Treatment | Days to callus | Color | Texture | After 2 week of culture | | After 4 week of culture | |
|---|---|---|---|---|---|---|---|
| | | | | % callus | callus fresh weight (g) | % callus | callus fresh weight (g) |
| MS+2 mg/l 2,4-D | 10-12 | LG | C | 41 | 0.247 | 57 | 0.523 |
| MS+4 mg/l 2,4-D | 10-12 | WG | C | 45 | 0.296 | 77 | 0.534 |
| MS+0.50 mg/l NAA | 8-10 | WG | C | 46 | 0.336 | 86 | 0.669 |
| MS+1 mg/l NAA | 10-12 | LB | F | 34 | 0.243 | 68 | 0.377 |
| MS+2 mg/l 2,4-D+0.50 mg/l NAA | 8-10 | LB | C | 53 | 0.287 | 81 | 0.598 |
| MS+4 mg/l 2,4-D+0.50 mg/l NAA | 8-10 | LG | C | 68 | 0.572 | 96 | 0.821 |
| MS+2 mg/l 2,4-D+1 mg/l NAA | 10-12 | WG | F | 48 | 0.378 | 88 | 0.545 |
| MS+4 mg/l 2,4-D+1 mg/l NAA | 10-12 | LG | F | 43 | 0.323 | 78 | 0.634 |
| MS+0.50 mg/l NAA+5 µM BAP | 8-10 | WG | C | 61 | 0.365 | 76 | 0.563 |
| MS+0.50 mg/l NAA+10 µM BAP | 8-10 | WG | C | 61 | 0.347 | 92 | 0.664 |
| MS+1 mg/l NAA+5 µM BAP | 10-12 | LG | C | 51 | 0.336 | 75 | 0.600 |
| MS+1 mg/l NAA+10 µM BAP | 10-12 | LB | C | 54 | 0.466 | 85 | 0.584 |
| MS+2 mg/l 2,4-D+5 µM BAP | 8-10 | LG | C | 42 | 0.211 | 76 | 0.356 |
| MS+2 mg/l 2,4-D+10 µM BAP | 8-10 | LG | C | 46 | 0.456 | 81 | 0.563 |
| MS+4 mg/l 2,4-D+5 µM BAP | 10-12 | LG | F | 59 | 0.345 | 82 | 0.567 |
| MS+4 mg/l 2,4-D+10 µM BAP | 10-12 | WG | F | 60 | 0.470 | 92 | 0.678 |
| **Mean** | | | | **50.75[a]** | **0.354[a]** | **80.62[b]** | **0.579[b]** |
| $B_5$+2 mg/l 2,4-D | 12-12 | LG | C | 39 | 0.245 | 53 | 0.567 |
| $B_5$+4 mg/l 2,4-D | 10-12 | WG | C | 47 | 0.309 | 81 | 0.511 |
| $B_5$+0.50 mg/l NAA | 8-10 | WG | C | 49 | 0.376 | 78 | 0.621 |
| $B_5$+1 mg/l NAA | 10-12 | LB | F | 45 | 0.289 | 62 | 0.364 |
| $B_5$+2 mg/l 2,4-D+0.50 mg/l NAA | 8-10 | LB | C | 56 | 0.267 | 79 | 0.545 |
| $B_5$+4 mg/l 2,4-D+0.50 mg/l NAA | 8-10 | LG | C | 65 | 0.520 | 94 | 0.800 |
| $B_5$+2 mg/l 2,4-D+1 mg/l NAA | 10-12 | WG | F | 44 | 0.334 | 83 | 0.513 |
| $B_5$+4 mg/l 2,4-D+1 mg/l NAA | 10-12 | LG | F | 46 | 0.387 | 81 | 0.667 |
| $B_5$+0.50 mg/l NAA+5 µM BAP | 8-10 | WG | C | 33 | 0.273 | 64 | 0.432 |
| $B_5$+0.50 mg/l NAA+10 µM BAP | 8-10 | WG | C | 45 | 0.285 | 78 | 0.523 |
| $B_5$+1 mg/l NAA+5µM BAP | 10-12 | LG | C | 43 | 0.259 | 71 | 0.506 |
| $B_5$+1 mg/l NAA+10 µM BAP | 10-12 | LB | C | 47 | 0.357 | 74 | 0.467 |
| $B_5$+2 mg/l 2,4-D+5 µM BAP | 8-10 | LG | C | 36 | 0.150 | 76 | 0.200 |
| $B_5$+2 mg/l 2,4-D+10 µM BAP | 8-10 | LG | C | 39 | 0.356 | 78 | 0.446 |
| $B_5$+4 mg/l 2,4-D+5 µM BAP | 10-12 | LG | F | 61 | 0.349 | 74 | 0.467 |
| $B_5$+4 mg/l 2,4-D+10 µM BAP | 10-12 | WG | F | 42 | O.310 | 82 | 0.558 |
| **Mean** | | | | **46.06[b]** | **0.316[a]** | **75.50[c]** | **0.511[b]** |
| LSD at 5% between treatment means | | | | 13.5 | 1.57 | 10 | 1.5 |

MS and $B_5$ means with same letters are not significantly different. F = Friable; C = compact; LB = light brown; LG = light green; WG = white green.

differences with respect to callus initiation were also observed in many other plants (Lee et al., 2004; Wang et al., 2004; Burbulis et al., 2007). Among all the growth regulators used, 2,4-D was found to be the most effective growth regulator for chick pea callus induction either when used alone or in combinations with cytokinins.

In conclusion, the standardize protocol established in this study for callus induction of chick pea on different concentrations and combination of phytohormones can get enough callus and plant regeneration efficiency to perform transgenic operation. Moreover, as the potentiality of shoot multiplication from callus continued for a

long time, regenerates may be characterized by soma-clonal variation and giving birth to traits of agronomic importance.

**REFRENCES**


Abdellatef E, Khalafallah MM (2008). Influence of growth regulators on callus induction from hypocotyls of medium staple cotton (*Gossypium hirsutum* L). Cultivar Barac B -67. J. Soil Nat. 2(1): 17-22.

Anju A, Chawla HS (2005). Organogenic plant regeneration via callus induction in chick pea (*Cicer arietinum* L.) Role of genotypes, growth regulators and explants. Indian J. Biotechnol. 4:251-256.

Barna KS, Wakhlu AK (1993). Somatic embryogenesis and plant regeneration from callus cultures of chickpea (*Cicer arietinum* L.). Plant cell Rep., 12(9): 521-524.

Burbulis N, Blinstrubiene A, Sliesaravicius A, Kupriene R (2007). Some factors affecting callus induction in ovary culture of flax (*Linum usitatissimum* L.). Biologia, 53: 21-23.

Castillo AM, Egana B, Sanz JM, Cistue L (1998). Somatic embryogenesisnand plant regeneration from barley cultivars grown in Spain. Plant Cell Rep. 17: 902-906.

Chee PP (1990). High frequency of somatic embryogenesis and recovery of fertile cucumber plants. Hort. Sci. 25: 792-793.

Evans DA, Sharp WR, Bravo JE (1984). Cell culture methods for crop improvement. In: Sharp WR, Evans DA, Ammirato PV, Yamada Y (Eds.). Hand Book of Plant Cell Culture. Vol. 2. Macmillan Pub. Company, New York.

Flick CE, Evans DE, Sharp WR (1983). Organogenesis in leguminosea. In: Handbook of Plant Cell Culture. McMillan Pub. New York.1: 29-33.

Gamborg OL, Miller RA, Ojima K (1968). Nutrient requirements for suspension cultures of soybean root cells. Exp Cell Res. 50: 151-158.

Geber-Medhin G, Traenkner A, Weltzien HC (1988). *In vitro* regeneration of *vicia faba* L. Fabis News-letter, 21: 14-16.

Gosal SS, Bajaj YPS (1979). Establishment of callus tissue culture and the induction of organogenesis in some grain legumes. Ind. J. Exp. Biol. 2: 154-160.

Hassan S, Khan I (1991). Improvement in chick pea production through induced mutations. Int. Chickpea Newslett. 25: 12-13.

Hita OL, Gaerra H (1997). Somatic embryogenesis from chickpea (*Cicer arietinum* L.) immature cotyledons: The effect of zeatin, giberellic acid and inole-3-butyrie acid. Acta Physiol. Plant, 19(B): 333-338.

Ho WO, Vasil IK (1983). Somatic embryogenesis in sugarcane (*Saccharum officinarum* L.) the morphology and physiology of callus formation and the ontogeny of somatic embryos. Protoplasm, 118: 169-180.

Huda S, Islam R, Bari MA, Asaduzzaman M (2003). Shoot differentiation from cotyledon derived callus of chick pea (*Cicer arietinum* L.). Plant Tissue Culture 13(1): 53-59.

Hulse JH (1991). Nature, composition and utilization of grain legumes. Uses of tropical Legumes: Proceedings of a Consultants, ICRISAT Center. ICRISAT, Patancheru, India, pp. 11-27.

Islam R, Riazuddin S (1994). Somatic embryogenesis from immature cotyledons of chickpea. Pak. J. Bot. 26(1): 197-199.

Jaiswal VS, Naryan P (1985). Regeneration of plantlets from the callus of stem segment of adult plants of *Fucus religosi* L. Plant Cell Rep., 4: 256-258.

Khaleda L, Al-Forkan M (2006). Genotypic variability in callus induction and plant regeneration through somatic embryogenesis of five deepwater rice (*Oryza sativa* L.) cultivars of Bangladesh. Afr. J. Biotechnol. 5(16): 1435-1440.

Lee S, Kim B, Won S, Jo J, Kim K, Park G, Sung B, Lee H, Lee B (2004). Factors affecting callus induction and plant regeneration from mature seed of zoysiagrass (*Zoysia japonica* Steud.) J. Kor. Soc. Gras. Sci. 24(1):29-36.

Malhotra RS, Pundir RPS, Slinkard AE (1987). Genetic resources of chick pea. The Chick pea. C.A.B. Inter. Cambrian News Ltd, Aberystwyth, UK. 67-81.

Mamun ANK, Islam R, Reza MA, Joadar OI (1996). In vitro differentiation of plantlet of tissue culture of *Samonea saman*. Plant Tissue Cult., 6: 1-5.

Muehlbauer FJ, Singh KB (1987). Genetics of chick pea. The Chick pea. CAB. Inter. Wallingford, Oxon, OX10 8DE, UK. pp. 99-125.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 443-497.

Panday R, Ganopathy PS (1984). The influence of media and organogenesis in chick pea. Int. Chickpea Newslett. 17: 7-10.

Rao BG, Chopra VL (1987). Genotypic and explant differences in callus initiation and maintenance in chick pea. Int. Chickpea Newslett. 17: 10-12.

Schnepf E (1965). Light and electron mikroskopi sche beobachtungen an den trichom hydothen von *Cicer arietinum* LZ. Pfl. Physiol. 53: 245-254.

Vesna K, Sibila J (1991). The influence of plant growth regulators on callus induction in pumpkin *(Cucurbita pepo* L.) hairy roots. Int. I. Ue. Iliol. 15: 265-268.

Wang J, Sun Y, Hu J, Cui G (2004). Factors affecting the frequencies of callus induction and plantlet regeneration in maize immature embryo culture. Acta Agron. Sin., 30: 398-402.