African Journal of Biotechnology Vol. 8 (4), pp. 591-596, 18 February, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Regeneration potential of seedling explants of chilli (*Capsicum annuum*)

M. Ashrafuzzaman[1,3]*, M. M. Hossain[2], M. Razi Ismail[3], M. Shahidul Haque[2], S. M. Shahidullah[3] and Shahin-uz-zaman[2]

[1]Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.
[2]Department of Biotechnology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.
[3]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Malaysia.

Accepted 27 January, 2009

A study was conducted with hypocotyl, cotyledon and shoot tip of chilli as explants for regeneration on MS medium supplemented with different concentrations and combinations of auxins and cytokinins. Regeneration potential was determined by two ways. One is regeneration of shoot via callus formation from hypocotyls and cotyledon explants; another was direct shoot regeneration from shoot tip explant. The highest callus was induced from hypocotyl in a combination of BAP (5.0 mgL^{-1}) with NAA (0.1 mgL^{-1}) and cotyledon in a combination of BAP (5.0 mgL^{-1}) with IAA ((1.0 mgL^{-1}). The callus induction as well as shoot initiation was higher in hypocotyls than cotyledon. Shoot tips regenerated into plantlets directly with sporadic small callus at the base. Shoot elongation was accelerated by using additional supplementation of GA_3 and $AgNO_3$. Regenerated shoots rooted best on the MS medium supplemented with 0.1 mg L^{-1} NAA + 0.05 mg L^{-1} IBA.

Key words: Chilli, hypocotyl, cotyledon, shoot tip, explants, hormone.

INTRODUCTION

Chilies are cultivated for vegetables as well as condiments and also used around the world as sweet peppers, pungent chili peppers, or as a source of dried powders of various colors (Ravishankar et al., 2003). To meet the increasing demand for the crops, faster propagation techniques for mass multiplication have become imperative. Conventional methods usually utilize huge amounts of seeds, which results in the wastage of the crop significantly. Cross pollination behavior of chilli plants is a constraint to propagation of agronomic traits and for commercial seed production. Short span of viability and low germination rate of the seeds are also the limitation of the propagation through seeds. Tissue culture methods provide a way for the asexual multiplication of chilli plants as the plants lack natural vegetative propagation. Therefore, *in vitro* tissue culture followed by gene transfer could be an easy, efficient and economic means for obtaining large number of disease-free, consistently uniform and true-to-type plants within a short span of time to improvement of yield and quality.

The genus capsicum is recalcitrant with regard to its *in vitro* regeneration potential (Liu et al., 1990), which in turn makes it difficult or efficient to apply recombinant DNA technologies via genetic transformation aimed at genetic improvement against pests and disease (Ochoa and Ramirez, 2001). *In vitro* plant regeneration of chilli has been achieved via protoplast, hypocotyls, cotyledons, young leaves, direct somatic embryogenesis and shoot organogenesis from seedling explants of different chilli cultivars (Christopher and Rajam, 1996; Gunay and Rao, 1978; Diaz et al., 1988). However, these procedures failed or to be modified when they were used to regenerate plants from other pepper varieties. Thus, the strong influence is pepper variety in regeneration from various explants and this makes it necessary to optimize *in vitro* propagation protocols for the specific cultivars (Ochoa-

*Corresponding author. E-mail: ashraf.u.zaman@gmail.com. Phone: +880 (0) 91 55 695 ext. 2460; GSM: + 880 (0) 171 738 8991; Fax: +880 (0) 91 55 810.

Abbreviations: BAP, 6-benzyle amino purine; **NAA**, α-naphthalene acetic acid; **IBA**, indole-butyric acid; **IAA**, indole-3-acetic acid; **MS**, Murashige and Skoog's (1962) medium.

Alejo and Ireta-Moreno, 1990). Low differentiation frequency, difficulty in shoot elongation, and low repeatability are also main barriers to the development of pepper gene engineering (Dabauza and Pena, 2001).

Therefore, in the present study, an attempt has been made to develop a simple, efficient *in vitro* propagation protocols for clonal propagation of the most popular cultivar of chilli plants by using cotyledon, hypocotyl and shoot-tips from *in vitro*-regenerated plants.

MATERIALS AND METHODS

Seeds of chilli (*Capsicum annuum*) were used to raise seedlings for the present study. Hypocotyls, cotyledon and shoot tip of chilli seedlings were used as explants. The seeds were collected from the local market of Mymensingh. They were first washed with tap water for 3 - 5 min to reduce the level of surface organisms and then treated with distilled water mixed with Twine 20. Later they were surface sterilized by immersion 70% ethanol for 5 min with vigorous shaking followed by 5 min in 4% NaOCl and rinsed 5 times with sterile distilled water. Sterilized seeds were then placed in Petri dishes for germination.

Seed germination was done in Petri dishes containing water soaked blotting paper. Twelve seeds were placed in each Petri dish. The Petri dishes were then incubated in an incubation room till the germination of seeds. Hypocotyls (2 - 3 mm in length) and cotyledons (1 - 2 mm length) from 11 day old seedlings were excised into pieces using sterilized surgical blades. Four segments were placed horizontally in each vial and gently pressed into the surface of the sterilized culture medium with various combinations of growth regulators like BAP, NAA, IAA, IBA and Kinetin. Shoot tips (1 - 2 mm length) were obtained from the seedlings by cut into pieces and placed vertically in the media supplemented with hormone combinations for the direct regeneration of shoots.

The culture vials containing explants were placed under fluorescent light in a room with controlled temperature (22 ± 2°C) using 16 h photoperiod. Callus was initiated 15 - 22 days after inoculation. Three weeks after inoculation of explants, calli attained convenient size and then they were removed aseptically from the vial on a sterilized glass plate inside the laminar air flow cabinet and were placed again on freshly prepared sterilized medium containing appropriate hormonal supplements for shoot initiation from callus. After shoot initiation, it was subcultured again on freshly prepared sterilized medium containing appropriate hormonal supplements for root initiation.

Parameters studied

Callus induction (%): Number of explants formed callus recorded and the percentage of callus induction was calculated as:

$$\text{Callus induction (\%)} = \frac{\text{No.of explants showing callus}}{\text{No.of explants inoculated}} \times 100$$

Days to callus initiation: Number of days required for initiation of callus from the day of inoculation was recorded. The number of callus initiated over a number of days recorded. The mean value of data provided the days required for callus initiation.

Days to shoot initiation: Shoot initiation started from 15 - 18 days on incubation of explants. The number of shoots proliferated over a number of days were recorded. The mean value of the data provided the days required for shoot initiation.

Number of roots per shoot: Number of roots per shoot was recorded and the mean was calculated.

Days to root initiation: Days required for initiation of root from the day of implantation was recorded. The number of roots proliferated over a number of days was recorded. The mean value of data provided the days required for root initiation.

Number of calli showing root: Number of calli with root was recorded and the percentage of root formation was calculated as:

$$\text{Root formation (\%)} = \frac{\text{No.of calli with root}}{\text{No.of inoculated calli}} \times 100$$

Number of roots per callus: Number of roots per callus was recorded and the mean was calculated.

The experiment was conducted with CRD design. Ten replicates were used for each treatment and all the experiments were repeated twice for confirmation. The mean values of different treatments were analyzed using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test.

RESULTS

Callus induction

Hypocotyl and cotyledon explants cultured on MS media supplemented with BAP in combinations with NAA, IAA or $AgNO_3$. Among the hormone combinations, callus formation potentiality of hypocotyl was the highest (95%) in 5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA while the potentiality of cotyledon was (80%) in 5.0 mgL^{-1} BAP + 1.0 IAA (Table 1 and Figure 1b). Minimum days required for callus initiation when cotyledon and hypocotyl cultured on MS media with 5.0 mgL^{-1} BAP + 3.0 mgL^{-1} $AgNO_3$. The size of calli was bigger and shiny on this medium (Figure 1a).

The callus induction from hypocotyl and cotyledon was studied on MS medium either with different concentrations (2 - 4 mg L^{-1}) of BAP and kinetin. The callus induction potentiality of the treatments was not significantly different at different concentrations of BAP. But in case of kinetin callus induction percentage was significantly different at various concentrations. The ability of callus induction from hypocotyl was higher with the growth regulator BAP (95%) than with kinetin (Table 2). The induction potentiality of cotyledon increased as the concentration of BAP increased and it was 85% at 4 mgL^{-1} BAP. On the other hand, the induction potentiality was only 50% when kinetin was used instead of BAP with equal concentration i.e. 4 mg L^{-1} (Table 3). So BAP is more effective hormone than kinetin. Between the two explants, hypocotyl had higher potentiality of callus induction than cotyledon.

Shoot regeneration

Calli derived from hypocotyl and cotyledon explants were transferred to the shooting media supplemented with 5.0 mgL^{-1} BAP in combination with NAA, IAA and $AgNO_3$ or (2 - 4 mgL^{-1}) BAP alone. The media containing 5.0 mg L^{-1} BAP in combination with 3.0 mg L^{-1} $AgNO_3$ had the best (90%) shoot regeneration (Table 4, Figure 1e) while the

Table 1. Combined effect of different supplements and explants on % callus induction and days to callus initiation.

Supplement	% Callus induction		Days to callus initiation	
	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon
5.0 mg L^{-1} BAP + 3.0 mg L^{-1} Ag NO_3	80.0ab	85.0ab	17	15
5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA	95.0a	80.0b	20	18
5.0 mg L^{-1} BAP + 1.0 mg L^{-1} IAA	70.0b	90.0a	20	22

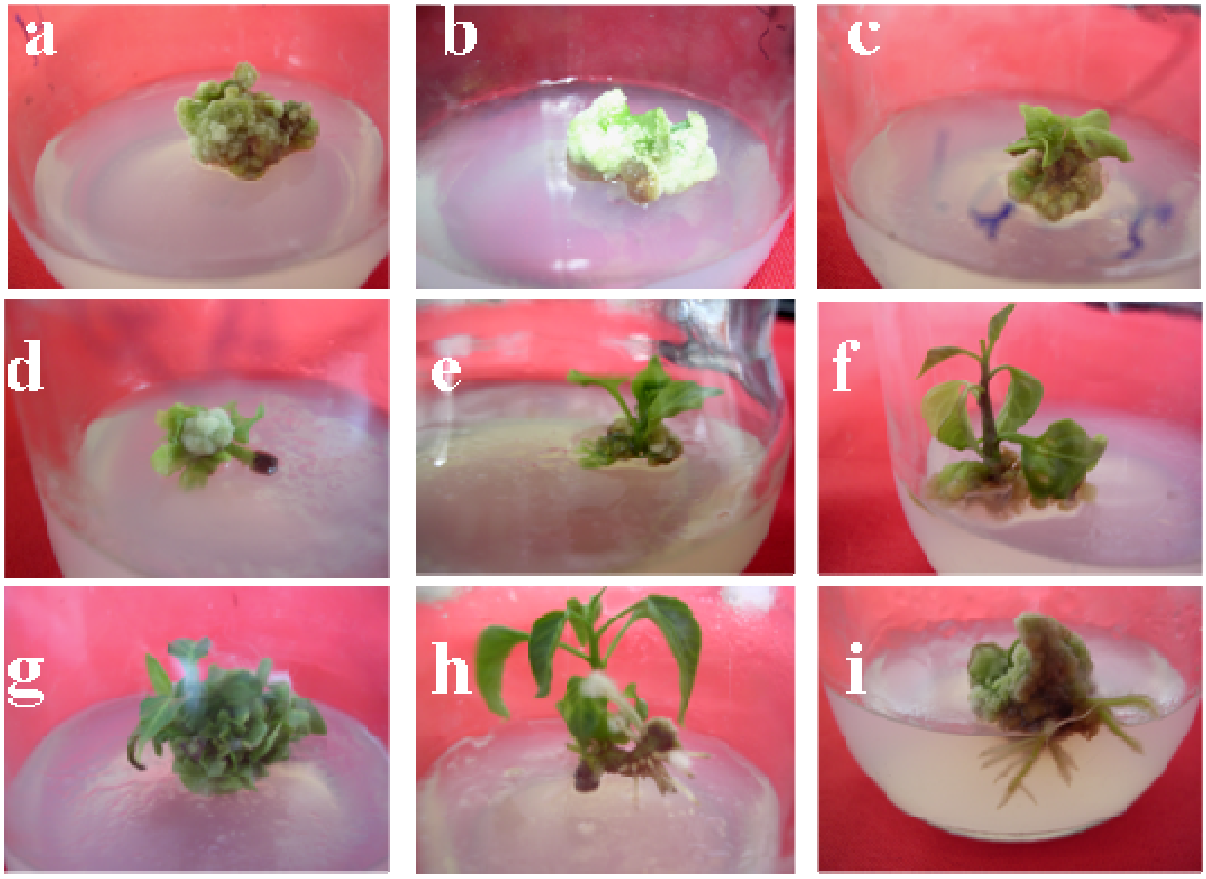

Figure 1. a. Callus derived from cotyledon explant cultured on MS+5.0 mgL^{-1} BAP + 3.0 mgL^{-1} $AgNO_3$. **b.** Callus derived from cotyledon explant cultured on MS+1.0 mgL^{-1} IAA +5.0 mgL^{-1} BAP. **c.** Shoot derived from cotyledonary explant callus on MS + 4.0 mg L^{-1}BAP. **d.** Shoot derived from hypocotyl explant callus on MS + 4.0 mgL^{-1} BAP. **e.** Shoot derived from hypocotyl explant callus on MS + 5.0 mgL^{-1}BAP+ 3.0 mgL^{-1} $AgNO_3$. **f.** Single shoot derived from shoot tips of chilli on MS + 5.0 mgL^{-1} BAP + 3.0 mgL^{-1} $AgNO_3$. **g.** Multiple shoot derived from shoot tips of chilli on MS + 5.0 mgL^{-1} BAP + 3.0 mgL^{-1} $AgNO_3$. **h.** Root derived from shoot tip on MS + 0.1 mg L^{-1} NAA + 0.05 mg L^{-1} IBA. **i.** Root and shoot derived from cotyledonary callus at a time on MS + 0.1 mg L^{-1} NAA + 2.0 mg L^{-1} BAP.

medium supplemented with 5.0 mg L^{-1} BAP and 1.0 mg L^{-1} IAA had the poorest performance where the calli became rusty in color. The rusty coloration of the calli might be due to the inhibitory effect of IAA on shoot regeneration. The effects of 5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA and 5.0 mg L^{-1} BAP +1.0 mg L^{-1} IAA on shoot regeneration are comparable.

The cut surface of cotyledons was swollen and after three weeks of subculture, multiple shoots (2 - 4) were found separately on the calli derived from BAP. But the cotyledonary calli derived from BAP in combination with auxins (IAA and NAA) found to produce multiple shoot primordia that covered the entire body of cotyledonary calli (Figure 1f and g). Shoot developed gradually with the advancement of culture period. Cotyledonary callus were also inoculated on the hormone medium. The medium containing $AgNO_3$ and BAP induced highest percentage (75%) of shoot regeneration from calli followed by media containing NAA and IAA, respectively (Table 4). IAA had can inhibitory effect on shoot regeneration from cotyledonary calli (Sripichitt et al.,

Table 2. Effect of different concentrations of BAP on callus induction and shoot regeneration from hypocotyl and cotyledon explants.

Explant	BAP conc. (mgL^{-1})	% Callus induction	Days to callus induction	% Shoot regeneration	Days to shoot regeneration
Hypocotyl	2.0	90.0b	20a	70.0c	20a
	3.0	95.0a	20a	75.0b	20a
	4.0	95.0a	18a	80.0a	18a
Cotyledon	2.0	85.0a	22a	50.0c	20a
	3.0	80.0b	20b	60.0b	20a
	4.0	85.0a	20b	65.0a	20a

Table 3. Different concentration of kinetin on callus induction from hypocotyl and cotyledon explants.

Explants	Kinetin conc. (mgL^{-1})	% Callus induction	Days to callus induction
Hypocotyl	2.0	65.0c	17b
	3.0	80.0a	20a
	4.0	70.0b	18b
Cotyledon	2.0	60.0c	22a
	3.0	75.0a	17c
	4.0	70.0b	19b

1987).

The percentage of shoot regeneration from hypocotyl and cotyledon derived calli was increased with the increase in BAP concentration from 2.0 to 4.0 mgL^{-1} (Figure 1c and d). The highest regeneration percent was 80% in hypocotyl derived callus while it was 65% in cotyledon derived explants. Hypocotyls explants had higher regeneration frequency then cotyledon. There were no significant influences observed in days required for shoot regeneration from hypocotyl and cotyledon explants derived calli.

Shoot elongation

Shoot buds obtained from different explants of chilli on media containing different concentration and combinations of BAP, NAA, and IAA did not elongate and resulted in a rosette of shoots when continued to culture on same medium. In most instances, shoots or shoot bud clusters were transferred to a shoot elongation medium *in vitro* because shoot elongation has repeatedly been found as major obstacle in obtaining normal pepper plant (Steinitz et al., 1999). Cytokinins commonly stimulate shoot proliferation and inhibit their elongation. The problem of shoot elongation can be overcome by transfer of shoot clusters on secondary medium (Peddaboina et al., 2003). $AgNO_3$ an ethylene inhibitor in plant tissue culture system, is found to be an essential compound in induction and elongation of shoots in pepper (Hyde and Phillips, 1996). In this study two different concentrations of BAP were used in addition to $AgNO_3$ (3 mgL^{-1}) and GA_3 (2 mgL^{-1}) separately as shoot elongation stimulant. The media supplemented with 3.0 mgL^{-1} $AgNO_3$ + 5.0 mgL^{-1} BAP and 2.0 mgL^{-1} GA_3 + 2.0 mgL^{-1} BAP (Wang et al., 1991) showed positive effect on shoot elongations compare to hormone free medium.

Rooting

The shoots derived from hypocotyl, cotyledon and shoot tip explants were implanted on MS media supplemented with NAA, IAA, BAP and IBA in four different combinations. Roots were developed from the shoots implanted on all the media supplemented with NAA, IAA, BAP and IBA. Number of roots per shoot and days to root initiation has been presented in Table 6. No hormonal influence was observed in days required for root initiation when shoot tip derived shoot placed on rooting media. On the other hand, callus derived shoot took longest time to root initiation on rooting media with 1.0 mgL^{-1} NAA+ 5.0 mgL^{-1} IBA. Other hormonal combinations were statistically similar and made root initiation faster. Callus derived shoot and shoot tip derived shoot responded differently to different hormonal combinations used. Number of roots per shoot varies from 4.0-6.0 in callus derived shoot while it was 8.8 - 12.0 in shoot tip derived shoot. The number of roots per shoot was higher in the medium 0.1 mgL^{-1} NAA+ 0.05 mgL^{-1} IBA (Figure 1h). This was true both for callus derived shoot and shoot tip derived shoot. The maximum number of roots per shoot was 6.0 and 12.0 in callus derived shoot and shoot tip derived shoot respec-

Table 4. Supplementation effects on shoot regeneration from hypocotyl and cotyledon explants.

Supplement	% Shoot induction		Days to shoot initiation	
	Hypocotyl	Cotyledon	Hypocotyl	Cotyledon
5.0 mg L^{-1} BAP + 3.0 mg L^{-1} Ag NO_3	90.0a	75.0a	20.0	20.0
5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA	45.0b	38.0b	20.0	20.0
5.0 mg L^{-1} BAP +1.0 mg L^{-1} IAA	40.0c	35.0c	20.0	20.0

Table 5. Direct shoot regeneration from shoot tips as influenced by supplements.

Supplements	% Shoot regenerated	Days to shoot initiation
5.0 mg L^{-1} BAP + 3.0 mg L^{-1} Ag NO_3	65.0a	28.0
5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA	50.0c	33.0
5.0 mg L^{-1} BAP	60.0b	33.0

Table 6. Supplementation effects on the number of root per shoot and days to root initiation.

Supplement	No. of roots per shoot		Days to root initiation	
	Callus derived shoot	Shoot tip derived shoot	Callus derived shoot	Shoot tip derived shoot
0.1 mgL^{-1} NAA + 0.05 mgL^{-1}IBA	6.0a	12.0a	16.5	20.0
1.0 mgL^{-1} NAA + 5.0 mgL^{-1} IBA	5.0b	9.0b	20.0	20.0
1.0 mgL^{-1} IAA + 2.0 mgL^{-1} BAP	4.0c	11.0a	16.75	21.0
0.5 mgL^{-1} NAA	4.0c	8.8b	16.5	19.0
Control	Nil	Nil	Nil	Nil

tively. However, shoots transferred to MS medium free of PGRs induced no roots at all. The callus developed roots later. Among the root initiating hormonal combination 0.1 mg L^{-1} NAA + 0.05 mgL^{-1} IBA was the most effective. Another combination 1.0 mg L^{-1} NAA + 2.0 mg L^{-1} BAP also had potential effect on root regeneration of chilli (Figure 1i).

DISCUSSION

Induction of callus from explants is an important step for successful plant regeneration. Three types of explants viz. cotyledon and hypocotyl were used. Explants were cultured on MS medium supplemented with different combinations of hormones. Assessment on callus induction was studied through days to callus initiation and per cent callus induction. The explants varied significantly for days to callus initiation and number of explants with callus. In case of per cent callus induction, the cotyledon and hypocotyl did not show any significant difference. On the contrary, media supplemented with different concentrations of hormones influenced callus induction significantly. The interactions with different hormone concentrations and explants for both the parameters were significant. The analysis of variance revealed that both explants and media supplemented with different hormone concentrations had conspicuous effect on callus induction ability of chilli. Among the hormone combinations, callus formation potentiality from hypocotyls explants was the highest (95%) in 5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA. Days to callus initiation was minimum in case of 5.0 mg L^{-1} BAP + 3.0 mg L^{-1} $AgNO_3$ (Table 1) and the size of callus was also bigger. Callus induction from cotyledon was the highest (80%) in 5.0 mgL^{-1} BAP + 1.0 IAA (Table 1) but 5.0 mgL^{-1} BAP + 3.0 mgL^{-1} $AgNO_3$ had the potential for callus formation within minimum days (15 days) and the size of callus was bigger and shiney on this medium.

The explants mean square for days to callus initiation, and per cent callus induction were significant indicating the presence of adequate variability between the explants for these parameters. Before callus initiation, the explants became bright and succulent after 5 - 7 days of culture. Callus developed within 14 - 22 days of placement of the explants on the media. Cotyledonary explants responded earlier in MS medium supplement with 5.0 mgL^{-1} BAP + 3.0 mgL^{-1} $AgNO_3$ than the other media.

Shoot regeneration is the ultimate goal to establish free living plantlets in *in vitro* techniques. The regeneration of shoot may be obtained in two ways; direct regeneration of shoot and shoot regeneration via callus. Cotyledonary explants and hypocotyl explants induced callus prior to

regeneration of shoots. The calli were transferred to the shoot regeneration media after 3 – 4 weeks of culture initiation. After 2 - 3 weeks in subculture media, multiple shoot primordia emerged mostly from both ends of hypocotyl and rarely on one end of hypocotyls (Figure 1d). Shoot primordia also emerged from cut ends of cotyledon. The calli from hypocotyl explants were transferred to the shooting media.

For direct shoot regeneration, shoot tip explants were cultured onto MS medium supplemented with 5.0 mg L^{-1} BAP + 3.0 mg L^{-1} AgN03, 5.0 mg L^{-1} BAP + 0.1 mg L^{-1} NAA. It might be concluded from the observation that 5 mg L^{-1} BAP + 3.0 mg L^{-1} AgN03 was better combination for regeneration from shoot tips (Table 5). This result comply with that of Ebida and Hu (1993) who reported that MS medium supplemented with additional component AgNO3 has the influential effect on shoot regeneration from different explants.

Conclusion

From the present study, we recommend to collect explants from 10-20 days old seedling. For direct regeneration from shoot tips, MS media supplemented with 0.1 mg L^{-1} NAA +5.0 mg L^{-1} BAP was the best. Callus induction from hypocotyl was the best on MS media supplemented with 0.1 mg L^{-1} NAA + 5.0 mg L^{-1} BAP while from cotyledon on MS media supplemented with 0.1 mg L^{-1} IAA + 5.0 mg L^{-1} BAP. MS media supplemented with 4.0 mg L^{-1} BAP was the best shoot regeneration from callus. The present research was successful to obtain shoot elongation. Plant regeneration through callus culture is a lengthy process but direct regeneration from shoot tips required less time and is less expensive.

REFERENCES

Christopher T, Rajam MY (1996). Effect of genotype, explants and medium on *in vitro* regeneration of red pepper. Plant Cell Tissue Organ Cult. 46: 245-250.

Dabauza M, Pena L (2001). High efficiency organogenesis in sweet pepper (*Capsicum annuum* L.) tissue from different seedling explants. Plant Growth Regul. 33. 221-229.

Diaz I, Moreno R, Power JB (1988). Plant regeneration from protoplasts of *Capsicum annuum*. Plant Cell Rep. 7: 210-212.

Ebida AIA, Hu C (1993). *In vitro* morphogenic responses and plant regeneration from pepper (*Capsicum annum* L. cv Early California Wonder) seedling explants. Plant Cell Rep. 13: 107-110.

Gunay S, Rao PS (1978). *In vitro* plant regeneration from hypocotyls and cotyledon explants of red pepper (*Capsicum annum*). Plant Sci. Lett. 11: 365-372.

Hyde CL, Phillips GC (1996). Silver nitrate promotes shootdevelopment and plant regeneration of chili pepper (*Capsicum annuum*L) via organogenesis. In Vitro Cell Dev. Biol. Plant. 32: 72-80.

Liu W, Parrott WA, Hildebrand DF, Collins GB, Williams EG (1990). *Agrobacterium*-induced gall formation in bell pepper (*Capsicum annuum* L) and formation of shoot like structures expressing induced genes. Plant Cell Rep. 9: 360-364.

Murashige T, Skoog F (1962). A revised medium for rapid growthand bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Ochoa-Alejo N, Ireta-Moreno L (1990). Cultivar differences in shoot-forming capacity of hypocotyl tissues of chili peppers (*Capsicum annuum* L.) cultured *in vitro*. Sci. Hort. 42: 21-28.

Ochoa-Alejo N, Ramirez-Malagon R (2001). *In vitro* chili pepper biotechnology. In Vitro Cell. Dev. Biol. Plant 37(6): 701-729.

Peddaboina V, Thamidala C, Karampuri S (2003). Thidiazuron induced high frequency adventitious shoot formation and Plant regeneration in *Capsicum annuum L.* J. Plant Biotech. 5(4): 245-250.

Ravishankar GA, Suresh B, Giridhar P, Rao SR, Johnson TS (2003). Biotechnological studies on *capsicum* for metabolite production and plant improvement. In: DE, Amit Krishna ed. *Capsicum: The genus Capsicum*. Harwood Academic Publishers, UK, pp. 96-128.

Sripichitt P, Nawata E, Shigenaga S (1987). *In vitro* shoot-forming capacity of cotyledon explants in red pepper (*Capsicum annum* L. cv. Yatsufusa). Japan J. Breed. 38: 27-34.

Steinitz B, Wolf D, Matzevitch T, Zelcer A (1999). Regeneration *in vitro* and genetic transformation of pepper. The current state-of-art. Capsicum and Eggplant Newsletter 18: 9-15.

Wang YW, Yang MZ, Pan NS, Chan ZL (1991). Plant regeneration and transformation of sweet pepper *(Capsicum frutescens).* Acta Bot. Sinica 33(10): 780-786.