Agrotechnology
ISSN: 2168-9881

Bhutia et al., Agrotechnol 2016, 5:1
DOI: 10.4172/2168-9881.1000142

Research Article Open Access

In Vitro Regeneration of Dalle Khursani, an Important Chilli Cultivar of Sikkim, using Various Explants

Karma Landup Bhutia, NG Tombisana Meetei and VK Khanna*

School of Crop Improvement, College of Post Graduate Studies, CAU, Umiam, Meghalaya, India

Abstract

An efficient micro propagation protocol was developed for Dalle Khursani, an important chilli cultivar of Sikkim. Aseptic cotyledon, shoot tip and hypocotyl explants of Dalle Khursani (*Capsicum annuum*) were cultured on Murashige and Skoog medium containing 16 different combinations of plant growth regulators for *in vitro* regeneration. Regeneration was observed only in 8 combinations of growth regulators among which medium containing 4 mg/l Thidiazuron (TDZ) showed the best result with an average of 2.95 shoots per explant and explant response of 73.95%. This was followed by MS medium containing 4 mg/l TDZ + 0.5 mg/l Gibberrellic Acid 3 (GA_3) + 0.5 mg/l Indole Acetic Acid (IAA) with an average of 1.94 shoots per explant and 66.66% explant response. Among the three explants used, cotyledons showed the best response in terms of number of shoots per explant with an average of 1.76. Regenerated shoots elongated and rooted well on MS medium containing 2 mg/l GA_3 + 1 mg/l IAA with an average shoot length of 3.10 cm and 6.35 ± 0.98 roots per shoot with explants response of 85% and 75%, respectively. The regenerated plants were acclimatized on a mixture of normal soil and artificial soil with 78% survival.

Keywords: Dalle Khursani; *In vitro* regeneration; Explants; cotyledon; Shoot tip; Hypocotyls; Thidiazuron; Artificial soil

Introduction

Chilli (*Capsicum sp.*) is a self-pollinated dicot plant and belongs to the family Solanaceae. Chilli has its centre of origin in American tropics. Capsicum is derived from the Greek word 'kapsimo', meaning 'to bite'. There are thought to be 25-30 species of Capsicum, of which 5 species; *C. annuum* L, *C. frutescens* mill, *C. chinense, C. baccatum* L. and *C. pubescens* have been domesticated and cultivated [1]. Capsicum is grown in the world on an area of 1.5 million hectare with the production of 10.60 million tons. In India, it is grown in an area of 0.775 million hectare with an average yield of 1.6 metric tonnes/hectare Indiastat.com, 2015.

Dalle Khursani belongs to *Capsicum annuum*. It is mostly grown in Sikkim and its surrounding regions like Darjeeling for its pungent fruits. It is one of the hottest chilli pepper with a Scoville rating of 100,000 to 350,000 SHU (For comparison Naga King chilli has Scoville rating of 330,000-1,000,000 SHU, Tabasco red pepper sauces has rating of 2500-5000 SHU and pure capsaicin has Scoville rating of 16,000,000 SHU).

In vitro regeneration is the process where a cell or group of cells differentiates to form organs in media containing required elements in aseptic environment. *In vitro* regeneration technique is generally used for micro propagation of elite crops and for the improvement of crops via genetic transformation as well to produce distant hybrids by using special techniques such as embryo culture, protoplast culture etc. The conventional method of chilli plant propagation using seeds is restricted by the short span of viability and low germination rate of seeds. Chilli plants are also highly susceptible to fungal and viral pathogens [2]. Capsicum species also has inherited problems like genotype dependence and recalcitrant nature (inability of plant cells, tissues and organs to respond to *in vitro* culture), etc. associated with *in vitro* regeneration system. Nevertheless, several reports are available for *in vitro* regeneration of different species / cultivars of chilli. Therefore, with the aim of developing an efficient *in vitro* regeneration protocol for Dalle Khursani, this research program was taken up as it will further help in micro propagation and improvement of the crop.

Materials and Methods

The seeds of the chilli cultivar Dalle Khursani were collected from the farmers' field at Temi, South Sikkim, and India. The seeds were first treated with 0.2% bavistin and washed with distilled water. The seeds are then taken inside the Laminar Flow chamber and immersed in 70%ethanol for 10 to 15 sec, followed by sterilization with 2% sodium hypochlorite (NaClO) for 15 to 20 minutes. They were then washed thoroughly with sterile distilled water for 5 times to remove the traces of NaClO. Sterilized seeds were then inoculated in culture bottles containing MS basal media without any growth regulators for germination. After 15 to 20 days of germination, the explants namely, the cotyledon, shoot tip and hypocotyl were excised from the seedlings.

Murashige and Skoog (MS) medium supplemented with different plant growth regulators (PGRs) was used in this experiment. The media used for shoot induction is given in (Table 1).

After 4 weeks of culture, the regenerated shoots were sub-cultured for elongation as well as for rooting on MS media supplemented with various concentrations and combinations of growth regulators as given in (Table 2). The cultures were maintained in the culture room at 25°C ± 0.5°C temperature, photoperiod regime of 16 hrs light and 8 hrs dark and with a relative humidity (RH) of 60%. The observations that were

***Corresponding author:** VK Khanna, School of Crop Improvement, College of Post Graduate Studies, CAU, Umiam, Meghalaya, India, Tel: 03642570614; Fax: 03642570030; E-mail: khannavk@rediffmail.com

Received November 09, 2015; **Accepted** March 04, 2016; **Published** March 10, 2016

Citation: Bhutia KL, Tombisana Meetei NG, Khanna VK (2016) *In Vitro* Regeneration of Dalle Khursani, an Important Chilli Cultivar of Sikkim, using Various Explants. Agrotechnol 5: 142. doi:10.4172/2168-9881.1000142

MEDIA	Plant growth regulators (mg/l)					
	TDZ	BAP	KIN	IAA	NAA	GA_3
MS_1	2.0	-	-	-	0.5	-
MS_2	-	2.0	-	-	0.5	-
MS_3	-	-	2.0	-	0.5	-
MS_4	4.0	-	-	-	-	-
MS_5	-	4.0	-	-	-	-
MS_6	-	-	40	-	-	-
MS_7	2.0	2.0	-	-	-	-
MS_8	2.0	-	2.0	-	-	-
MS_9	-	2.0	2.0	-	-	-
MS_{10}	4.0	4.0	-	-	-	-
MS_{11}	4.0	-	4.0	-	-	-
MS_{12}	-	4.0	4.0	-	-	-
MS_{13}	6.0	-	2.0	-	-	-
MS_{14}	-	8.0	-	-	-	2.0
MS_{15}	-	4.0	-	0.5	-	0.5
MS_{16}	4.0	-	-	0.5	-	0.5
MS_{17}	Control without any growth hormones					

(TDZ = Thidiazuron; BAP = Benzyl Amino Purine; IAA = Indole Acetic Acid; KIN = Kinetin; NAA = Naphthalene Acetic Acid, GA_3 = Giberrellic Acid 3)

Table 1: Different combinations of growth regulators for direct regeneration.

MS MEDIA	Plant growth regulators (mg/l)			
	KIN	GA_3	NAA	IAA
ER_1	0.0	0.0	0.0	0.0
ER_2	2.0	0.5	0.0	0.0
ER_3	0.5	2.0	0.0	0.0
ER_4	0.0	2.0	0.5	0.0
ER_5	0.0	0.0	2.0	0.0
ER_6	0.0	2.0	0.0	0.5
ER_7	0.0	0.5	0.0	2.0

Table 2: Different concentrations and combinations of growth regulators for shoot elongation and rooting.

recorded from the *in vitro* culture included the explants' response i.e., the percentage of explants responding to *in vitro* culture, the number of shoots per explants, shoot length, root length and the number of roots. After 8 to 9 weeks of culture, the seedlings were transferred into artificial soil for acclimatization.

Artificial soil was prepared by mixing Perlite, Vermiculite and Peat (1:1:1 ratio) and was further mixed with autoclaved soil at 1:1 ratio. Fully developed elongated shoots with roots were taken out from the culture tubes or bottles and the base portion was washed thoroughly with running water to remove the medium attached on it. Regenerated seedlings were transplanted on disposable cups containing artificial soil and sterilized soil and then transferred to greenhouse for hardening.

For each treatment, 10 replications were used and the experiment was repeated three times. Completely Randomized Design (CRD) was used as the experimental design and for statistical analysis statistical software SPSS version 17.0 was used.

Results and Discussion

Among the 18 different media combinations used initially for in vitro culture, adventitious shoot induction was observed in only eight combinations of the growth regulators listed in (Table 3). Only these eight media combinations were used for further study.

Effect of explants

The present study indicated the effect of explants on the *in vitro* regeneration of Dalle Khursani to be highly significant (Table 4). Among three types of explants used *viz.* cotyledon, shoot tip and hypocotyl, the maximum number of shoots per explant was observed in cotyledons (3.76 ± 0.58 in MS_4). On the other hand, shoot tip explants showed better results in terms of percentage of explants response (81% in MS_4) (Figure 1).

In earlier available reports, the best response in terms of number of shoots per explant was obtained from cotyledon explants as in the case of sweet pepper [3] and *Capsicum annuum* L. cv. Kaddi B [4]. Other reports are also available which show successful regeneration from cotyledon explants [4-8]. Shoot tips have also successfully used as explants [9-11]. In some reports, the hypocotyl explants showed better result both in terms of number of shoots per explant as well as percentage of explant which responded [7,12,13]. These results suggested that the response of the different explants to *in vitro* culture varies for different cultivars/species. In the case of Kharsani D, also, the cotyledon was found to be the explant of choice as it gave the maximum number of shoots per explant (3.76 ± 0.58) when 4 mg/L TDZ alone was used in the medium.

Effects of media

The effect of media for *in vitro* culture of Dalle Khursani was also found to highly significant (Table 5). Types and levels of plant growth regulators in growing media play an important role during *in vitro* regeneration of plants. In the present study, among different concentrations and combinations of growth regulators used, MS medium supplemented with 4 mg/l TDZ (MS_4) showed the best results both in terms of number of shoots per explant as well as explant

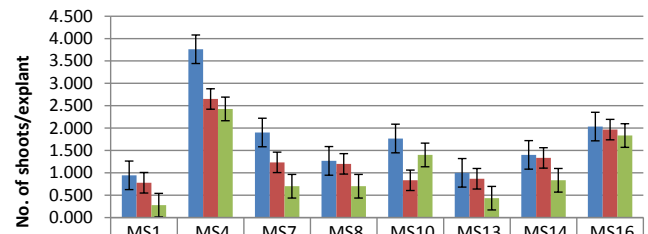
Sl. No.		Mean no. of shoots per explant (Mean ± SE_M)			**Media**
	Media	Cotyledon	Shoot tip	Hypocotyl	Mean
1	MS_1	0.95 ± 0.33	0.78 ± 0.13	0.28 ± 0.12	0.667
2	MS_4	3.76 ± 0.58	2.70 ± 0.29	2.40 ± 0.33	2.946
3	MS_7	1.90 ± 0.34	1.20 ± 0.19	0.70 ± 0.20	1.278
4	MS_8	1.27 ± 0.32	1.20 ± 0.17	0.70 ± 0.19	1.056
5	MS_{10}	1.77 ± 0.32	0.83 ± 0.14	1.40 ± 0.29	1.333
6	MS_{13}	1.00 ± 0.40	0.87 ± 0.15	0.43 ± 0.18	0.767
7	MS_{14}	1.40 ± 0.29	1.30 ± 0.16	0.83 ± 0.22	1.189
8	MS_{16}	2.00 ± 0.42	1.97 ± 0.21	1.83 ± 0.28`	1.944
Explant Mean		**1.759**	**1.358**	**1.076**	

C.D	0.05% or 5%	0.01% or 1%
Explant	1.05	1.67
Media	0.7	1

Table 3: Effect of different media treatments on regeneration of shoots from cotyledon, shoot-tip and hypocotyl explants after 4 weeks of culture.

Sl. No.	Media	Explant Response (%)			Mean
		Cotyledon	Shoot tip	Hypocotyl	
1	MS_1	22.22	61.11	16.66	33.35
2	MS_4	65.6	81.25	75	73.95
3	MS_7	56.66	70	30	52.22
4	MS_8	36.66	73.33	33.33	47.77
5	MS_{10}	56.66	66.66	50	57.77
6	MS_{13}	20	60	23.33	34.44
7	MS_{14}	46.66	76.66	36.66	53.33
8	MS_{16}	50	86.66	63.33	66.66
	Mean	44.3	71.95	41.03	

Table 4: Response percentage of cotyledon, shoot-tip and hypocotyls explants to different growth hormones for direct regeneration after 4 weeks of culture.



	MS1	MS4	MS7	MS8	MS10	MS13	MS14	MS16
Cotyledon	0.944	3.761	1.900	1.267	1.767	1.000	1.400	2.033
Shoot tip	0.778	2.650	1.233	1.200	0.833	0.867	1.333	1.967
Hypocotyl	0.278	2.428	0.700	0.700	1.400	0.433	0.833	1.833

Figure 1: Effect of different media combinations on direct *in vitro* shoot regeneration from cotyledon, shoot tip and hypocotyl explants.

Source of Variation	Degrees of freedom	Sum of square	Mean sum of square	$F_{calculated}$	$F_{tabular}$	
					5%	1%
Replication	2	0.558	0.279			
Treatment	23	43.299	1.882			
Explant (E)	2	5.66	2.83	17.453	3.2	5.1
Media (M)	7	34.279	4.897	30.2	2.22	3.05
(E x M)	14	3.359	0.239	1.479	1.91	2.5
Error	46	7.458	0.162			
Total	71	51.316				

Table 5: Analysis of variance (ANOVA) among different explants, combination of growth regulator and interaction between explants and media.

response (Tables 3 and 4). This concentration of TDZ was many times higher than the concentration of TDZ reported by Channappagaudar [4] where the highest frequency of shoot regeneration was obtained on MS medium containing 0.5 mg/l TDZ. Results of present study show that callus formation at petiolar end of cotyledon (Figure 2A) basal end of shoot tip and cut ends of hypocotyl explants on media containing lower concentration of BAP interferes with the shoot formation. However, higher concentration of BAP (8 mg/L) showed direct regeneration of shoots from explants. These results are similar to the previous reports where higher concentration of BAP showed enhanced shoot induction [6,14-20]. TDZ has a high efficiency in stimulating cytokinin dependent shoot regeneration from a wide variety of plants [17]. Previous reports suggest that MS media supplemented with 0.5 to 2 mg/L TDZ are best for shoot induction [17,21-25]. In contrast to the previous reports, results of the present study show that, supplementing MS media with a slightly higher concentration of TDZ (4 to 6 mg/L) enhanced shoot induction.

Effect of explant x media (interaction)

Irrespective of the growth media, shoot tip explants showed high explants response ranging from 61% to 81% response with an average of 1.36 shoots per explant and irrespective of explants, MS media containing 4 mg/L TDZ showed the highest average number of shoots (2.95) per explant in all the three types of explants used. Analysis of variance showed no significant differences among the interaction between explants x media at 1% and 5% level of significance (Table 5).

Shoot elongation

In the present study, among different combinations of growth regulators tried, medium containing a combination of GA_3 (2 mg/L) and IAA (0.5 mg/L) was found to be the best for shoot elongation with an average shoot length of 3.1 ± 0.33 and 85% response. Similar combination was used by Kumar et al. [26], but the concentration of growth regulators was higher in the present case. Other findings also suggested the addition of GA_3 alone or in combination with either cytokinin or auxin in media to be suitable for enhancing elongation of regenerated shoots [4,6,7,13,26-28]. Another study reported that sub culturing of regenerated shoots on MS media without any growth regulators enhanced the shoot elongation [18,21,22,29]. In our study also, shoot elongation was observed in media devoid of growth regulators with an adequate shoot length of 3.00 ± 0.36 cm and 80% response.

Rooting

In tissue culture generally shoots are allowed to regenerate first than roots by manipulating growth regulators in the growing media. In the present study, results showed that MS media containing 2 mg/L GA_3 and 0.5 mg/L IAA was best for root induction with an average of 6.35 ± 0.98 roots per explant and 70% response (Table 6). Similar results were reported earlier where presence of IAA in media was suitable for root induction [27,30,31]. In previous reports MS media amended with NAA was best for root induction [18,31]. In the present study, MS medium containing 2 mg/L NAA showed the second best results in terms of number of roots per shoot (5.55 ± 0.94) with a 55% response. MS medium without any growth regulators also gave good results for root induction with an average of 4.60 ± 0.87 roots per shoot and 65% response. This result was similar to those reported by Song et al. [21] and Arous et al. [32].

Hardening

In vitro regenerated, elongated and rooted seedlings of Dalle Khursani (*Capsicum annuum*) were taken out from the culture tubes after 8-9 weeks of culture and the traces of media from the basal region or roots were removed by washing thoroughly with running tap water. Plantlets were than transplanted in cups containing a mixture of artificial soil and normal soil at 1:1 ratio. The cultures were kept inside the culture room under controlled conditions for at least 2 - 3 weeks after which they were transferred to the Greenhouse (Figure 3). In this experiment, 78% of *in vitro* regenerated seedlings survived hardening.

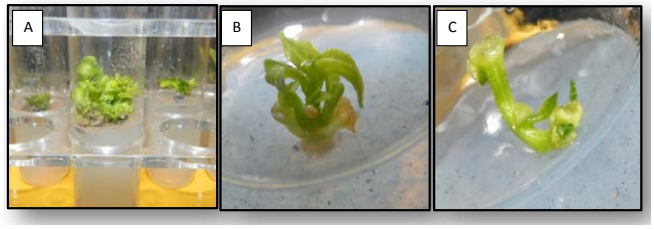


Figure 2: *In vitro* direct regeneration. (A) Rosette like structure formed at petiolar end of cotyledon explants. (B) Shoots regenerated from shoot tip explants. (C) Hypocotyl explants showing direct regeneration.

Media	Shoot length (cm) (Mean ± SEM)	Response (%)	Root No. (Mean ± SEM)	Root length (cm) (Mean ± SEM)	Response (%)
ER_1	3.00 ± 0.36	80	4.60 ± 0.87	1.30 ± 0.25	65
ER_2	1.10 ± 0.25	55	2.70 ± 0.62	1.15 ± 0.26	55
ER_3	2.00 ± 0.30	75	1.45 ± 0.53	0.55 ± 0.18	35
ER_4	2.00 ± 0.32	65	4.05 ± 0.92	1.38 ± 0.30	55
ER_5	0.80 ± 0.22	45	5.55 ± 0.94	1.80 ± 0.34	65
ER_6	3.10 ± 0.33	85	6.35 ± 0.98	1.68 ± 0.29	70
ER_7	1.55 ± 0.24	75	4.20 ± 0.74	2.15 ± 0.41	65

Table 6: Effect of different combinations and concentration of growth hormones on shoot elongation, root induction and root elongation.

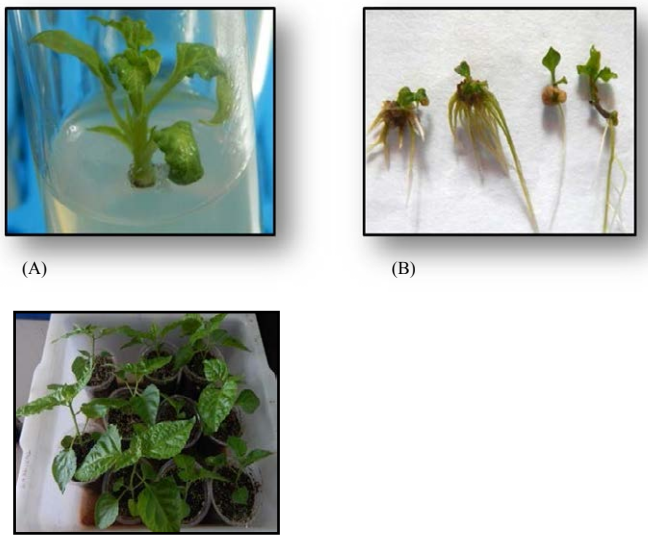


Figure 3: Shoot elongation (A), rooting (B) and hardening (C).

References

1. Kothari SL, Joshi A, Kachhawaha S, Ochoa-Alejo N (2010) Chilli Pepper A review on tissue culture and transgenics. Biotechnology advances 28: 35-48.
2. Morrison RA, Koning RE, Evans DA (1986) Anther culture of an interspecific hybrid of Capsicum. Journal of Plant Physiology 126: 1-9.
3. Otroshi M, Ghehsareh NM (2013) The effect of plant growth regulators and different type of explants on organogenesis of pepper (*Capsicum annuum* L.) in in-vitro. Journal of Applied Science and Engineering Technology 3: 271-278.
4. Channappagoudar SB (2007) Studies on in vitro regeneration and genetic transformation in chilli (*Capsicum annuum* L.). Ph.D thesis submitted to University of Agriculture Science (Dept of Gen and Pl Breeding, Agri College, Dharwad, UAS) India.
5. Kehie M, Kumaria S, Tandon P (2013) *In vitro* plantlet regeneration from cotyledon segments of *Capsicum chinense* Jacq. cv. Naga King Chili and determination of capsaicin content in fruits of in vitro propagated plants by high performance liquid chromatography. Scientia Horticulturae 164: 1-8.
6. Verma S, Dhiman K, Srivastava DK (2013) Efficient *in vitro* regeneration from cotyledon explants in bell pepper (*Capsicum annuum* L. cv. california wonder). International Journal of Advanced Biotechnology and Research 4: 391-396.
7. Pishbin N, Mousavi A, Kalatejari S, Shariatpanahi M, Jahromi BB (2014) The effect of plant growth regulators and different types of explants on *in vitro* regeneration of sweet pepper (*Capsicum annuum* L.). International Journal of Bioscience 5: 139-146.
8. Husain S, Jain A, Kothari S L (1999) Phenylacetic acid improves bud elongation and In vitro plant regeneration efficiency in *Capsicum annuum* L. Plant Cell Reports 19: 64-68.
9. Gururaj HB, Giridhar P, Sharma A, Prasad BCN, Ravishankar GA (2004) *In vitro* clonal propagation of bird eye chilli (*Capsicum frutescens* Mill.). Indian Journal of Experimental Biology 42: 1136-1140.
10. Sanatombi K, Sharma GJ (2008) *In vitro* plant regeneration in six cultivars of *Capsicum* sp. using different explants. Biologia Plantarum 52: 141-145.
11. Sanatombi K, Sharma GJ (2012) *In vitro* regeneration of *Capsicum chinense* Jacq. Current Trends in Biotechnology and Pharmacy 6: 66-72.
12. Bustos MGV, Aguado-Santacruz GA, Carrillo-Castañeda C, Aguilar-Rincón VH, Espitia-Rangel E, et al. (2009) *In vitro* propagation and agronomic performance of regenerated chili pepper (*Capsicum* sp.) plants from commercially important genotypes. In Vitro Cellular and Developmental Biology-Plant 45: 650-658.
13. Kumar V, Gururaj HB, Prasad BC, Giridhar P, Ravishankar GA (2005) Direct shoot organogenesis on shoot apex from seedling explants of *Capsicum annuum* L. Scientia Horticulturae 106: 237-246.
14. Rizwan M, Sharma R, Soni P, Gupta NK, Singh G (2013) Regeneration protocol for chili (*Capsicum annuum* L.) variety Mathania. *Journal of Cell and Tissue* Research 13: 3513-3517.
15. Sanatombi K, Sharma GJ (2006) *In vitro* regeneration and mass multiplication of *Capsicum annuum* L. Journal of Food, Agriculture and Environment 4: 205-206.
16. Ashajyothi SS (2004) Regeneration and transformation studies in chilli (*Capsicum annuum* L.). M. Sc. (Agri.) Thesis, submitted to University of Agricultural Sciences (Dept. of Gen. and Pl. Breeding, Agri. College, Dharwad, UAS) India.
17. Manoharan M, Vidya CSS, Sita GL (1998) Agrobacterium-mediated genetic transformation in hot chilli (*Capsicum annuum* L. var. Pusajwala). Plant Science 131: 77-83.
18. Hyde CL, Phillips GC (1996) Silver nitrate promotes shoot development and plant regeneration of Chilli pepper (*Capsicum annuum* L.) *via.* organogenesis. *In Vitro* Cellular and Developmental Biology-Plant 32:72-80.
19. Christopher T, Rajam MV (1994) *In vitro* clonal propagation of *Capsicum* spp. Plant Cell, Tissue and Organ Culture 38: 25-29.
20. Gunay AL, Rao PS (1978) *In vitro* plant regeneration from hypocotyl and cotyledon explants of red pepper (*Capsicum* Spp). Plant Science Letters 11: 365-72.
21. Song JY, Sivanesan I, An CG, Jeong BR (2010) Adventitious shoot regeneration from leaf explants of miniature paprika (*Capsicum annuum*) 'Hivita Red' and 'Hivita Yellow'. African Journal of Biotechnology 9: 2768-2773.
22. Ahmad N, Siddique I and Anis M (2006) Improved plant regeneration in *Capsicum annuum* L. from nodal segments. Biologia Plantarum 50: 701-704.
23. Santana N, Canto A, Barahona F, Miranda M (2005) Regeneration of Habarno peppers (*Capsicum chinense* Jacq.) *via.* organogenesis. HortScience 40: 1829-1831.
24. Dabauza M, Pena L (2001) High efficiency organogenesis in sweet pepper (*Capsicum annuum* L.) tissues from different seedling explants. Plant Growth Regulation 33: 221-229.
25. Szasz A, Nervo G, Fasi M (1995) Screening for *In vitro* shoot forming capacity of seedling explants in bell pepper (*Capsicum annuum* L.) genotypes and efficient plant regeneration using thidiazuron. Plant Cell Reports 14: 666-669.
26. Kumar OA, Rupavathi T, Tata SS (2012) Adventitious shoot bud induction in chili pepper (*Capsicum annuum* L. cv. x-235). International Journal of Science and Nature 3: 192-196.
27. Joshi A, Kothari SL (2006) High copper levels in the medium improves shoot bud differentiation and elongation from the cultured cotyledons of *Capsicum annuum* L. Plant Cell, Tissue Organ culture 12: 71-76.
28. Golegaonkar PG, Kantharajah GS (2006) High-frequency adventitious shoot bud induction and shoot elongation of chilli pepper (*Capsicum annuum* L.). *In Vitro* Cellular and Developmental Biology-Plant 42:341-344.
29. Siddique I, Anis M (2006) Thidiazuron induced high frequency shoot bud formation and plant regeneration from cotyledonary node explant of *Capsicum annuum* L. Indian Journal of Biotechnology 5: 303-308.
30. Kehie M, Kumaria S, Tandon P (2012) *In vitro* plantlet regeneration from nodal segments and shoot tips of *Capsicum chinense* Jacq. Cv. Naga King Chilli. Biotech 2: 31-35.
31. Dafadar A, Das A, Bandopadhyay B, Jha TB (2012) In vitro propagation and molecular evaluation of *Capsicum annuum* L. cultivar with high chromosome number (2n = 48). Scientia Horticulturae 140: 119-124.
32. Arous S, Boussaid M, Marrakchi M (2001) Plant regeneration from zygotic embryo hypocotyls of Tunisian chili (*Capsicum annuum* L.) Journal of Applied Horticulture 3:17-22.