

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.605.026>

In Vitro Regeneration of Capsicum (*Capsicum annuum* L.) from Cotyledon Explants

Vivek Hegde^{1,3*}, P.S. Partap¹ and R.C. Yadav²

¹Department of Vegetable Science,

CCS Haryana Agricultural University, Hisar-125 004, Haryana, India

²Department of Molecular Biology and Biotechnology

CCS Haryana Agricultural University, Hisar-125 004, Haryana, India

³Division of Crop Improvement,

ICAR- Central Tuber Crops Institute, Thiruvananthapuram, Kerala, India

*Corresponding author

ABSTRACT

Pre-requisite for any plant biotechnological approach is development of an efficient plant regeneration system in that crop. In this regard the *in vitro* regeneration was achieved by using cotyledon explants from aseptically raised seedlings of popular Capsicum F₁ hybrids Bharat and Indra. Seeds of both hybrids were exposed to different treatments for proper germination, were decontaminated and sown *in vitro* on half-strength MS medium. The seed soaked in distilled water along with 2 mg/l GA₃ for two days prior to sowing had more pronounced effect on both capsicum hybrids recording maximum germination (90.45 and 84.59 %, respectively) in minimum number of days (9.67 days and 10.33 days, respectively). Regeneration potential of cotyledonary explants touching the medium both abaxial and adaxial sides was examined. Tissue culture responses varied with the genotypes, side of explants touching the medium and combinations of growth regulators used. Regeneration per cent (96.30%), number of shoots per explants (4.56) and per cent shoot elongation (82.10%) was maximum in hybrid Indra from cotyledon explant having abaxial side in contact with growing medium supplemented with 7.5 mg/l zeatin along with 2.0 mg/l GA₃. When regenerated shoots cultured on MS media supplemented with 0.5 mg/l IBA produced 100% rooting in both the hybrids. The survival percentage of regenerated plantlets during hardening in pots containing sterile mixture of 1:1 coco-peat and vermiculite was higher in Indra (92.31%) as compared to Bharat (85.71%).

Keywords

In vitro regeneration, Cotyledon, Capsicum, Tissue culture.

Article Info

Accepted:

04 April 2017

Available Online:

10 May 2017

Introduction

Capsicum (*Capsicum annuum* L.) is one of the most important vegetable crops in the world and commonly known as pepper, sweet pepper or bell pepper, belongs to the family Solanaceae. This indispensable item of kitchen occupying an important place in Indian diet is consumed daily in one or the

other form as a condiment and spice, vegetable, adding colour, flavour, pungency and piquancy to various foodstuffs. It is an excellent source of vitamins A, B-complex, C, E and is rich in minerals like molybdenum, manganese, folate and potassium (Simonne *et al.*, 1997).

In spite of 400 different capsicum varieties found grown all over the world, the vulnerability of these genotypes consequent to the abiotic and biotic stresses including the extremes of temperature, moisture, light, nutrients and pH among others and the insect pests, fungi, bacteria and viruses has drastically restricted their yield potential and quality (Ochoa and Ramirez, 2001; Egea *et al.*, 2002; Suzuki and Mori, 2003 and Venkataiah *et al.*, 2003). Although, the conventional plant breeding combined with improved agricultural practices has upgraded capsicum crop production, quality and use remarkably, yet the restricted gene pool or range of organisms among which genes can be transferred, has placed a limit to this technology.

Hence, gradually the biotechnology encompassing plant tissue culture and genetic engineering is becoming a functional tool of classical plant breeding to boost the crop improvement programs. *In vitro* plant regeneration from cells, tissues and organ cultures, which is a fundamental process of biotechnology application in plant propagation, plant breeding and genetic improvement would serve the main purposes of micro-propagation of elite plants, maintenance of male sterile lines, selection of soma-clonal variants, mass multiplication of pollen-derived haploid plants, selection of plants against biotic and abiotic stresses and the genetic transformation.

The regeneration responses depend on various factors like genotype, explants, growth regulators and their concentrations etc. (Dabauza and Pena, 2001). However, despite the economic significance of capsicum, its whole plant regeneration has not progressed akin to vegetables crops like tomato and potato having greater plant regeneration capability from cells, tissues or organs.

Materials and Methods

Among the popular F₁ hybrids of sweet pepper largely being grown by the farmers of the region, the hybrids Bharat and Indra were chosen for the present study. The seeds were first washed with tween-20 and exposed to different treatments for proper germination. Seeds soaked in distilled water for 2 days at room temperature and at 4°C, distilled water with 2mg/l GA₃ for 2days at room temperature and at 4°C, also fresh seeds were taken to laminar-airflow cabinet and surface sterilized with 0.1 per cent mercuric chloride (HgCl₂) for 5 to 6 minutes and finally rinsed 4 to 5 times with sterile double distilled water to remove the traces of HgCl₂. Surface sterilized seeds were aseptically inoculated on half strength solid MS basal medium and incubated at 25±2 °C under 16h light and 8h dark photoperiod for raising the seedlings. These *in vitro* grown, 5-7 day old seedlings were used to harvest explants for regeneration purpose.

The 0.4 to 0.6 cm cotyledon explants were inoculated horizontally on MS basal medium containing three per cent sucrose, 0.8 per cent agar supplemented with different concentrations and combinations of growth regulators. Regeneration potential of both abaxial and adaxial sides of the cotyledonary explants was examined (Plate 1A). After inoculation of explants in Petri-dishes containing media with different treatments, these were sealed properly with the parafilm strips to protect from contamination.

Cultures were incubated in culture room at 25±2 °C temperature under a photoperiod of 16 h light and 8 h dark. The well elongated individual shoots generated and obtained from shooting medium were separated and transferred to MS medium supplemented with 0.5 mg/l IBA for root induction.

Plantlets grown were carefully removed from the culture bottles without damaging their root systems and washed properly under running tap water to remove the traces of medium sticking to the roots. The rooted shoots (plantlets) were transferred to pots containing the sterile mixture of coco-peat and vermiculite (1:1) for proper establishment. The nutrients were supplied through the application of liquid MS basal medium without sucrose, at 5 day intervals. Each pot was covered with a polythene bag to maintain humidity around the plants and kept in culture room at $25\pm 2^{\circ}\text{C}$ temperature, under photoperiod of 16 h light and 8 h dark. Within 6-7 days, the polythene bags were removed and after 14 days of acclimatization under laboratory condition, plantlets were transferred to shade net in big size pot containing soil: sand: farmyard manure (1:1:1).

Seed germination percentage and the number of days taken from the date of seed inoculation to germination in various treatments were expressed as mean number of days. The per cent regeneration, elongated shoots per explants, per cent shoot elongation and per cent survivals of regenerated plants in soil were assessed. The mean and standard errors were worked out from triplicate data obtained from various treatments. The per cent data transformed using angular transformation and analyzed following Completely Randomized Design (CRD).

Results and Discussion

In the present study when fresh seeds of two F_1 capsicum hybrids namely, Bharat and Indra, were placed *in vitro* on MS culture medium without any pre-sowing treatment, exhibited delayed and very low seed germination i.e. 23.67 and 20.00 per cent, respectively. The seed germination due to the pre-sowing seed treatments improved and

ranged from 42.44 % to 90.45%. Seeds soaked in distilled water before sowing had positive influence on germination percentage as well as number of days taken for germination while, seed soaked in distilled water along with GA_3 at 2 mg/l for two days prior to sowing had more pronounced effect on both capsicum hybrids recording maximum germination in minimum number of days (Table 1). In earlier studies, Watkins and Cantlife (1983) and Watkins *et al.*, (1985) found that enclosure of radical tips by non starchy endospermic tissues might placed mechanical barrier to the growing embryo and contributed to slow and erratic germination of pepper seeds. External application of GA_3 might weaken the endosperm and triggered the germination. Watkins *et al.*, (1985) and Groot and Karssen (1987) reported that GA_3 mediated enzymic activity might participate in weakening process of endosperms in seeds prior to germination. Similarly, Andreoli and Khan (1999) described that seed treatment with GA_3 might efficiently digest the endosperm cells by GA_3 induced enzymes and reduced the mechanical restraints against germination of pepper seeds.

Genotype is one of the main factors that influence the organogenic response of *in vitro* cultures in different plant species. Per cent regeneration, number of shoots per explants and per cent elongation was maximum in hybrid Indra as compared to Bharat, on MS medium supplemented with 7.5 mg/l zeatin along with 2.0 mg/l GA_3 and 7.5 mg/l kinetin along with 2.0 mg/l GA_3 (Tables 3 and 5). But, hybrid Bharat responded better than Indra on MS medium containing 10.0 mg/l BAP along with 2.0 mg/l GA_3 and 5.0 mg/l TDZ along with 2.0 mg/l GA_3 (Table 2). These results are in conformity with the previous reports. Organogenic capacity differences have been observed in different chilli pepper genotypes (Christopher and Rajam, 1994), cultivars (Ezura *et al.*, 1993;

Szasz *et al.*, 1995; Ramirez and Ochoa, 1996), and species (Christopher and Rajam, 1996). Recent studies have also supported the influence of genotype on organogenic capacity, for example, Mathew (2002) observed better regeneration response in cv. Byadagi Dabbi compared to Arka Lohit. Venkataiah *et al.*, (2003) reported TDZ mediated organogenesis in 10 pepper cultivars and the extent of the response depended upon the genotype specifically. Valadez *et al.*, (2009) reported a protocol for *in vitro* regeneration of four different types of chilli genotypes that varied in their organogenic responses. Kumar and Tata (2010) obtained better *in vitro* plant regeneration from variety X-235. Kumar *et al.*, (2012) studied regeneration of red pepper cultivars and observed variable degree of regeneration. Marta and Pawel (2015) observed organogenesis in the 3 genotypes representing *C. annuum* L. was similar and considerably lower for line SF9 derived from an interspecific hybrid (*C. frutescens* L. × *C. annuum*). Orientation of cotyledon explants resulted significant effect on plant regeneration. Abaxial side of cotyledonary

explants having the contact with growing medium responded better than adaxial side of cotyledons (Plate 1). However, the plant regeneration response was variable on MS medium supplemented with different concentrations of growth regulators. Cotyledon explants of hybrid Indra having abaxial side in contact with the growing medium supplemented with 7.5 mg/l zeatin along with 2.0 mg/l GA₃ recorded maximum per cent regeneration (78.9%), more number of elongated shoots per explant (2.4) and highest per cent shoot elongation (65.0%) (Table 5). While, the hybrid Bharat reported maximum per cent regeneration (75.7%), more number of elongated shoots per explant (2.4) and highest per cent shoot elongation (64.7%) on cotyledon explants having abaxial side in contact with growing medium containing 10.0 at mg/l zeatin along with 2.0 mg/l GA₃ (Table 5). In a comparative study of *in vitro* capsicum regeneratin, Mathew (2002), Shivegowda *et al.*, (2002), Mok and Norzulaani (2007) and Khurana *et al.*, (2011) reported best regeneration in cotyledonary explants.

Table.1 Effect of different seed treatments on *in vitro* seed germination of two Capsicum hybrids used in the study

Seed treatments before sowing seeds <i>in vitro</i>	Days taken to seed germination		Seed germination percentage	
	Bharat	Indra	Bharat	Indra
Sowing of fresh seeds <i>in vitro</i> - (no seed treatment)- as a Control	29.00 ±0.58	32.67 ±0.67	23.67 ±1.45	20.00 ±2.31
Seed soaked in distilled water for two days at room temperature	25.00 ±0.58	26.33 ±0.88	53.89 ±1.10	42.44 ±1.24
Seed soaked in distilled water for two days at 4°C	24.33 ±0.33	25.00 ±0.58	55.14 ±0.94	42.59 ±1.33
Seed soaked in distilled water with GA ₃ at 2 mg/l for 2 days at room temperature	10.67 ±0.33	11.67 ±0.33	83.49 ±0.97	89.14 ±1.51
Seed soaked in distilled water with GA ₃ at 2 mg/l for two days at 4°C	9.67 ±0.33	10.33 ±0.33	84.59 ±1.09	90.45 ±0.81
CD at 5%	1.43	1.90	3.58	4.86

Table.2 Effect of MS medium with BAP ± NAA/GA₃ on regeneration of cotyledon explants of Capsicum hybrids Bharat and Indra having abaxial as well as adaxial side in contact with the growing medium

MS + growth regulators (mg/l)			Bharat						Indra					
			Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation		Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation	
BAP	NAA	GA ₃	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial
2.5	0.0	0.0	27.16 (31.4±0.8)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	17.28 (24.5±1.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
2.5	0.2	0.0	12.35 (20.5±1.1)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	7.41 (15.8±0.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
2.5	0.0	2.0	28.40 (32.2±0.8)	0.00 (0.0±0.0)	0.33 (1.2±0.0)	0.00 (1.0±0.0)	18.52 (25.2±2.9)	0.00 (0.0±0.0)	17.28 (24.5±1.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
5.0	0.0	0.0	51.85 (46.1±0.0)	12.35 (20.5±1.1)	0.89 (1.4±0.0)	0.00 (1.0±0.0)	28.33 (32.2±0.0)	0.00 (0.0±0.0)	45.68 (42.5±0.7)	19.75 (26.4±0.9)	0.56 (1.3±0.1)	0.00 (1.0±0.0)	24.44 (29.6±0.4)	0.00 (0.0±0.0)
5.0	0.2	0.0	37.04 (37.5±0.0)	4.94 (12.7±1.6)	0.33 (1.2±0.0)	0.00 (1.0±0.0)	9.26 (17.7±0.9)	0.00 (0.0±0.0)	29.63 (33.0±0.0)	6.17 (14.2±1.6)	0.11 (1.1±0.1)	0.00 (1.0±0.0)	8.33 (16.8±0.0)	0.00 (0.0±0.0)
5.0	0.0	2.0	53.09 (46.8±0.7)	13.58 (21.6±1.1)	2.33 (1.8±0.0)	0.44 (1.2±0.1)	57.78 (49.5±0.7)	50.00 (44.9±0.0)	46.91 (43.2±0.7)	20.99 (27.2±0.9)	1.56 (1.6±0.0)	0.33 (1.2±0.0)	50.00 (45.0±1.1)	42.59 (40.7±1.1)
7.5	0.0	0.0	86.42 (68.4±1.1)	37.04 (37.5±0.0)	2.11 (1.8±0.0)	0.89 (1.4±0.0)	55.75 (48.3±0.5)	46.30 (42.8±2.1)	82.72 (65.4±1.0)	38.27 (38.2±0.7)	1.22 (1.5±0.0)	0.89 (1.4±0.0)	47.62 (43.6±0.8)	40.74 (39.6±1.1)
7.5	0.2	0.0	61.73 (51.8±0.7)	12.35 (20.5±1.1)	1.00 (1.4±0.1)	0.33 (1.2±0.0)	30.37 (33.4±0.6)	27.78 (31.5±3.7)	59.26 (50.3±0.0)	13.58 (21.6±1.1)	0.89 (1.4±0.0)	0.22 (1.1±0.1)	28.89 (32.5±1.4)	20.37 (26.8±1.3)
7.5	0.0	2.0	87.65 (69.5±1.1)	37.04 (37.5±0.0)	3.78 (2.2±0.0)	1.67 (1.6±0.0)	73.21 (58.8±0.8)	62.96 (52.5±1.1)	83.95 (66.4±1.0)	39.51 (38.9±0.7)	2.67 (1.9±0.0)	1.56 (1.6±0.0)	66.47 (54.6±0.6)	62.96 (52.5±0.6)
10.0	0.0	0.0	87.65 (69.5±1.1)	38.27 (38.2±0.7)	2.22 (1.8±0.0)	0.89 (1.4±0.0)	56.15 (48.5±0.7)	48.15 (43.9±1.1)	81.48 (64.5±0.0)	37.04 (37.5±0.0)	1.11 (1.5±0.0)	0.78 (1.3±0.0)	45.24 (42.3±0.0)	38.89 (38.6±0.0)
10.0	0.2	0.0	61.73 (51.8±0.7)	12.35 (20.5±1.1)	1.22 (1.5±0.0)	0.44 (1.2±0.1)	31.48 (34.1±0.6)	27.78 (31.5±3.7)	58.02 (49.6±0.7)	12.35 (20.5±1.1)	0.89 (1.4±0.0)	0.22 (1.1±0.1)	29.63 (33.0±0.9)	20.37 (26.8±1.3)
10.0	0.0	2.0	88.89 (70.5±0.0)	38.27 (38.2±0.7)	3.89 (2.2±0.0)	1.78 (1.7±0.0)	73.61 (59.1±0.9)	62.96 (52.5±1.1)	82.72 (65.4±1.0)	37.04 (37.5±0.0)	2.56 (1.9±0.0)	1.44 (1.6±0.0)	65.87 (54.3±1.6)	61.11 (51.4±0.0)
CD at 5%			2.28	2.39	0.09	0.08	2.99	5.07	2.08	2.29	0.10	0.09	2.34	2.11

*,** Figures in parenthesis are angular and square root transformed values, respectively

Table.3 Effect of MS medium with Kinetin ± NAA/GA₃ on regeneration of cotyledon explants of Capsicum hybrids Bharat and Indra having abaxial as well as adaxial side in contact with the growing medium

MS + growth regulators (mg/l)			Bharat						Indra					
Kinetin	NAA	GA ₃	Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation		Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation	
			abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial
2.5	0.0	0.0	29.63 (33.3±0.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	35.80 (36.7±0.7)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
2.5	0.2	0.0	16.05 (23.6±1.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	14.81 (22.6±0.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
2.5	0.0	2.0	30.86 (33.7±0.8)	0.00 (0.0±0.0)	0.89 (1.4±0.0)	0.00 (1.0±0.0)	22.22 (28.1±0.0)	0.00 (0.0±0.0)	35.80 (36.7±0.7)	0.00 (0.0±0.0)	1.00 (1.4±0.0)	0.00 (1.0±0.0)	22.22 (28.1±0.0)	0.00 (0.0±0.0)
5.0	0.0	0.0	53.09 (46.8±0.7)	14.81 (22.6±0.0)	1.00 (1.4±0.1)	0.22 (1.1±0.1)	36.67 (37.2±1.0)	27.78 (31.5±3.7)	58.02 (49.6±0.7)	23.46 (28.9±0.8)	1.11 (1.5±0.0)	0.44 (1.2±0.1)	40.37 (39.4±0.0)	33.33 (35.3±0.0)
5.0	0.2	0.0	39.51 (38.9±0.7)	8.64 (17.0±1.2)	0.78 (1.3±0.0)	0.00 (1.0±0.0)	16.67 (24.1±0.0)	0.00 (0.0±0.0)	39.51 (38.9±0.7)	11.11 (19.5±0.0)	0.78 (1.3±0.0)	0.00 (1.0±0.0)	20.37 (26.8±0.7)	0.00 (0.0±0.0)
5.0	0.0	2.0	54.32 (47.5±0.7)	14.81 (22.6±0.0)	2.56 (1.9±0.1)	0.67 (1.3±0.0)	58.89 (50.1±0.7)	50.00 (45.0±0.0)	59.26 (50.3±0.0)	24.69 (29.8±0.8)	2.78 (1.9±0.0)	0.78 (1.3±0.0)	60.37 (51.0±1.1)	51.85 (46.1±1.1)
7.5	0.0	0.0	90.12 (71.7±1.2)	38.27 (38.2±1.5)	2.78 (1.9±0.0)	1.11 (1.5±0.0)	57.56 (49.3±0.5)	48.15 (43.9±2.1)	91.36 (73.0±1.2)	41.98 (40.4±0.7)	2.89 (2.0±0.1)	1.22 (1.5±0.0)	58.18 (49.7±1.3)	50.00 (45.0±1.8)
7.5	0.2	0.0	62.96 (52.5±0.0)	13.58 (21.6±1.1)	1.33 (1.5±0.0)	0.67 (1.3±0.0)	33.33 (35.2±1.4)	27.78 (31.5±3.7)	64.20 (53.2±0.7)	18.52 (25.4±1.6)	1.44 (1.6±0.0)	0.78 (1.3±0.0)	38.52 (38.3±1.0)	27.78 (31.8±0.0)
7.5	0.0	2.0	91.36 (73.0±1.2)	38.27 (38.2±1.5)	3.89 (2.2±0.0)	1.78 (1.7±0.0)	75.62 (60.4±0.2)	64.81 (53.6±0.6)	92.59 (74.5±2.4)	43.21 (41.1±0.7)	4.11 (2.3±0.0)	2.00 (1.7±0.1)	76.08 (60.7±1.2)	65.93 (54.3±0.6)
10.0	0.0	0.0	90.12 (71.7±1.2)	39.51 (38.9±0.7)	2.89 (2.0±0.0)	1.22 (1.5±0.0)	57.56 (49.3±0.5)	50.00 (45.0±1.8)	91.36 (73.0±1.2)	40.74 (39.7±0.0)	2.78 (1.9±0.0)	1.22 (1.5±0.0)	56.79 (48.9±0.5)	48.15 (43.9±2.3)
10.0	0.2	0.0	62.96 (52.5±0.0)	14.81 (22.6±0.0)	1.44 (1.6±0.0)	0.78 (1.3±0.0)	33.33 (35.2±1.4)	27.78 (31.5±3.7)	62.96 (52.5±0.0)	18.52 (25.4±1.6)	1.33 (1.5±0.0)	0.78 (1.3±0.0)	37.41 (37.7±1.1)	27.78 (31.8±0.0)
10.0	0.0	2.0	91.36 (73.0±1.2)	40.74 (39.7±0.0)	4.00 (2.2±0.0)	1.89 (1.7±0.0)	75.77 (60.5±0.3)	65.00 (53.7±0.7)	91.36 (73.0±1.2)	41.98 (40.4±0.7)	4.00 (2.2±0.0)	2.00 (1.7±0.0)	75.93 (60.6±1.3)	64.81 (53.6±0.6)
CD at 5%			2.55	2.37	0.11	0.08	2.01	6.01	3.06	2.39	0.09	0.10	2.59	2.65

*,** Figures in parenthesis are angular and square root transformed values, respectively

Table.4 Effect of MS medium with TDZ ± NAA/GA₃ on regeneration of cotyledon explants of Capsicum hybrids Bharat and Indra having abaxial as well as adaxial side in contact with the growing medium

MS + growth regulators (mg/l)			Bharat						Indra					
			Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation		Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation	
TDZ	NAA	GA ₃	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial
2.5	0.0	0.0	62.96 (52.5±0.0)	8.64 (17.0±1.2)	0.56 (1.3±0.1)	0.00 (1.0±0.0)	23.33 (28.9±0.0)	0.00 (0.0±0.0)	58.02 (49.6±0.7)	12.35 (20.5±1.1)	0.33 (1.2±0.0)	0.00 (1.0±0.0)	19.26 (26.0±0.3)	0.00 (0.0±0.0)
2.5	0.2	0.0	39.51 (38.9±0.7)	3.70 (11.1±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	35.80 (36.7±0.7)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
2.5	0.0	2.0	64.20 (53.2±0.7)	8.64 (17.0±1.2)	1.78 (1.7±0.0)	0.44 (1.2±0.1)	40.37 (39.4±0.4)	33.33 (35.3±0.0)	59.26 (50.3±0.0)	13.58 (21.6±1.1)	1.44 (1.6±0.0)	0.33 (1.2±0.0)	37.78 (37.9±0.0)	33.33 (35.3±0.0)
5.0	0.0	0.0	91.36 (73.0±1.2)	37.04 (37.5±0.0)	0.89 (1.4±0.0)	0.33 (1.2±0.0)	31.02 (33.8±0.6)	27.78 (28.6±0.0)	83.95 (66.4±1.0)	39.51 (38.9±0.7)	0.78 (1.3±0.0)	0.22 (1.1±0.1)	30.75 (33.7±0.3)	27.78 (31.8±0.0)
5.0	0.2	0.0	59.26 (50.3±1.3)	13.58 (21.6±1.1)	0.56 (1.3±0.1)	0.00 (1.0±0.0)	18.89 (25.8±0.5)	0.00 (0.0±0.0)	56.79 (48.9±0.7)	13.58 (21.6±1.1)	0.44 (1.2±0.1)	0.00 (1.0±0.0)	19.63 (26.3±0.3)	0.00 (0.0±0.0)
5.0	0.0	2.0	92.59 (74.2±0.0)	37.04 (37.5±0.0)	2.44 (1.9±0.0)	1.11 (1.5±0.1)	57.41 (49.3±0.8)	50.00 (45.0±0.0)	85.19 (67.4±1.7)	40.74 (39.7±0.0)	1.89 (1.7±0.0)	1.00 (1.4±0.0)	55.16 (47.9±0.4)	49.07 (44.5±1.9)
7.5	0.0	0.0	51.85 (46.1±1.2)	9.88 (18.2±1.2)	0.56 (1.3±0.1)	0.00 (1.0±0.0)	21.67 (27.7±0.7)	0.00 (0.0±0.0)	39.51 (38.9±0.7)	8.64 (17.0±1.2)	0.44 (1.2±0.1)	0.00 (1.0±0.0)	17.59 (24.8±0.7)	0.00 (0.0±0.0)
7.5	0.2	0.0	35.80 (36.7±0.7)	3.70 (11.1±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	28.40 (32.2±0.8)	3.70 (11.1±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
7.5	0.0	2.0	53.09 (46.8±0.7)	11.11 (19.5±0.0)	1.33 (1.5±0.1)	0.00 (1.0±0.0)	34.44 (35.9±0.3)	0.00 (0.0±0.0)	40.74 (39.7±0.0)	9.88 (18.2±1.2)	1.00 (1.4±0.0)	0.00 (1.0±0.0)	27.78 (31.8±0.0)	0.00 (0.0±0.0)
10.0	0.0	0.0	8.64 (17.0±1.2)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
10.0	0.2	0.0	3.70 (11.1±0.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
10.0	0.0	2.0	8.64 (16.7±2.8)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
CD at 5%			3.39	2.01	0.10	0.05	1.18	3.15	2.19	2.22	0.08	0.04	0.78	1.63

*,** Figures in parenthesis are angular and square root transformed values, respectively

Table.5 Effect of MS medium with Zeatin ± NAA/GA₃ on regeneration of cotyledon explants of Capsicum hybrids Bharat and Indra having abaxial as well as adaxial side in contact with the growing medium

MS + growth regulators (mg/l)			Bharat						Indra					
			Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation		Per cent Regeneration		Elongated shoots per explant		Per cent shoot elongation	
Zeatin	NAA	GA ₃	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial	abaxial	adaxial
2.5	0.0	0.0	30.86 (33.7±0.8)	6.17 (14.2±1.6)	0.33 (1.2±0.1)	0.00 (1.0±0.0)	11.11 (19.5±0.0)	0.00 (0.0±0.0)	39.51 (38.9±0.7)	7.41 (15.8±0.0)	0.44 (1.2±0.1)	0.33 (1.2±0.0)	16.67 (24.1±0.0)	0.00 (11.8±0.0)
2.5	0.2	0.0	17.28 (24.5±1.0)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)	24.69 (29.8±0.8)	0.00 (0.0±0.0)	0.00 (1.0±0.0)	0.00 (1.0±0.0)	0.00 (0.0±0.0)	0.00 (0.0±0.0)
2.5	0.0	2.0	32.10 (34.5±0.8)	6.17 (14.2±1.6)	1.00 (1.4±0.0)	0.22 (1.1±0.1)	25.00 (29.9±1.8)	22.22 (27.8±3.7)	40.74 (39.7±0.0)	8.64 (17.0±1.2)	1.00 (1.4±0.1)	0.56 (1.3±0.1)	33.33 (35.3±0.0)	33.33 (31.3±0.7)
5.0	0.0	0.0	58.02 (49.6±0.7)	19.75 (26.4±0.9)	1.44 (1.6±0.0)	0.44 (1.2±0.1)	38.52 (38.4±0.4)	29.63 (33.0±1.2)	61.73 (51.8±0.7)	28.40 (32.2±0.8)	1.56 (1.6±0.0)	0.67 (1.3±0.1)	47.78 (43.7±0.6)	31.48 (36.4±1.1)
5.0	0.2	0.0	39.51 (38.9±0.7)	9.88 (18.2±1.2)	0.89 (1.4±0.0)	0.11 (1.1±0.1)	24.44 (29.6±0.4)	16.67 (24.1±0.0)	43.21 (41.1±0.7)	12.35 (20.5±1.1)	1.00 (1.4±0.0)	0.22 (1.1±0.1)	25.93 (30.6±0.6)	16.67 (26.7±2.6)
5.0	0.0	2.0	59.26 (50.3±0.0)	20.99 (27.2±0.9)	2.78 (1.9±0.0)	0.89 (1.4±0.0)	62.22 (52.1±0.1)	51.85 (46.1±1.1)	61.73 (51.8±0.7)	28.40 (32.2±0.8)	2.89 (2.0±0.0)	1.11 (1.5±0.0)	63.70 (52.9±0.4)	40.74 (46.1±1.1)
7.5	0.0	0.0	92.59 (74.2±0.0)	41.98 (40.4±0.7)	2.89 (2.0±0.0)	1.33 (1.5±0.0)	58.80 (50.1±0.8)	50.00 (45.0±1.8)	95.06 (77.3±1.6)	45.68 (42.5±0.7)	3.00 (2.0±0.1)	1.56 (1.6±0.0)	59.88 (50.7±0.6)	40.56 (45.6±0.6)
7.5	0.2	0.0	62.96 (52.5±0.0)	17.28 (24.5±1.0)	1.56 (1.6±0.0)	0.78 (1.3±0.0)	35.56 (36.6±0.0)	33.33 (35.3±0.0)	67.90 (55.5±0.8)	19.75 (26.4±0.9)	1.67 (1.6±0.1)	1.00 (1.4±0.0)	39.95 (39.2±0.6)	33.33 (36.4±1.1)
7.5	0.0	2.0	93.83 (75.7±1.6)	41.98 (40.4±0.7)	4.44 (2.3±0.0)	1.89 (1.7±0.0)	80.40 (63.7±0.2)	67.59 (55.3±1.5)	96.30 (78.9±0.0)	46.91 (43.2±0.7)	4.56 (2.4±0.0)	2.33 (1.8±0.0)	82.10 (65.0±1.0)	63.33 (57.8±1.1)
10.0	0.0	0.0	93.83 (75.7±1.6)	43.21 (41.1±0.7)	2.89 (2.0±0.0)	1.33 (1.5±0.1)	59.41 (50.4±0.5)	51.85 (46.1±1.1)	95.06 (77.3±1.6)	44.44 (41.8±0.0)	2.89 (2.0±0.0)	1.56 (1.6±0.0)	58.64 (50.0±0.5)	41.67 (46.1±1.1)
10.0	0.2	0.0	62.96 (52.5±0.0)	18.52 (25.5±0.0)	1.78 (1.7±0.0)	0.89 (1.4±0.0)	35.56 (36.6±0.0)	33.33 (35.3±0.0)	64.20 (53.2±0.7)	19.75 (26.4±0.9)	1.56 (1.6±0.0)	1.00 (1.4±0.0)	38.52 (38.3±1.0)	33.33 (36.4±1.1)
10.0	0.0	2.0	93.83 (75.7±1.6)	43.21 (41.1±0.7)	4.56 (2.4±0.0)	2.11 (1.8±0.0)	81.79 (64.7±1.0)	68.52 (55.9±1.2)	95.06 (77.3±1.6)	45.68 (42.5±0.7)	4.44 (2.3±0.0)	2.22 (1.8±0.0)	80.71 (64.0±1.6)	62.22 (57.8±1.1)
CD at 5%			2.74	2.80	0.11	0.12	1.97	4.19	2.85	2.23	0.11	0.11	2.12	2.64

*,** Figures in parenthesis are angular and square root transformed values, respectively

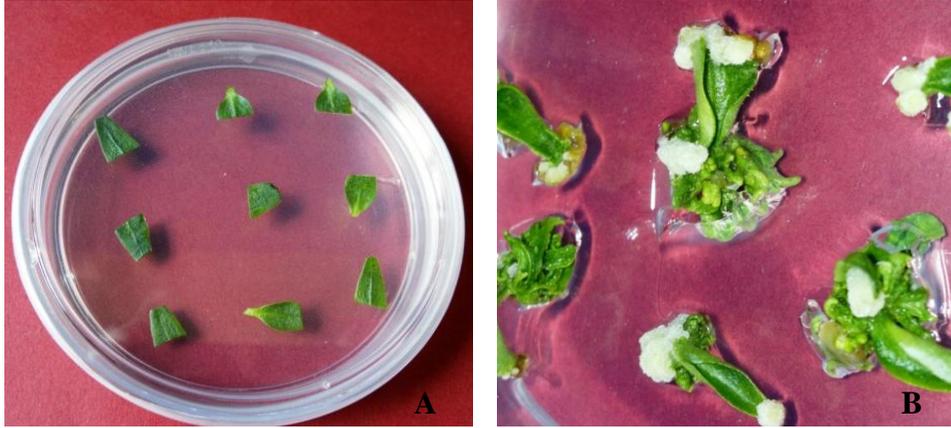


Plate.1 (A) Cotyledonary explants, adaxial side contact with regeneration medium, (B) Multiple shoots induction at the cut ends of cotyledonary explants



Plate.2 (A) Root induction on rooting medium, (B) Complete plant before hardening



Plate.3 (A) Initial hardening of regenerated plantlets and, (B) Hardened plants of capsicum grown in pots

Plant hormones play a crucial role in controlling the plants growth and development. Both auxins and cytokinins are synergistically required to induce cell division and growth in plant tissue cultures. In the study, BAP, kinetin, TDZ and zeatin along with GA₃ were used for developing efficient *in vitro* plant regeneration in selected capsicum hybrids Bharat and Indra. Among the cytokinins tested, zeatin followed by kinetin, was found superior with maximum per cent shoot elongation but minimum was observed in thidiazuron (TDZ). Inhibition of shoot elongation is a common problem with TDZ and it may be consistent with its super-optimal cytokinin activity, whereas the presence of a phenyl group in TDZ might be the possible cause of shoot-bud fasciation (Huetteman and Preece, 1993; Steinitz *et al.*, 2003). In the study, shoot buds induced from explants on a medium containing TDZ, did not elongate properly and resulted in a rosette of shoots when continued to be cultured on the same medium. Thus, in order to elongate the shoot, it needs to culture the explants on medium containing TDZ along with GA₃.

Among plant growth regulators concentrations, BAP, kinetin and zeatin at 7.5 and 10.0 mg/l along with 2.0 mg/l GA₃ and TDZ at 5.0 mg/l along with 2.0 mg/l GA₃ gave good response in all the explants studied in both the hybrids. The higher concentrations of TDZ lead to death of explants (Table 4). The growing medium supplemented with 7.5 mg/l zeatin along with 2.0 mg/l GA₃ recorded maximum per cent regeneration (78.9%), more number of elongated shoots per explant (2.4) and highest per cent shoot elongation (65%) in cotyledon explants of hybrid Indra having abaxial side in contact with medium (Plate 1B) and in hybrid Bharat same medium reported maximum per cent regeneration (75.7%), more number of elongated shoots per explant (2.4) and highest per cent shoot elongation (64.7%) on cotyledon explants

having abaxial side in contact with growing medium (Table 5). A higher concentration of BAP at 10.0 mg/l was used by Ebida and Hu (1993) to proliferate multiple shoots from cut surfaces of capsicum hypocotyls. Christopher and Rajam (1994) observed maximum shoots on MS medium with BAP at 88.8 µM or kinetin at 116.2 µM. Regeneration from cotyledonary explants of chilli was obtained by Shivegowda *et al.*, (2002) on MS medium supplemented with zeatin 9-18 µM in combination with GA₃ (2.89 µM). Nancy *et al.*, (2005) observed multiple shoots from Habanero pepper with nodes cultured on MS medium supplemented with 3.4 µM TDZ. Sanatombi and Sharma (2008) obtained maximum number of shoots in shoot-tip of *Capsicum chinense* Jacq. cv. Umorok, on medium containing 91.2 µM BAP and 31.1 µM TDZ with 4.7 µM Kinetin. Shoot multiplication in four chilli cultivars was obtained in MS medium with 6.0 mg/l BAP, 1.0 mg/l kinetin and 0.5 mg/l GA₃ (Ranjan *et al.*, 2010). Khurana *et al.*, (2011) developed a method of plant regeneration in male sterile line MS-12 of chilli on MS media supplemented with 9.0 mg/l BAP, 2.0 mg/l kinetin and 2.0 mg/l IAA. Dafadar *et al.*, (2012) obtained maximum number of shoots per leaf explants on medium containing 8.87 µM BAP and 2.85 µM IAA. Rahul *et al.*, (2015) obtained more numbers of shoot buds from Naga chili in MS medium containing 5 mg/l BAP and 0.5 mg/l IAA.

Root induction was done by sub-culturing the shoots on MS medium supplemented with 0.5 mg/l IBA. Cent per cent rooting was observed in regenerated shoots of capsicum hybrids Bharat and Indira on MS medium containing 0.5 mg/l IBA (Plate 2). MS medium with IBA at 0.1 mg/l was used for root induction of pepper by Agarwal *et al.*, (1989). Garcia and Bahilla (1990) and Arroyo and Revilla (1991) proposed supplementation of the rooting medium with IBA at 0.05 mg/l and NAA at

0.1 mg/l. Rahul *et al.*, (2015) achieved shoot elongation and rooting in MS basal medium.

Plantlets with well developed roots were transferred to pots containing sterile mixture of coco-peat and vermiculite (1:1) and kept under culture room for hardening (Plate 3A). After 14 days of acclimatization under laboratory condition, plantlets were transferred to shade net in big size pot containing 1:1:1 mixture of soil: sand: farmyard manure (Plate 3B). Similarly, *In vitro* rooted pepper plants were acclimatized soil: vermiculite 50:50 v/v mix (Christopher and Rajam, 1994), perlite: soil 1:1 mix (Szasz *et al.*, 1995) and then transferred to soil for establishment and grow as normal plant.

The present investigation showed that plant regeneration is highly dependent on genotypes, type of explants, plant growth regulators. Hence, before conducting the transformation experiment or experiments on creation of somaclonal variation or *in vitro* selection etc., it becomes necessary to standardize the regeneration system for the targeted genotypes. Here, an efficient and reproducible regeneration protocol using capsicum hybrids Bharat and Indra was developed, which can be exploited for mass production and further research.

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How to cite this article:

Vivek Hegde, P.S. Partap and Yadav, R.C. 2017. *In Vitro* Regeneration of *Capsicum* (*Capsicum annuum* L.) from Cotyledon Explants. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 225-237. doi: <http://dx.doi.org/10.20546/ijcmas.2017.605.026>