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In Vitro Shoot Bud Differentiation from Hypocotyl Explants of Chili Peppers (*Capsicum annuum* L.)

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Abstract

Chili pepper (*Capsicum annuum* L.) is an economically important spice crop in tropical and subtropical countries. *In vitro* plant regeneration was obtained from 15th day old hypocotyl explants of three chili pepper cultivars (*Capsicum annuum* L., var. 'X-235', var. 'PC-1' and var. 'Pusa Jwala'). Among the genotypes of *Capsicum* L. var. 'X-235' responded better than the var. 'PC-1' and var. 'Pusa Jwala'. MS medium containing BAP (4.0 mg/l) and IAA (0.5 mg/l) was found to be the best medium for the production of maximum number of shoot buds in all the genotypes of chili pepper i.e., 6.80±0.16 (var. 'X-235'), 5.00±0.19 (var. 'PC-1') and 4.80±0.12 (var. 'Pusa Jwala'). The shoots were rooted on MS medium fortified with IBA (0.5 mg/l). Rooted plants were hardened and transplanted to the soil. The plants showed 80-90% survival during transplantation.

Keywords: BAP, hypocotyl, IAA, IBA, MS medium, rooting

Introduction

Capsicum annuum L. is an economically important crop plant belonging to the family *Solanaceae*; two main consumption types of pepper- spice and vegetable are prevalent throughout the world. A major constraint facing the chili crop is the high incidence of pests and diseases (Morrison *et al.*, 1986). In order to facilitate the development of plant biotechnology based cultivar improvement for this crop, considerable effort has been devoted in developing and optimizing efficient in vitro regeneration protocols. Even though other *Solanaceae* members easily undergo morphogenesis, chili was found to be highly recalcitrant (Ochoa-Alejo and Ramirez-Malagon, 2001; Steinitz *et al.*, 1999). The regeneration capacity of chili plants has been carried out successfully from hypocotyl explants (Borychowski *et al.*, 2002; Christopher and Rajam, 1994, 1996; Fari and Czako, 1981; Gunay and Rao, 1978; Mok and Norzulaani, 2007; Rodeva *et al.*, 2006; Sanatombi and Sharma, 2008; Singh and Shukla, 2001). However, many of these reports suggest a strong influence of the genotype on the regeneration process (Christopher and Rajam, 1996; Ochoa-Alejo and Ireta-Moreno, 1990; Ramirez-Malagon and Ochoa-Alejo, 1996; Szasz *et al.*, 1995). The present investigation, describes the culture of hypocotyl explants of three cultivars of *C. annuum* L. The study was undertaken to determine the regeneration potential and to develop efficient in vitro plant regeneration protocol for the three genotypes of *C. annuum* L.

Materials and methods

Seeds of three genotypes of *C. annuum* L. viz., var. 'X-235', var. 'PC-1' and var. 'Pusa Jwala' were obtained from Sutton and Seeds, Calcutta, India. The seeds were surface sterilized with 0.1% $HgCl_2$, repeatedly washed in sterile distilled water and inoculated on MS (Murashige and Skoog, 1962) basal medium for germination. The hypocotyl explants were about 2-3 (cm) in length, derived from 15 days old *in vitro* germinated seedlings cultured on MS medium supplemented with various concentrations of auxins like IAA (indole-3-acetic acid) and IBA (indole-3-butyric acid) and cytokinin BAP (6- benzyl aminopurine). The pH of the media was adjusted to 5.8 and solidified with agar, before autoclaving. For shoot bud induction the explants were placed on MS medium supplemented with BAP (1.0-5.0 mg/l) alone or combination with IAA (0.5 mg/l) and subculture at two weeks to the same medium. The number of shoot buds were recorded after eight weeks of culture. To test their rooting, capacity the elongated shoots, were excised and transferred to different concentrations of IAA (0.25-1.0 mg/l) and IBA (0.25-1.0 mg/l). The rooting i.e., number of roots and length of the root per shoot were noted after four weeks of culture. Plants with roots were transferred during four weeks, after washing of the agar with distilled water, to pot with a mixture of soil-rite (1:1). Potted plantlets were covered with transparent polythene membrane to ensure high humidity and watered every third day with half strength MS salt solution for two weeks in order to acclimatize plants to field conditions. After four weeks, acclimatized plants were transferred to pots containing normal garden soil and maintained in greenhouse under natural day length conditions. All the cultures were maintained in a growth chamber at a temperature of 25±2°C and 16 hours photoperiod provided by white fluorescent tubes (30 μmol $m^{-2}S^{-1}$). All the experiments were repeated thrice; each treatment for shoot bud

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Tab. 1. Effect of BAP and IAA on shoot bud induction from hypocotyl explants of three genotypes of *C. annuum* L.

No.	Growth regulators (mg/l)		Genotype		
	BAP	IAA	*C. annuum* var. 'X-235'	*C. annuum* var. 'PC-1'	*C. annuum* var. 'Pusa Jwala'
1	-	-	-	-	-
2	1.0	-	0.83 ± 0.02	0.52 ± 0.04	0.40 ± 0.09
3	2.0	-	1.00 ± 0.05	0.64 ± 0.02	0.57 ± 0.03
4	3.0	-	1.06 ± 0.02	1.00 ± 0.07	0.94 ± 0.01
5	4.0	-	1.10 ± 0.06	1.08 ± 0.02	1.00 ± 0.08
6	5.0	-	1.00 ± 0.03	0.90 ± 0.07	0.85 ± 0.05
7	1.0	0.5	2.20 ± 0.04	2.00 ± 0.08	1.83 ± 0.18
8	2.0	0.5	3.10 ± 0.20	2.90 ± 0.25	1.97 ± 0.27
9	3.0	0.5	4.90 ± 0.12	3.82 ± 0.20	3.62 ± 0.19
10	4.0	0.5	6.80 ± 0.16	5.00 ± 0.19	4.80 ± 0.12
11	5.0	0.5	5.20 ± 0.25	4.80 ± 0.30	4.50 ± 0.20

* Results are mean of twenty replicates (20X3) ± SE

induction from hypocotyl explants and rooting of shoot buds consisted of twenty and ten replicates respectively.

Results and discussions

The data from (Tab. 1) show that the cultivated hypocotyl explants from the three studied *C. annuum* L. genotypes develop shoot buds with reference to the media composition. In this study, we obtained successful regeneration with 15th day old explants of the three genotypes. The results obtained were agreed according to data reported by Borychowski *et al*. (2002) and Mok and Norzulaani (2007). The genotypes showed considerable variation in multiple shoot bud number ranged from 0.4 to 6.8 shoot buds per explant. The results are in conformity with the findings of Gunay and Rao (1978); Christopher and Rajam (1996); Ramirez-Malagon and Ochoa-Alejo (1996); Sanatombi and Sharma (2008) that reveals the regeneration of plants from hypocotyl explants in diverse cultivars of *Capsicum* L. The maximum number of shoot buds per explant was registered on an MS medium supplemented with BAP (4.0 mg/l) and IAA (0.5 mg/l) (Fig. 1 a) for all three genotypes i.e., 6.80±0.16 (var. 'X-235'), 5.00±0.19 (var. 'PC-1') and 4.80±0.12 (var. 'Pusa Jwala') while, the lowest number of shoots per explant were recorded in MS medium fortified with BAP (1.0 mg/l) i.e., 0.83±0.02 (var. 'X-235'), 0.52±0.04 (var. 'PC-1') and 0.40±0.09 (var. 'Pusa Jwala') (Tab. 1). The results of the above indicate that the plant hormonal combination of 4 mg/l (BAP) and 0.5 mg/l (IAA) was better than any other combination. The results are in agreement with earlier reports of Gunay and Rao (1978); Christopher and Rajam (1994, 1996); Ramage and Leung (1996); Sanatombi and Sharma (2008). This may be due to auxins or cytokinins used alone or in combination, which is supposed to be the result of the promotion of biosynthesis or inhibition of degradative metabolism, theory shared also by Singh and Shukla (2001). The shoots derived from hypocotyl explants were excised and implanted on MS medium supplemented with IAA and IBA in three different concentrations (Tab. 2). The better response of rooting was higher in the MS medium supplemented with IBA (0.5 mg/l). The best rooting (i.e., number of roots (11.0±0.25) and length of root (5.20±0.32) per shoot bud were achieved in the genotype 'X-235' (Fig. 1 b). Similar results were obtained by Sanatombi and Sharma (2006) on cultivars of *C. annuum* L. Our results show that 4.8 to 6.8 shoots per hypocotyl, which can be considered as a very good regeneration in comparison to 2-5 shoots reported by Gunay and Rao (1978); Christopher and Rajam (1996); Sanatombi and Sharma (2008). The regenerated plants showed 80-90% survival during hardening and acclimatization (Fig. 1 c and d) and there are no observable differences between the parent plant and in vitro raised plants. The transplanted plantlets established well in pots and in the field.

Tab. 2. Effect of Auxins on rooting of *in vitro* induced shoot buds from hypocotyl explants of three genotypes of *C. annuum* L.

S. No.	Auxins (mg/l)		Genotype					
	IAA	IBA	*C. annuum* var. 'X-235'		*C. annuum* var. 'PC-1'		*C. annuum* var. 'Pusa Jwala'	
			No. of Roots/ Shoot	Root length (cm)	No. of Roots/ Shoot	Root length (cm)	No. of Roots/ Shoot	Root length (cm)
1	-	-	-	-	-	-	-	-
2	0.25	-	7.00 ± 0.28	2.60 ± 0.28	5.00 ± 0.20	1.80 ± 0.30	5.00 ± 0.18	1.60 ± 0.20
3	0.50	-	9.00 ± 0.11	3.20 ± 0.20	8.00 ± 0.19	2.70 ± 0.16	7.00 ± 0.14	2.80 ± 0.11
4	1.00	-	6.50 ± 0.34	2.40 ± 0.25	6.00 ± 0.28	2.00 ± 0.20	4.80 ± 0.24	1.80 ± 0.13
5	-	0.25	9.00 ± 0.49	4.60 ± 0.20	8.20 ± 0.35	4.10 ± 0.25	7.60 ± 0.37	3.80 ± 0.27
6	-	0.50	11.00 ± 0.25	5.20 ± 0.32	9.00 ± 0.20	4.80 ± 0.26	9.00 ± 0.15	4.60 ± 0.30
7	-	1.00	8.00 ± 0.45	3.40 ± 0.26	6.00 ± 0.22	3.00 ± 0.19	5.50 ± 0.29	4.00 ± 0.19

* Results are mean of ten replicates (10X3) ± SE

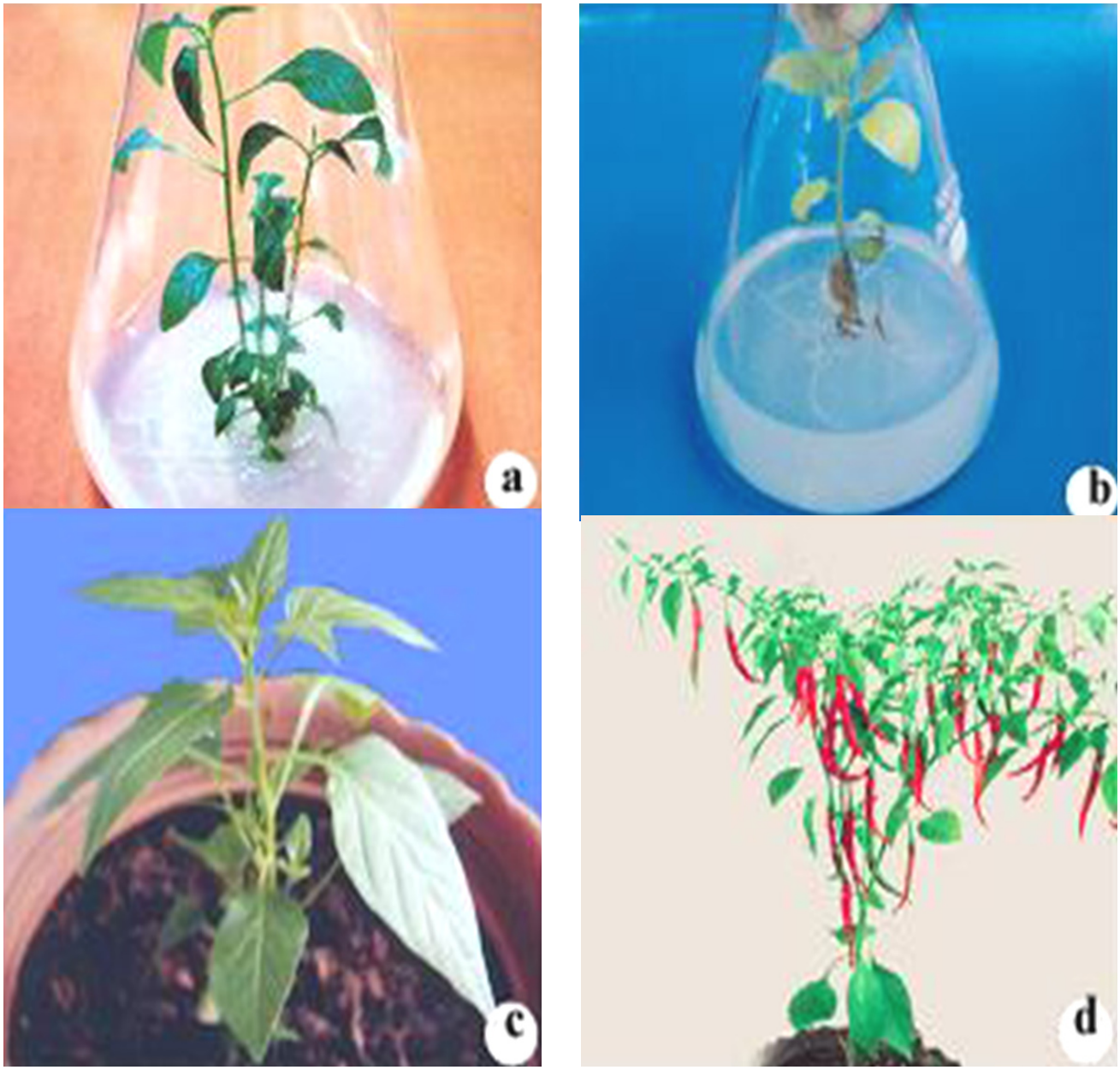

Fig. 1. *In vitro* shoot bud differentiation on *Capsicum annuum* L. var. 'X-235': (a) Induction of multiple shoot buds from hypocotyl explant; (b) Rooted plantlet; (c) Growth of transplanted plantlet in pot; (d) Fully developed plant with good fruit set

Conclusions

The present study reports that 15th day old hypocotyl explants of three genotypes of *C. annuum* L. showed variation from multiple shoot bud number from one another, among the genotypes var. 'X-235' responded better than the var. 'PC-1' and var. 'Pusa Jwala'. The study revealed that 4.8 to 6.8 shoot buds per explant were achieved in genotypes of *C. annuum* L. in MS medium supplemented with BAP (4.0 mg/l) and IAA (0.5 mg/l). The genotypes selected for the study and the methods used for *in vitro* regeneration and rooting, can be applied to increase the efficiency of transformation protocols using hypocotyls as explant source.

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