Standardization of *in vitro* rooting medium for root induction of regenerated chili shoots system

M.M. Uddin, M.S. Haque[1] and M.B. Miah[2]
Department of Agricultural Extension, Khamarbari, Dhaka, [1]Department of Biotechnology and [2]Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202

Abstract: A study was designed with the aim to establish high frequency root induction in chili cultivars (balujuri marich, bogra marich and deshi marich) which is limited by a number of biotic and abiotic factors. Explants were collected from *in vitro* culture. The effects of genotype, various explant types and hormones were evaluated. It was shown that MS medium with o.5mg/L IBA was suitable for root differentiation. The different pepper materials differed greatly in their root regeneration capacities. Per cent of root initiation was significantly different between the two studied explants and shoot tip based shoot ensured higher (31.94%) root initiation. Shoot tip based shoot of Bogra marich cultured on MS supplemented with 0.5 mg/L IBA and produced the highest per cent of root (91.67%).
Key words: Chili, genotype, root induction.

Introduction

The yield of chili (*Capsicum spp*. belonging to the family Solanaceae) is very low compared to the yield of chili in other countries of the world. The most important factors that affect chili cultivation drastically are the insect and diseases problems. In Bangladesh, the crop suffers from as many as 15 diseases of which 12 are caused by fungi (Ahmed and Hossain, 1985). The use of transgenic plants is a method to improve existing chili cultivar. Hence, for commercial cultivation with profitable yield, it required to develop insect and diseases resistant variety. *In vitro* plant regeneration from cells, tissues and organ cultures is a prerequisite for the application of plant biotechnology to plant propagation, plant breeding and genetic improvement. Tissue culture aspects of the chili plant have been well studied (Hussain *et al*., 1999). The establishment of efficient and promising protocol for in vitro plantlet regeneration of *Capsicum* is required for the application of modern biotechnological tools, such as asexual reproduction of elite stocks, recovery of useful somaclonal variants, germplasm preservation as well as the production of transgenic plants with improved agronomic traits, interspecific hybrids, and haploid plants (Aguado-Santacruz *et al.,* 2004). In tissue cultures of chili on the shoot induction medium normally roots are not induced. Therefore, rooting has to be induced by subculturing on medium with auxins. Roots were produced in the rosette buds that developed into plantlets using rooting media containing IBA, according to earlier reports (Ebida and Hu, 1993). Agrawal *et al*. (1988) reported that IBA was successfully employed for rooting in *Capsicum.* Ahmad *et al*. (2006) conducted an experiment to observe the effect of different Auxin (IAA, IBA, NAA) in various concentrations for root initiation. Regenerated shoots rooted within 2 weeks of culture but with different rooting frequency in each treatment. The maximum rooting was observed at 1.0 µM IBA. The regenerated plantlets could be maintained on MS basal medium for 2 weeks and then transferred to vermiculite irrigated with mineral nutrient solution to establish regenerated plant (Subhash and Christopher, 1988). *In vitro* rooted plants were acclimatized in a Terrulite vegetable plug mix (Ediba and Hu, 1993), 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. The well rooted plantlets were hardened in plastic cups containing sterilized vermiculite, garden soil and farmyard soil (1:1:1) by Nithiya and Arockiasamy (2007). In this study, presenting the protocol for *in vitro* root induction of regenerated chili plantlet resulting in higher number of roots per cultured explants.

Materials and Methods

The study was conducted in the Biotechnology Laboratory, BAU, Mymensingh during January/10 to July/10 to establish high frequency root induction in chili cultivars. Three local varieties of chili (Balujuri marich, Bogra marich and Deshi marich), two explant (shoot tip based shoot and callus mediated shoot) and five concentrations of IBA (0.0, 0.3, 0.5, 0.7, 1.0 mg/L) were used in the experiment. Micro shoots (explants) were collected from the plantlets (45 days after culture) acquired from the experiment studied in the laboratory. One explant was placed in one test tube/vial. Total 30 treatments were set in CRD design and each treatment consisted of six test tubes/vials and replicated 4 times. MS medium added with Indole-3-Butyric Acid (IBA) were used in the experiment. For the preparation of stock solution of IBA, 10 mg of the hormone powder was taken in a clean watch glass and dissolved in 1 ml of the particular solvent (0.1 N NaOH). The mixture was then transferred in a 100 ml measuring cylinder and volume was made up to 100 ml by the further addition of ddH_2O. The solution was then poured into a clean plastic or glass container and stored at 4^0C as stock solution. Data of days to root initiation, % root initiation (number of shoots produced root/total number of cultured shoots X 100) and number of roots per shoot was counted after required days (25 days) of culture and arithmetic mean was worked out. The regenerated rooted plantlets transferred during three weeks after washing off the agar with deionized water to pots with a mixture of Sawdust and Peat soil (1:1). The regenerated planlets were acclimatized for 72 hours before they were transferred. The data was analyzed following standard statistical procedures (Gomez and Gomez, 1984) and mean differences were adjusted by Duncan's Multiple Range Test (DMRT) using a computer operated programme named MSTST-C.

Results and Discussion

Effect of variety: Introduction of roots on regenerated shoots are essential for successful establishment of the plantlet on the soil. Hence root characteristics from shoot were investigated in the present experiment. Per cent root

initiation was significantly different among the studied three chili varieties, where 29.58% root initiation was observed in case of Balujuri marich and Bogra marich but the lowest (26.67%) root initiation was estimated from Deshi marich (Table 1 and Plate 1). The findings of the present study agreed by Aniel Kumar and Subba Tata (2010). Effect of variety on days required for root initiation was non-significant. Deshi marich required more time to initiate root than that of other two varieties.

Table 1. Effect of variety, explants and IBA on root induction, days to root initiation and number of root/shoot

Variety/explant/ IBA (mg/L)	Root induction (%)	Days to root initiation	Number of roots/shoot
Balujuri marich	29.58 a	5.98	2.78 b
Bogra marich	29.58 a	6.00	3.15 b
Deshi marich	26.67 a	6.10	3.38 a
CV (%)	19.90	7.16	14.82
Shoot tip based shoot	31.94	5.68	3.42
Callus mediated shoot	25.28	6.37	2.78
CV (%)	19.90	7.16	14.82
0.0	0.00 c	0.00	0.00 c
0.3	0.00 c	0.00	0.00 c
0.5	88.56 a	14.88	9.25 a
0.7	62.5 b	15,25	6.25 b
1.0	0.00 c	0.00	0.00 c
CV (%)	19.90	7.16	14.82

Figures followed by same letter(s) are statistically similar as per DMRT

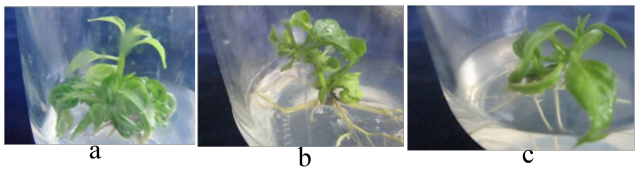

Plate 1. Root derived from different variety of chili on MS medium supplemented with 0.5 mg/L IBA; a) Balujuri marich, b) Bogra marich and c) Deshi marich.

A certain amount of roots is prerequisite condition for successful micro propagation hence root from explant of different chili varieties in different hormone concentration were also widely investigated. It was shown that variety exerted significant influences on number of roots per shoot. The highest number (3.38) of roots per shoot was counted in case of Deshi marich and the lowest (2.78) was observed in Balujuri marich followed by Bogra marich (Table 1). The results are in conformity with the findings of Sanatombi and Sharma (2008). Number of roots may be varied from variety to variety which was proved earlier by Aniel kumar and Subba Tata (2010).

Effect of explant: Per cent root initiation was investigated from the two types of explants namely shoot tip based shoot and callus mediated shoot and the results are shown in Table 1. It was found from the investigation that per cent of root initiation was significantly different between the two studied explants and shoot tip based shoot ensured higher (31.94%) root initiation. Root regeneration from different explants varied significantly which is supported by Berljak *et al*. (1985). Explants showed significant effects on days required to root initiation. Between the two explants, shoot tip based shoot started root initiation earlier (5.68 days) than shoot derived from callus-mediated shoot (6.37 days) for root initiation (Table 1). Similar findings were observed By Song *et al*. (2010) where they recorded that roots initiated from the shoot base within a week and a well developed shoot (> 10 cm in length) with a root system was achieved after four weeks of culture. The number of roots producing from shoot tip based explant was higher (3.42) than the shoot derived from callus mediated shoot (Table 1). Significantly different number of roots in different explants was reported by Berljak *et al.* (1985).

Effect of IBA: Different hormonal combinations of IBA showed significant variation on per cent of root induction. The better response (80.56%) of rooting was observed in the MS medium supplemented with 0.5 mg/L IBA and the minimum per cent of roots (62.50%) was found with 0.7 mg/L IBA. No roots were produced at IBA below 0.3 mg/L and 1.0 mg/L. Similar findings were stated by Sanatombi and Sharma (2008). Zhang *et al*. (2007) reported that the rooting efficiency with 0.5 mg/L IBA reached up to 100% within 2 weeks, strong roots formed within 3 weeks, while other hormones gave lower rooting efficiency of below 80%. Chen *et al.* (2005) also stated that the elongated shoots cultured on MS basal medium with IBA were easier to root than that on MS basal medium without IBA. There was also significant effect on days required for rooting due to different hormonal concentrations.

The minimum number (14.75) of days required for root initiation was observed in the concentration of 0.5 mg/L IBA. The maximum number (15.25) of days required for root initiation was estimated from the concentration of 0.7 mg/L IBA (Table 1). Chen *et al.* (2005) reported that rooting in the medium containing IBA was faster than that in the medium without phytohormones. The effects of IBA on number of roots were also significantly different. MS media containing 0.5 mg/L IBA was found to be most effective in producing maximum number (9.25) of roots and the minimum number (6.25) of roots was found with 0.7 mg/L IBA while no roots were produced with 0, 0.3 and 1.0 mg/L IBA (Table 1).

Combined and interaction effect of variety, explant and IBA: The interaction effect of variety, explant and different concentration of IBA was found as non-significant on per cent of root initiation. Shoot tip based shoot of Bogra marich cultured on MS supplemented with 0.5 mg/L IBA and produced the highest per cent of root

(91.67%). But when explants of the studied three varieties cultured with IBA at concentration below 0.3 or 1.0 mg/L exhibited no root initiation (Table 2). The interaction effects of variety, explant and different combinations of IBA was also non-significant in terms of days to root initiation. Considering all the three factors, it was observed that shoot tip of Balujuri marich in 0.5 mg/L IBA required minimum days (14.0) for root initiation followed by Deshi marich. Maximum days (16.75) were required for root initiation in case of callus based shoot of Deshi marich with 0.7 mg/L IBA (Table 2). No roots were observed when explants of Balujuri marich, Bogra marich and Deshi marich cultured with 0, 0.3 and 1.0 mg/L IBA. The above result showed that suitable combination of IBA was essential for the best performance in respect of root initiation. Significant effects from the interaction of variety, explant and IBA were also observed in terms of number of roots /shoot. Explant of Balujuri marich, Bogra marich and Deshi marich culture with 0.0, 0.3, 0.5, 0.7 and 1.0 mg/L IBA exhibited that shoot tip based shoot of Deshi marich cultured with 0.5 mg/L IBA was the best concentration favorably for induction of maximum number of roots (11.25) among the explants (Shoot tip based shoot and callus mediated shoot) of the studied chili varieties treating with different concentrations of IBA (Table 2).

Table 2. Combined effect of variety, explant and different IBA concentrations on root initiation, days to root initiation and number of roots/shoot

Variety	Explant	IBA (mg/L)	Root induction (%)	Days to root initiation	Number of roots/shoot
Balujuri marich	Shoot tip based shoot	0.0	0.00	0.00	0.00 h
		0.3	0.00	0.00	0.00 h
		0.5	87.50	14.00	9.50 b
		0.7	75.00	14.25	4.50 g
		1.0	0.00	0.00	0.00 h
	Callus mediated shoot	0.0	0.00	0.00	0.00 h
		0.3	0.00	0.00	0.00 h
		0.5	75.00	15.50	7.50 cd
		0.7	58.34	16.00	6.25 ef
		1.0	0.00	0.00	0.00 h
Bogra marich	Shoot tip based shoot	0.0	0.00	0.00	0.00 h
		0.3	0.00	0.00	0.00 h
		0.5	91.67	14.25	11.00 a
		0.7	75.00	14.50	7.00 de
		1.0	0.00	0.00	0.00 h
	Callus mediated shoot	0.0	0.00	0.00	0.00 h
		0.3	0.00	0.00	0.00 h
		0.5	70.84	15.50	8.00 c
		0.7	58.34	15.75	5.50 f
		1.0	0.00	0.00	0.00 h
Deshi marich	Shoot tip based shoot	0.0	0.00	0.00	0.00 h
		0.3	0.00	0.00	0.00 h
		0.5	87.50	14.00	11.25 a
		0.7	62.50	14.25	8.00 c
		1.0	0.00	0.00	0.00 h
	Callus mediated shoot	0.0	0.00	0.00	0.00 h
		0.3	0.00	0.00	0.00 h
		0.5	70.84	16.00	8.25 c
		0.7	45.83	16.75	6.25 f
		1.0	0.00	0.00	0.00 h
CV (%)			19.90	7.16	14.82

Figures followed by same letter(s) are statistically similar as per DMRT

Plate 2. Growth of chili plantlet of different varieties after transferred to earthen pot (30 days after transplant) .

Establishment of plantlets: The regenerated plantlets needs to be transplanted on appropriate soil media in *ex vitro* condition for ensuring successful adaptation with normal environment (Hasnat *et al*., 2008) The approaches increase the survival rate of plantlets up to 90% and also should prove to be highly promising to produce large quantities of plants through micropropagation within a shortest period of time. The study revealed that 96.25% plantlets where established successfully when plantlets of three variety were transplanted on the soil media consisting equal amount of Sawdust and Peat soil (Plate 2).

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