Veg. Sci. 37(1) : 30-34 (2010)

MICRO-PROPAGATION OF CHILLI (*CAPSICUM ANNUUM* L.) PLANTS THROUGH SHOOT TIP CULTURE

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Summary

Varietal response of four Indian chilli (*Capsicum annuum* L.) cultivars *viz*. KtPL-19, Pusa Sadabahar, ArCH-001 and Salem, to *in vitro* shoot tip culture was examined and protocol for *in vitro* cloning was standardized. Among different concentration combinations of BAP and IAA in MS medium, the maximum number of shoots was obtained on MS medium with 7.0 mg l^{-1} of BAP and 0.25 mg l^{-1} of IAA after 4 weeks of culture. Interaction between cultivars and media were significant with reference to the number of shoots per explant. Shoot proliferation and elongation was maximum in MS medium with combination of 6.0 mg l^{-1} BAP + 1.0 mg l^{-1} KIN + 0.5 mg l^{-1} GA_3. Supplementation with 40 gl^{-1} of sucrose resulted maximum shoot proliferation.

सारांश

मिर्च में KtPL-19, Pusa Sadabahar, ArCH-001 और Salem प्रजातियों को टीशू कल्चर में उगाने का प्रयास किया गया। बीएपी 7.0 एमजी. l^{-1} तथा IAA 0.25 एमजी l^{-1} में ज्यादा तने पाये गये। बीएपी 6.0 एमजी. l^{-1}, KIN 1.0 एमजी l^{-1} तथा GA_3 0.5 एमजी l^{-1} में तने की लम्बाई ज्यादा पाई गयी।

Introduction

Chilli (*Capsicum annuum* L.) is an important vegetable/ spice crop grown worldwide. It is an indispensable commodity in every household in India. It is conventionally propagated by seeds. However, chilli plants show high level of cross pollination (Tanksley,1984), which leads to heterogeneity in seed population which is a constraint in commercial seed production. Propagation through seeds is further restricted by short viability and low germination rate. Since the plants lack natural vegetative propagation, the *in vitro* shoot tip culture provides a novel way for asexual rapid multiplication. Furthermore, chilli plants are highly susceptible to fungal and viral diseases (Morrison *et al*.,1986). Therefore, *in vitro* shoot tip culture is an effective way for producing large number of disease-free, true-to-the type plant materials. Thus, advantage in employing this technique is the efficient clonal propagation of a large number of disease-free plants that can be used in commercial agriculture. Furthermore, *in vitro* technique also provides a tool for employing genetic transformation in this crop. Therefore, the present study was undertaken to standardize the *in vitro* clonal multiplication technique in some common chilli genotypes.

Materials and Methods

Aseptic plant establishment: Four chilli cultivars *viz*. KtPL-19 (an open pollinated cultivar good for oleoresin and red powder), Pusa Sadabahar (a cultivar resistant to LCV, CMV, and TMV), ArCH-001 (a F_1 hybrid from private seed company, good for preparing pungent red powder) and a local cultivar Salem were collected from Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi, India. The dried seeds of all the cultivars were soaked in sterile double-distilled water for 12 hour prior to *in vitro* culture for germination. Surface sterilization was carried out with 0.1 per cent $HgCl_2$ with agitation for two minutes followed by three rinses in sterile double-distilled water. Forty five seeds of each cultivar were then inoculated in 250 ml conical flask, each containing 50 ml MS (Murashige and Skoog, 1962) medium with 30 gl^{-1} sucrose and gelled with 7.0 gl^{-1} agar. The inoculated seeds were kept in dark at 25^0C temperature for germination. After germination, seedlings were exposed to light for 16/8 light and dark cycle with 45 μmol m^{-2} s^{-1} light intensity.

Explant, culture media and growth factors: Shoot tip was excised from 21-day-old in *vitro* raised seedlings

of each cultivar and used as explant for *in vitro* multiplication. MS medium was used as a basal medium supplemented with 30 gl^{-1} sucrose, 7.0 gl^{-1} agar and 0.5 mg l^{-1} BAP for establishment of culture. Eight different shoot bud inducing media were utilized *viz*. M_1 = MS + 2.0 BAP + 0.25 IAA; M_2 = MS + 3.0 BAP + 0.25 IAA; M_3 = MS + 4.0 BAP + 0.25 IAA; M_4 = MS + 5.0 BAP + 0.25 IAA; M_5 = MS + 6.0 BAP + 0.25 IAA; M_6 = MS + 7.0 BAP + 0.25 IAA; M_7 = MS + 8.0 BAP + 0.25 IAA; M_8 = MS + 9.0 BAP + 0.25 IAA. For shoot multiplication, following growth regulator combinations on MS medium were tried. Medium 1= MS + 1.0 BAP + 1.0 KIN; Medium 2 = MS + 2.0 BAP + 1.0 KIN; Medium 3 = MS + 3.0 BAP + 1.0 KIN; Medium 4 = MS + 4.0 BAP + 1.0 KIN; Medium 5 = MS + 5.0 BAP + 1.0 KIN + 0.5 GA_3; Medium 6 = MS + 6.0 BAP + 1.0 KIN + 0.5 GA_3; Medium 7 = MS + 7.0 BAP + 1.0 KIN + 0.5 GA_3; Medium 8 = MS + 8.0 BAP + 1.0 KIN + 0.5 GA_3. The effect of three different level of sucrose (30,40 and 50 gl^{-1}), light intensity (3000, 4000 and 5000 lux) and light and dark cycle (16/8, 12/12, 24/0) were tested for shoot multiplication .

In vitro rooting and hardening of plantlets: For *in vitro* rooting, the regenerated shoots were separated and transferred in test tube containing half-strength MS medium supplemented with 1.0 mg l^{-1} IBA as per previously standardized media (Ranjan *et al,* 2006). After proper root development, *i.e.* after 30 days of inoculation, the rooted plantlets were transferred to hardening medium containing peat and soilrite® in 1:1 ratio. Three hardening strategies, *viz*. plastic pot with inverted glass beaker (Borosil^{R}500 ml), glass jar with Poly Propylene cap and plastic pot covered with white polyethylene cover of 100μ thickness were tested for maximum recovery of healthy plantlets.

Statistical analysis: All the experiments were laid out in completely randomized design. Fifteen test tubes were maintained in each replication and treatment for observation recording. The statistical analysis was done as per the factorial CRD in Statistical Package for Social Sciences (SPSS 10.0)

Results and Discussion

Culture initiation: Shoot tip explants of all the cultivars were inoculated onto MS medium for culture initiation. More than 85% of culture was established in all the cultivars (Figure 1). The maximum culture was established in cultivar KtPL-19 followed by ArCH-001. However, both the cultivars were on a par with respect to culture establishment. Murashige and Skoog (1962) medium was found to be most effective. It is the most common culture medium used since the first endeavour in micro-propagation of chilli (Gunay and Rao, 1978; Agrawal *et al*.,1989; Venkataiah, *et al*., 2001). However, modifications of MS medium were later employed for culture initiation (Christopher, *et al*., 1986; Fari *et al*., 1990; Ochoa-Alejo and Ireta-Monero, 1990). The similar results were obtained when the MS medium was supplemented with 0.5 mg l^{-1} BAP. The genotypic differences were also significant. The endogenous level of growth regulators in different genotypes might be responsible for the differences in culture establishment (Gupta *et al*., 1990).

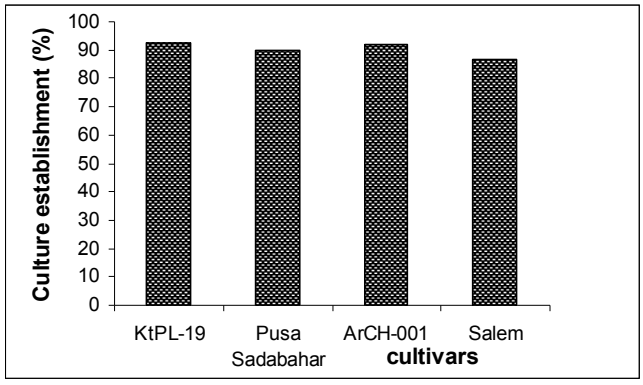

Fig.1 Culture establishment in different chilli cultivars inoculated onto MS medium supplemented with 0.5 mg l^{-1} BAP.

Multiple shoot formation from shoot tip **:** Data on different concentrations of growth regulator combination on multiple shoot formation from shoot tip in terms of number of multiple shoot per explant and number of days required for multiple shoot formation revealed that with increase in cytokinin (BAP) level upto 7.0 mg l^{-1}, number of shoots increased significantly and decreased thereafter. The best growth regulator combination was 7.0 mg l^{-1} BAP and 0.25 mg l^{-1} IAA where maximum number of shoots per explant (4.67) was recorded. Cultivars also showed significant difference with respect to number of shoots per explant. The maximum number of shoots (3.66) was recorded in cultivar Pusa Sadabahar. The interaction of cultivar and treatment indicates that cultivars differ significantly with regard to BAP

concentration. The numbers of shoots were maximum in cultivar Pusa Sadabahar when shoot tip was inoculated on to MS medium supplemented with 7.0 mg l^{-1} BAP and 0.25 mg l^{-1} IAA (Table 1). MS medium supplemented with 7.0 mg l^{-1} BAP and 0.25 mg l^{-1} IAA gave the maximum shoot bud induction (80.5 per cent). However, minimum was on medium containing 2.0 mg l^{-1} BAP and 0.25 mg l^{-1} IAA (Figure 2).

Table 1. Effect of different growth regulators on multiple shoot formation on shoot tip explant

Treatment No.	Growth regulator (mg/l)	Number of shoots/ explant				
		V_1	V_2	V_3	V_4	Mean
T_1	2.0 BAP + 0.25 IAA	2.23	2.25	1.98	1.10	1.89
T_2	3.0 BAP + 0.25 IAA	2.85	2.85	2.50	1.25	2.36
T_3	4.0 BAP + 0.25 IAA	3.12	2.98	3.00	1.95	2.76
T_4	5.0 BAP + 0.25 IAA	3.20	3.00	3.75	2.28	3.06
T_5	6.0 BAP + 0.25 IAA	3.80	5.10	4.25	3.60	4.19
T_6	7.0 BAP + 0.25 IAA	4.95	5.19	4.70	3.85	4.67
T_7	8.0 BAP + 0.25 IAA	4.10	4.80	3.80	2.90	3.90
T_8	9.0 BAP + 0.25 IAA	2.35	3.10	2.95	2.75	3.01
Mean		3.66	3.37	3.44	2.46	

CD0.05 Treatment: 0.08; Cultivar: 0.06; Interaction :0.17 V1 = KtPL-19; V2 = Pusa Sadabahar; V3 = ArCH-001; V4 = Salem

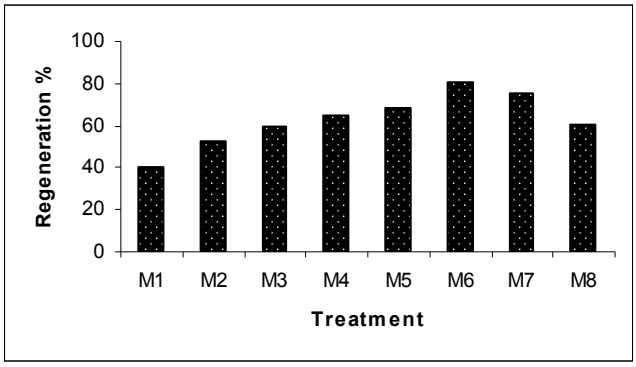

Fig.2 Effect of growth regulator combinations on shoot regeneration frequency

Days required for shoot initiation: Days required for shoot initiation decreased with the increase in BAP concentration up to 7.0 mg l^{-1} and increased thereafter. Shoot initiation was earliest (15-20 days) with medium containing 7.0 mg l^{-1} BAP and 0.25 mg l^{-1} IAA. The combined effect of cytokinin and auxin gave better response on multiple shoot initiation from shoot tip. The results are in agreement with that of Agrawal *et al*. (1988) and Mirza and Namkhede (1996).

Shoot multiplication

Effect of growth regulators: The effect of different growth regulator combinations on shoot multiplication has been presented in Figure 3. Among the eight different growth regulator combinations, the maximum number of shoots (5.38) developed in the medium containing 6.0 mg l^{-1} BAP, 1.0 mg l^{-1} KIN and 0.5 mg l^{-1} GA_3. It was also observed that with the increase in cytokinin level, the number of shoots increased significantly up to 6.0 mg l^{-1} and decreased thereafter. The mean shoot length also increased significantly with the increase in BAP and GA_3 level. The maximum shoot length (5.03 cm) was recorded in treatment containing 8.0 mg l^{-1} BAP, 1.0 mg l^{-1} KIN and 0.5 mg l^{-1} GA_3.

Table 2. Effect of different growth regulators on number of days taken for shoot initiation

Treatment No.	Growth regulator (mg/l)	Number of shoots/ explant			
		V_1	V_2	V_3	V_4
T_1	2.0 BAP + 0.25 IAA	+	+	+	+
T_2	3.0 BAP + 0.25 IAA	+	+	+	+
T_3	4.0 BAP + 0.25 IAA	+	++	+	+
T_4	5.0 BAP + 0.25 IAA	++	++	++	+
T_5	6.0 BAP + 0.25 IAA	++	+++	++	++
T_6	7.0 BAP + 0.25 IAA	+++	+++	+++	+++
T_7	8.0 BAP + 0.25 IAA	+++	++	++	++
T_8	9.0 BAP + 0.25 IAA	++	+	+	+

\+ = 30-35 days; ++ = 25-30 days; +++ = 15-20 days V1 = KtPL-19; V2 = Pusa Sadabahar; V3 = ArCH-001; V4 = Salem

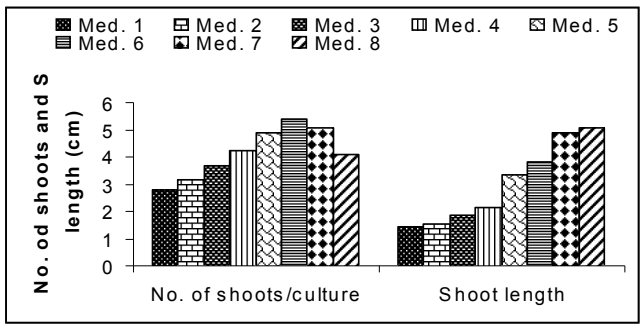

Fig.3 Effect of growth regulator combinations on number of shoots and shoot length.

Effect of sucrose level: Varying sucrose levels have often given either increase or decrease in shoot multiplication rate and accordingly *in vitro* requirement of sucrose varies greatly with the morphogenetic stages (Damino *et al*., 1987). The number of shoots varied with the level of sucrose added to the medium. With the increase in sucrose level from 30 to 40 g l^{-1}, the number of shoot per culture increased significantly and decreased there after (Table 3). Maximum number of shoots (5.96) was recorded in the medium

Table 3. Effect of sucrose level on shoot multiplication

Treatment No.	Sucrose level (g/l)	Number of shoots				Mean
		V_1	V_2	V_3	V_4	
T_1	30.0	5.25	6.50	5.05	4.25	5.26
T_2	40.0	6.00	6.90	6.20	4.75	5.96
T_3	50.0	4.75	5.25	4.95	3.70	4.66
T_4	60.0	3.50	4.10	3.85	2.80	3.56
Mean		4.88	5.69	5.09	3.88	

CD0.05 : Treatment = 0.13; Cultivar = 0.13; Interaction = 0.25 V1 = KtPL-19; V2 = Pusa Sadabahar; V3 = ArCH-001; V4 = Salem

containing 40 g l^{-1} sucrose. Interaction between sucrose level and cultivar was found to be significant with respect to number of shoots. Maximum number of shoots (6.90) was recorded in cultivar Pusa Sadabahar at 40 g l^{-1} sucrose level. In tissue culture experiments, generally 2 to 4 % (20-40 g l^{-1}) sucrose level (w/v) is optimum (George, 1993).

Effect of light intensity: Shoot proliferation is greatly influenced by light intensity and accordingly light intensity requirement varies with the genotypes. In the present study the best light intensity was found to be the 5000 lux for all the cultivar. Interaction between light intensity and cultivar indicated that the maximum number of shoots (6.50) was formed at 5000 lux in cultivar Pusa Sadabahar and those of minimum (1.98) at 3000 lux in cultivar Salem (Table 4). Different morphogenetic processes require specific light intensity and both axillary and adventitious shoot buds proliferation are higher when intensity is increased from level at which culture were initiated (Lazzeri and Dunwell,1986).

Table 4. Effect of light intensity on shoot multiplication

Treatment No.	Light Intensity (Lux)	Number of shoots				Mean
		V1	V2	V3	V4	
T1	3000	3.50	4.95	2.50	1.98	3.23
T2	4000	4.80	5.82	4.95	3.15	4.68
T3	5000	5.75	6.50	5.50	4.50	5.56
Mean		4.68	5.76	4.32	3.21	

CD0.05 : Treatment = 0.14; Cultivar = 0.16; Interaction = 0.28 V1 = KtPL-19; V2 = Pusa Sadabahar; V3 = ArCH-001; V4 = Salem

Effect of light and dark cycle: The best photoperiod was observed to be 16/8 hours of light and dark cycle(Table 5). With increase in light period from 16 to 24 hour or decrease from 16 to 12 hour, the number of shoots formed per culture decreased. The maximum number of shoots (5.27) were recorded when the culture were provided with 16/8 hour of light and dark

Table 5. Effect of light and dark cycle on shoot multiplication

Treatment No.	Light/dark cycle (hr)	Number of shoots				Mean
		V1	V2	V3	V4	
T1	16/8	4.80	6.62	5.40	4.25	5.27
T2	12/12	4.50	4.80	3.50	2.22	3.76
T3	24/0	3.20	3.52	2.51	1.50	2.68
	Mean	4.17	4.98	3.80	2.66	

CD0.05 : Treatment = 0.10; Cultivar = 0.12; Interaction = 0.21 V1 = KtPL-19; V2 = Pusa Sadabahar; V3 = ArCH-001; V4 = Salem

cycle. The interaction between cultivar and photoperiod was also significant. The number of shoots recorded were maximum in cultivar Pusa Sadabahar at 16/8 hour of light and dark cycle.

Shoot morphogenesis is generally stimulated by light and photoperiod coupled with total irradiance, which had profound effect during the above processes. In some plants, shoot proliferation is enhanced by high irradiation during stage-II (Hammerschag, 1978). Earlier, Haramaki (1971) suggested the use of light maintained at 3000-1000 lux for higher shoot proliferation in *Sinniongia*, while in Ulmus hybrid 45 μmol m^{-2} s^{-1} proved beneficial (Fink *et al*., 1986). An optimum light condition is also known to improve the quality of micro-shoots (Mcgranachau *et al*., 1987).

***In vitro* rooting:** Rooting under *in vitro* conditions is a time consuming and expensive process in many recalcitrant species. This stage is also very important as proper root initiation and growth could help in the future performance of *in vitro* raised plantlets. The *in vitro* multiplied shoots were excised and subjected to *in vitro* rooting on earlier standardized half-strength MS medium supplemented with 1.0 mgl^{-1} IBA (Ranjan *et al*., 2006).

Hardening: Commercial tissue culture venture is successful when the tissue cultured plantlets survive in *ex vitro* conditions in the field or glasshouse. For better survival of tissue culture raised plants three hardening strategies were tested *viz*. plastic pot covered with inverted glass beaker, glass jar with polypropylene cap and plastic pot with polythene (100 μ thick) cover. Of these three hardening strategies plastic pot covered with inverted glass beaker was found to be the best in terms of survival percentage, height of plants and root growth (figure 4 and 5). The maximum survival (93.80%) was observed in this hardening strategy which differed significantly with other two strategies. Survival was recorded to be best

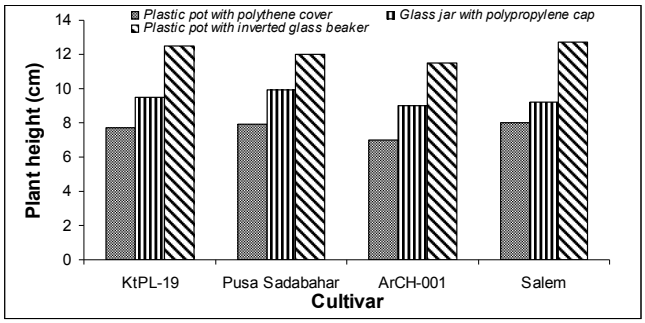

Fig.4 Effect of different hardening methods on height of *in vitro* raised plantlets of different cultivars

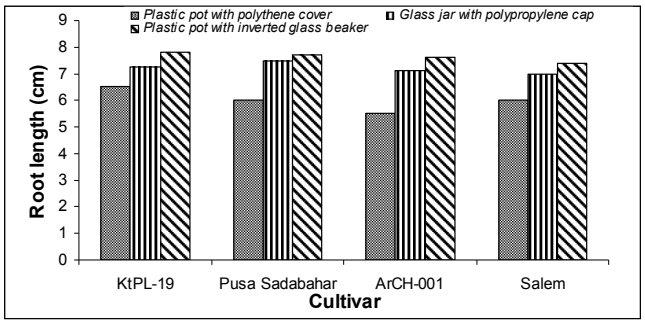

Fig.5 Effect of different hardening method on root length of *in vitro* raised plantlets of different cultivars

in cultivar Salem. Interaction between cultivar and hardening strategy was significant. Likewise, plant height and root length was highest in the plants covered with inverted glass beaker. In the plastic pots covered with polythene, the plants were in touch with the polythene surface and during first 7-10 days, rotting was observed on foliage parts. The merit of glass beaker was that the plants were free standing along with the better transmission of light and favourable micro-climate for growth (Kumar, 2002).

The standardized protocol for micro-propagation of chilli plants through shoot tip culture would permit mass multiplication of elite or difficult-to-isolate stocks as well as production of virus-free planting material. The developed protocol may be tested in other popular cultivars and their utility in genetic transformation in chilli.

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