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Article

In vitro regeneration protocol development via callus formation from stem explant of tomato

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Abstract: The experiment was conducted on *in vitro* regeneration of tomato at the Plant Biotechnology Laboratory, Department of Horticulture, Patuakhali Science and Technology University, Patuakhali. The objective was to develop an efficient regeneration protocol in tomato through callus induction for subsequent plantlet regeneration. Seeds were inoculated on MS medium where germination rate was 78.4%. The stems of *in vitro*cultured seedlings were used as explants. Different concentrations and combinations of growth regulators were added to MS medium to observe their efficacy on callus induction, shoot initiation and root formation. Stem explants cultured on MS medium fortified with 2 mg/L BAPgave the highest number of shoots (3.0) at 45 DAC. Among the concentrations of PGRs, 0.25 mg/L IAA produced the highest length (4.064 cm) of plantlets, number (5.0) of leaves and fresh weight (0.663 g) of plantlets with the stem explants at 45 DAC. The concentration of 0.5 mg/L IAA produced the highest number (21.00) of roots/plantlet, length (7.676 cm) of roots at 45 DAC, from the same explants. The highest survival rate of in vitro regenerated plantlets in the pot was 70.00 % with the stem explants. The results of the current study showed significant increase in the growth of callus of *Solanumlycopersicon* Mill. Indicating a good efficiency of the optimized media composition and the experimental model used in comparison to other studies of similar nature.

Keywords: regeneration; tomato; callus; explants

1. Introduction

Tomato (*Solanumlycopersicon* Mill.) belonging to the family Solanaceae, is one of the most popular, important and nutritious vegetables in the world. Tomato is considered as the second most popular and highly nutritive vegetable crop after potato (Mamidala and Nanna, 2011) and is a model species for introduction of agronomically important genes into dicotyledonous crop plants (Wing *et al.*, 1994). It is one of the most important protective foods as it possesses appreciable quantities of vitamins and minerals and sometimes rightly referred to as the poor men's orange (Devi *et al*., 2008). Hundred grams of edible parts of tomato contains 0.9 g protein, 0.1 g fat, 0.7 g fibre, 3.5 g carbohydrates, 15-20 calorie energy, 500-1500 IU vitamin A, 0.1 mg thiamine, 0.02 mg riboflavin, 0.6 mg niacin, 20-25 mg vitamin C, 6-9 mg calcium and 0.1-0.3 mg iron (Uddin*et al*., 2004). Tomato is also an excellent source of lycopene (approximately 20–50 mg /100g of fruit weight), a powerful antioxidant in the carotenoid family which protects humans from free radicals that degrade many parts of the body; lycopene is also known to prevent cancer (Rao and Agarwal, 2000). Tomato is cultivated all over Bangladesh due to its adaptability to wide range of soil and climate (Ahamed*et al.*, 1995). The technique of plant tissue culture has been emerged as a new and powerful tool for crop improvement like potato (Shahriyar*et al.*, 2015). The plant regeneration in tomato is genotype, explant, growth regulator and medium dependent. Many kinds of plant growth regulators are used with varying concentrations for tomato regeneration. Different types of cytokinin and auxin combinations could induce callus induction in tomato from different source of

explants (Mohamed *et al*., 2010). Furthermore, the hormones auxins and cytokinins have a multitude of complex interactions, which control plant development. The hormonal balance between auxins and cytokinins can regulate the formation of roots, shoots and callus tissue *in vitro.* There are, however, considerable difficulties in predicting the effects of plant growth regulators. This is because of the great differences in culture response between species, cultivars and even on the type of tissue in which the interaction occurs. *In vitro* regeneration of tomato is a subject of research because of the commercial value of the crop and its potential of amenability to further improvement. Efficient plantlet regeneration in tomato was reported from meristems (Mirghis *et al.,* 1995), leaf (Ajenifujah-Solebo *et al.,* 2012), stems (Liu *et al.,* 2003), anthers, root (Singh and Bezei, 2002), shoot tip (Selvi and Khader, 1993) and hypocotyls (Mamidala and Nanna, 2011) in other countries. But very little studies are attempted in Bangladesh on protocol development for high frequency plant regeneration of tomato. Tomato seed production program in Bangladesh is practiced with imported virus free seeds which are expensive. It is possible to bring down the cost of production by developing virus free seeds through tissue culture. Moreover, maintenance of valuable germplasm in disease free conditions may be obtained by meristem culture. But a standard tissue culture technique for tomato with suitable explants and plant growth regulators in Bangladesh is yet to be established. On the above mentioned perspective, the present study was undertaken to develop a suitable protocol for *in vitro* regeneration of tomato plantlets via callus formation of stem explants.

2. Materials and Methods

The present research work was conducted at the Plant Biotechnology Laboratory, Department of Horticulture, Patuakhali Science and Technology University. The seeds were collected from the Regional Horticulture Research Station (RHRS), Lebukhali, Patuakhali. Winter variety of BARI tomato-14 was used as the plant material. Stem from *in vitro* grown tomato plants were cultured on MS medium and used as explants. P^H of the medium was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCI. All the media were autoclaved for 20 minutes with 15 psi at 121°C.Collected tomato seeds were washed in tap water and surface sterilized with 70% ethanol for five minutes with vigorous shaking followed by washing with sterile distilled water, surface disinfected with sodium hypochlorite 5.25% for 10 minutes and rinsed 4-5 times with sterile water. Then they were washed with Tween 20 for 2-3 minutes and rinsed with sterilized water till the foam was completely removed. The surface sterilized seeds were then allowed to soak overnight to break dormancy. Then the seeds were placed in test tubes containing 10 ml MS medium and later transferred to growth room at 25 ± 1°C temperature under 16 hours photoperiod with a light intensity 1500 lux and relative humidity 60-70%. Sterilized seeds were placed onto seed germination medium in test tubes. In each test tube, 4 seeds were inoculated. The culture was then incubated in incubation room till the germination of seeds. It was noticed that seeds started growing in dark and later they were transferred to light. Thirty days old seedlings were used as the source of explants.

2.1. Callus proliferation

The seedlings raised *in vitro* culture were used as the source of stem explants. Stemparts were placed on the sterile culture medium with various concentrations and combinations of BAP (1, 2, 3 mg/L) and NAA (.25, .5 mg/L) and subsequent fresh weight and dry weight of the callus and changes in colors were recorded visually after 15, 30 and 45 DAC.

2.2. Dry weight of the callus

The calli were kept in an oven (Model no.: NIIVE FN-400) for drying for 72 hours at 50^0C after taking fresh weights. After 72 hours, dried calli were weighed and the means were calculated.

2.3. Subculture of the callus for shoot regeneration

When the calli turned into green to yellow color, those were removed aseptically. The pieces were again cultured on freshly prepared medium supplemented with 0, 0.5, 1, 2 and 3 mg/L BAP for shoot induction from callus and subsequent fresh weight and number of shoot were recorded after15, 30 and 45 DAC.

2.4. Number of shoots /plantlet

The number of shoots emerged in each cultured bottle was calculated by counting the number of shoots emerged. The data were recorded at 15 days of interval up to 45 days of culture and the means were calculated.

2.5. In vitro plantlet regeneration with leaves

Initially 1.5 cm of plantlets were transferred to ½ strength MS media containing 0.0, 0.1, 0.25, 0.50, 1.0 mg/L IAA and average length of plantlets and number of leaves per plantlet were counted at 15, 30 and 45 DAC.

2.6. Subculture of the shoots for root induction

The sub cultured calli continued to proliferate and differentiated into shoots. When these shoots grew about 2-3 cm in length, those were rescued aseptically from the bottle and were separated from each other and again cultured on freshly prepared half strength MS medium containing 0.0, 0.1, 0.25, 0.50, 1.0 mg/L IAA supplements for root induction. Number of roots/plantlet formed was recorded at 15 days interval up to 45 days of culture and the means were recorded. The length of root was also measured at 45 days of culture using a scale and the mean values of data provided the root length. After 45 days of inoculation, the fresh weight (g) of plantlet was taken with the electric balance.

Plantlets of the 5-7 cm length with well-developed roots were removed from culture vessel with the forceps and transferred into a small earthen pot containing garden soil, sand and well rotten cowdung at the ratio of 1:2:1. The pots were kept in the laboratory and irrigated regularly at an interval of 2 days. The plantlets established within 5 to 7 days and the polythene bags were removed.

2.7. Statistical analyses

Data collected on different parameters under study were statistically analyzed to ascertain the significance of the experimental results. The Analysis of Variance was performed and means were compared by Least Significant Difference (LSD) test for interpretation of results. The significance of the difference between the pair of means was evaluated using MSTAT-C computer package programs.

3. Results

An experiment was conducted to assess the performance of the stem explants of tomato for callus induction and plantlet regeneration.

3.1. Seed germination

After surface sterilization, seeds were inoculated on plain MS medium. The germination rate was 78.4 wherein 2.4% seeds were contaminated and remaining was unable to grow.

3.2. Callus proliferation from explants

The effects of different concentrations and combinations of plant growth regulators (PGRs) in MS medium for stemexplants of tomato (var. BARI tomato-14) for callus proliferation was observed.

3.3. Fresh weight of callus

The fresh weights of calli were recorded at 15, 30 and 45 days after culture (DAC) of stem explants. The maximum fresh weights of calli was 0.638, 1.305 and 2.061g produced by stem explants at 15, 30 and 45 DAC at 3 mg/L BAP + 0.25 mg/L NAA (Table 1).The fresh weights of calli varied significantly due to different concentrations and combinations of PGR at all observing dates. The minimum fresh weights of calli (0.373, 0.971 and 1.268 g) were produced in control (hormone free medium) at 15, 30 and 45 DAC, respectively.

3.4. Dry weight of callus

The dry weights of calli varied significantly due to different concentrations and combinations of PGRs. The stem explants cultured on the MS medium containing 2 mg/L BAP + 0.25 mg/L NAA produced the maximum dry weight (0.1957 g) of callus and the minimum dry weight (0.2050 g) of callus was in control (hormone free medium).

3.5. Changes in colors of explants

After inoculation of explants to culture media, the color changes of stem segments were observed gradually with the advancement of culture period. The extent of color change was recorded at l5, 30 and 45 DAC (Figure 1).After 45 days of inoculation, the stem explants became yellow at 1 mg/L BAP + 0.5 mg/L NAA, 2 mg/L BAP + 0.25 mg/L NAA and 3 mg/L BAP + 0.25 mg/L NAA treatment and became green at 1 mg/L BAP + 0.25 mg/L NAA, 2 mg/L BAP + 0.5 mg/L NAA and 3 mg/L BAP + 0.5 mg/L NAA combinations of PGRs and the explants became brown at control (Table 2).

Table 1. Effect of BAP on stem explants for callus proliferation at different DAC.

Concentrations and combinations of PGRs	Fresh weight (g) of explants inoculated	Fresh weight (g) of callus at different DAC			Dry weight (g) of callus at 45 DAC
		15	30	45	
1 mg/L BAP + 0.25 mg/L NAA	0.005	0.4850 b-g	1.107 c-f	1.632 fgh	0.1760 def
1 mg/L BAP + 0.50 mg/L NAA	0.005	0.5070 bcd	1.137 c-f	1.722 ef	0.1890 cd
2 mg/L BAP + 0.25 mg/L NAA	0.005	0.5170 bc	1.229 ab	1.825 cd	0.2050 b
2 mg/L BAP + 0.50 mg/L NAA	0.005	0.4920 b-e	1.155 b-e	1.717 ef	0.1880 cd
3 mg/L BAP + 0.25 mg/L NAA	0.005	0.6380 a	1.305 a	2.061 a	0.2270 a
3 mg/L BAP + 0.50 mg/L NAA	0.005	0.4737 c-g	1.093 def	1.579 hi	0.1730 d-g
Control	0.005	0.3730 j	0.971 g	1.268 j	0.1140 i
$LSD_{0.01}$ value		0.02612	0.04664	0.05234	0.008258
CV (%)		5.08	3.88	3.00	4.52
Level of significance		**	**	**	**

In a column, values having different letter (s) differ significantly at the 1% level of probability according to LSD.
** denotes significant at the 1% level of probability.

Table 2. Relative color change of callus from stem explants of tomato at different concentration and combination of BAP and NAA.

Concentrations and combinations of PGRs	Explant source and color at different DAC		
	Stem		
	15	30	45
1 mg/L BAP + 0.25 mg/L NAA	Lye	Lye	Gre
1 mg/L BAP + 0.5 mg/L NAA	Lye	Lye	Ye
2 mg/L BAP + 0.25 mg/L NAA	Lye	Lye	Ye
2 mg/L BAP + 0.5 mg/L NAA	Lye	Lye	Gre
3 mg/L BAP + 0.25 mg/L NAA	Lye	Lye	Ye
3 mg/L BAP + 0.5 mg/L NAA	Lye	Lye	Gre
Control	Lye	Lye	Br

Ye = Yellow, Lye=Light yellow, Gre= Green, Br= Brown

3.6. Shoot induction from callus

The effects of different concentrations and combinations of BAP on stem explant of tomato for shoot induction were observed.

3.7. Fresh weight of callus with shoots

The highest fresh weights of calli with shoots were 0.5810, 1.410 and 2.173 g obtained from stem explants at 15, 30 and 45 DAC, respectively at the hormonal concentration of 2 mg/L BAP (Table 3).There was significant difference among the different concentrations and combinations of PGRs in MS medium in respect of fresh weight of callus with shoots at all sampling dates. The minimum fresh weights of calli with shoots 0.3630, 0.752 and 1.259 g were produced from control (hormone free medium) at 15, 30 and 45 DAC, respectively.

3.8. Number of shoots

The maximum number of shoots were 2 and 3.0produced by stem explants at 30 and 45 DAC, respectively at 2.0 mg/L BAP (Figure 2). In the present work, the number of shoots gradually increased with the advancement of culture duration in all hormonal treatments. The increasing of BAP concentration up to 2 mg/L caused the number of shoots to continue developing, but it fell down in presence of BAP (3 mg/L) that indicates the toxic effect of growth regulators due to their accumulation.

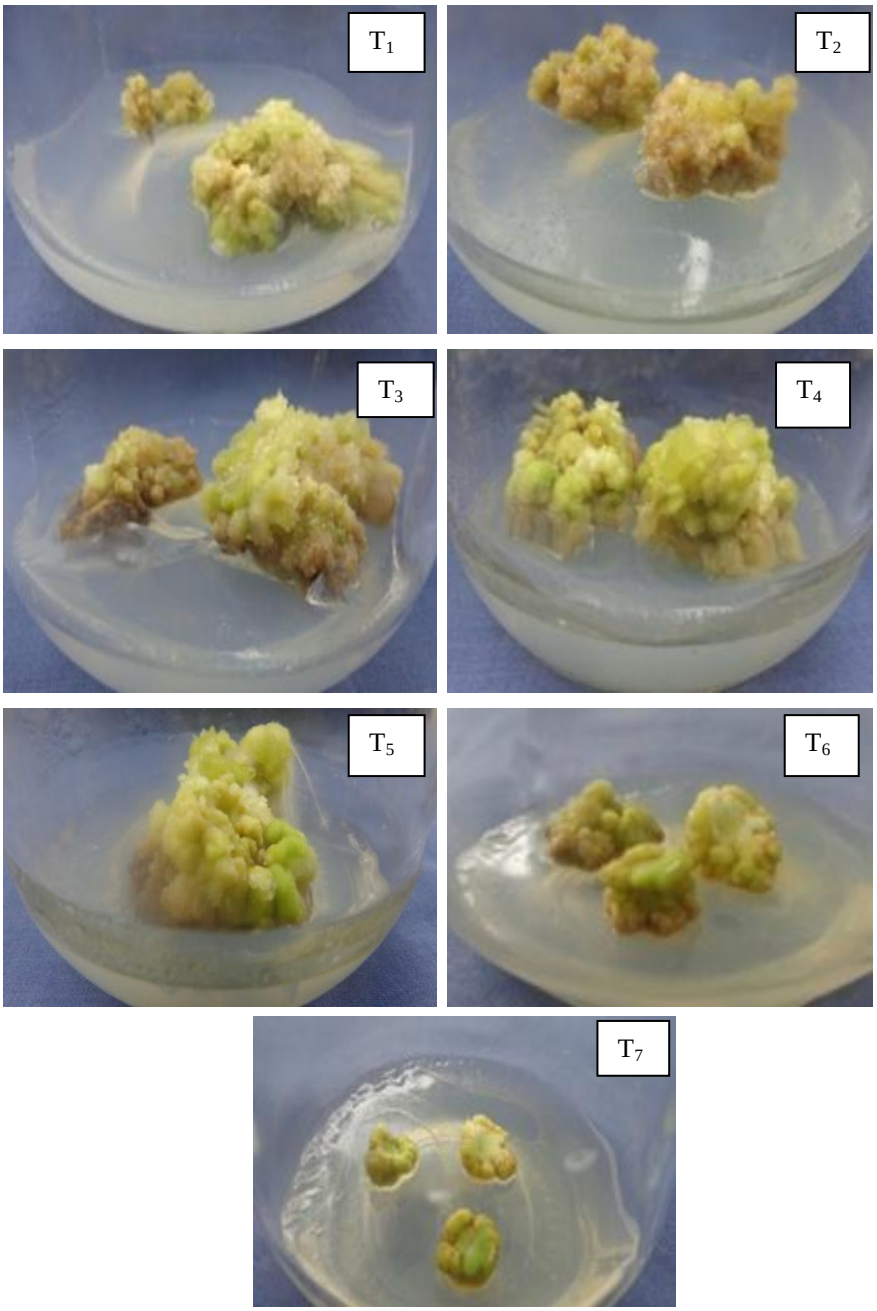

Figure 1. Effect of NAA and BAP on callus formation from stem explants of tomato at 45 DAC. Here,

T_1 = 1 mg/L BAP + 0.25 mg/L NAA
T_2 = 1 mg/L BAP + 0.50 mg/L NAA
T_3 = 2 mg/L BAP + 0.25 mg/L NAA
T_4 = 2 mg/L BAP + 0.50 mg/L NAA
T_5 = 3 mg/L BAP + 0.25 mg/L NAA
T_6 = 3 mg/L BAP + 0.50 mg/L NAA
T_7 = Control

Table 3. Interaction effects of stem and BAP on the fresh weight of callus with shoot and average number of shoots at different DAC.

Explants	Concentrations of PGRs	Fresh weight (g) of explants inoculated	Fresh weight (g) of callus with shoot at different DAC			Average no. of shoots at different DAC		
			15	30	45	15	30	45
Stem	0.5 mg/L BAP	0.25	0.5450 cd	1.246 cd	1.958 bc	-	1.25 de	1.750
	1.0 mg/L BAP	0.25	0.6100 a	1.229 cde	2.019 b	-	1.75 cd	2.417
	2.0 mg/L BAP	0.25	0.5810abc	1.410 a	2.173 a	-	2.00 c	3.000
	3.0 mg/L BAP	0.25	0.5120 de	1.160 def	1.740 d	-	1.25 de	1.500
	Control	0.25	0.3630 f	0.752 h	1.259 f	-	-	-
$LSD_{0.01}$ value			0.03481	0.08024	0.09988	-	0.4959	0.5642
CV (%)			3.64	3.80	3.00	-	18.18	15.50
Level of significance			**	*	**		**	ns

In a column, values having different letter(s) differ significantly at the 1% and 5% levels of probabilities according to LSD. **, *, ns denotes significant at the 1%, 5% level and non-significant, respectively.

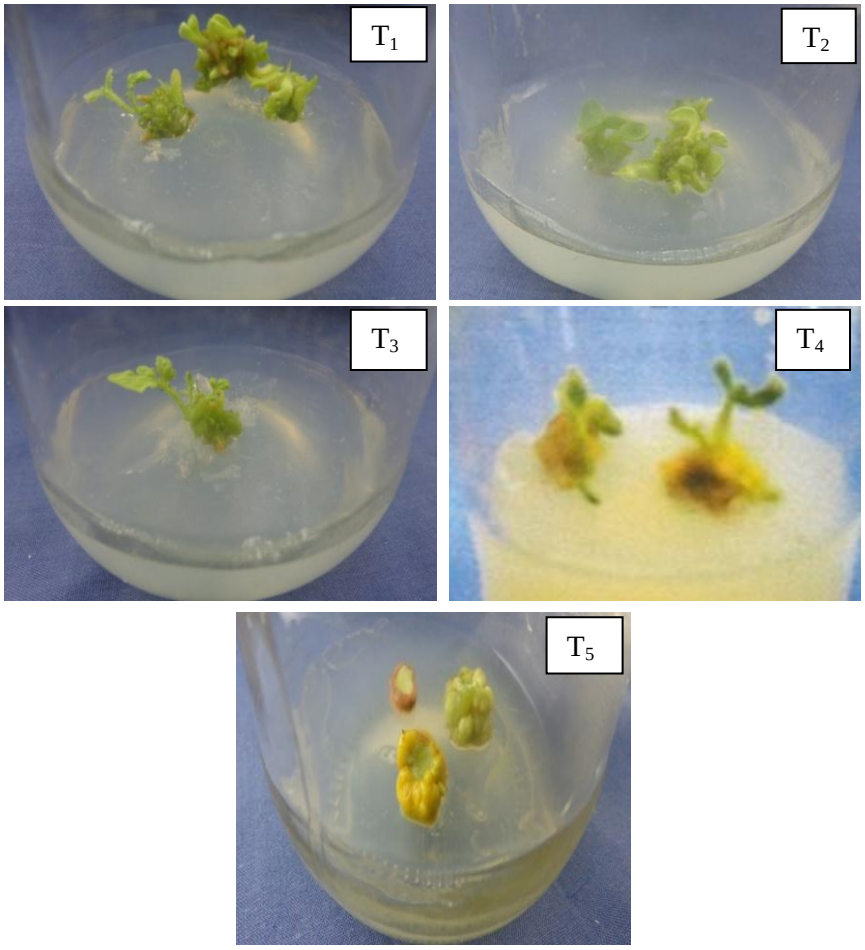

Figure 2. Effect of BAP on callus with shoot production from stem explants of tomato at 45 DAC.

Here,

T_1 = 0.5 mg/L BAP
T_2 = 1 mg/L BAP
T_3 = 2 mg/L BAP
T_4 = 3 mg/L BAP
T_5 = Control

3.9. Root development

The effects of different concentrations of IAA in ½ MS medium on root formation were observed.

3.10. Length of the plantlets

The lengths of the plantlet were recorded at 15, 30 and 45 DAC. The tallest plantlet 0.6210, 1.39 and 4.064 cm from stem explants at 15, 30 and 45 DAC, respectively at 0.25 mg/L IAA (Table 4).The smallest plantlets were recorded at control (hormone free medium).

3.11. Number of leaves/plantlet

The highest numbers of leaves (3.5, 4.25 and 5.0) were produced from stem explants at 15, 30 and 45 DAC, respectively on the half strength medium supplemented with 0.25 mg/L IAA and the lowest number of leaves 2.333, 2.50 and 3.25 was for the hormone free medium (control) at 15, 30 and 45 DAC, respectively.

3.12. Number of roots/plantlet

Number of roots/plantlet was recorded at 15, 30 and 45 DAC. The highest numbers of roots (13.5, 17.75 and 21.00) were produced from stemex plants at 15, 30 and 45 DAC, respectively on ½ MS medium supplemented with 0.5 mg/L IAA(Table 5).The lowest numbers of roots were observed on ½ MS medium supplemented with 0.1 mg/L IAA. No root formation was observed in control treatment at 15, 30 and 45 DAC.

Table 5. Interaction effects of stem and IAA in ½ MS medium on the average no. of roots/plantlet, length of roots and fresh weight of plantlets at different DAC.

Explants	Concentrations of PGRs	Average no. of roots/plantlet at different DAC			Length of roots (cm) at 45 DAC	Fresh weight (g) of plantlets at 45 DAC
		15	30	45		
	½ MS + 0.10 mg/L IAA	6.750 h	11.25 g	13.50 i	5.001 g	0.4030 h
	½ MS + 0.25 mg/L IAA	10.83 fg	14.67 e	17.25 ef	5.943 e	0.6630 c
	½ MS + 0.50 mg/L IAA	13.50 c	17.75 c	21.00 c	7.676 c	0.5820 e
	½ MS + 1.00 mg/L IAA	9.750 g	13.25 f	16.00 g	5.456 f	0.3880 h
Stem	Control	-	-	-	-	0.2480 j
$LSD_{0.01}$ value		1.140	1.047	0.9414	0.2250	0.03327
CV (%)		6.06	4.32	3.26	2.32	3.57
Level of significance		**	**	**	**	**

In a column, values having different letter(s) differ significantly at the 1% level of probability according to LSD.
** denotes significant at the 1% level of probability.

3.13. Length of roots

The results revealed that the explants differed significantly in respect of root length. Stem explants produced the longest root (7.676 cm) at 45 DAC on 0.5 mg/L in ½ MS medium. The shortest root (5.001 cm) was observed on ½ MS medium supplemented with 0.1 mg/L IAA. No root was formed in control (Figure 3).

Figure 3. Root initiation from stem explants of tomato in ½ MS + 0.25 mg/L IAA.

3.14. Fresh weight of plantlets

The highest fresh weight of plantlet was 0.6630 g produced from stem explants at 45 DAC on 0.25 mg/L IAA in ½ MS medium. The fresh weights of plantlets were significantly influenced by the application of IAA. Oppositely, the lowest weight 0.2480 g was produced by control (hormone free medium) at 45 DAC.

3.15. Survival rate of regenerated plants

The regenerated plantlets were transferred into pots, which were filled with soil, sand and cowdung (1: 2: 1). The percentage of survival rate of regenerated plants from stemwas 70 (Figure 4).

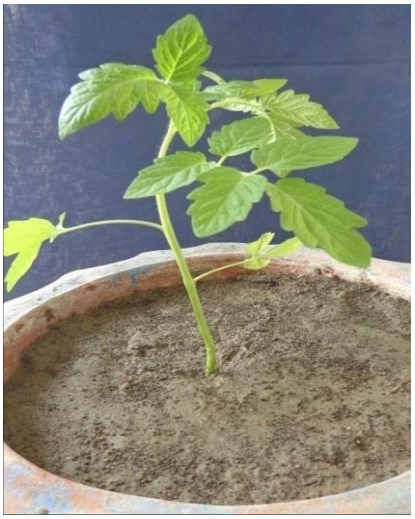

Figure 4. Establishment of tomato plantlets in pots containing a mixture of garden soil, sand and cowdung at the ratio of 1:2:1 from stem explants.

Table 4. Interaction effects of stem and IAA in ½ MS medium on the average length of plantlets and average no. of leaves/plantlet at different DAC.

Explants	Concentration of PGRs	Initial length of plantlet (cm) inoculated	Average length of plantlets (cm) at different DAC			Average no. of leaves/plantlet at different DAC		
			15	30	45	15	30	45
	½ MS + 0.10 mg/L IAA	1.5	0.3870 de	1.1800 e	3.612 f	2.750 e	3.500 cd	4.000 f
Stem	½ MS + 0.25 mg/L IAA	1.5	0.6210 a	1.3900 c	4.064 cd	3.500 bc	4.250 b	5.000 bc
	½ MS + 0.50 mg/L IAA	1.5	0.4320 cd	1.3010 d	3.972 de	3.000 de	4.250 b	4.750 cd
	½ MS + 1.00 mg/L IAA	1.5	0.3420 e	1.1530 e	3.232 g	2.750 e	3.500 cd	3.500 g
	Control	1.5	0.2000 g	0.6560 g	1.303 i	1.750 g	2.250 e	2.500 i
$LSD_{0.01}$ value			0.04770	0. 07415	0.1119	0.3007	0.2480	0.4079
CV (%)			5.84	3.15	1.70	5.22	3.66	5.01
Level of significance			**	**	**	**	**	**

In a column, values having different letter(s) differ significantly at the 1% level of probability according to LSD.
**denotes significant at the 1% level of probability.

4. Discussion

4.1. Callus proliferation from explants

The effects of different concentrations and combinations of plant growth regulators (PGRs) in MS medium for stem explants of tomato (var. BARI tomato-14) for callus proliferation was observed.

4.2. Fresh weight of callus

As the maximum and the minimum fresh weight of calli of stem explants were2.061 g and 1.579 g at 45 DAC at 2 mg/L BAP + 0.25 mg/L NAA and control respectively. Liu *et al.* (2003) reported similar results while working with leaf and stem explants with 3 mg/L BAP + 0.25 mg/L NAA. The fresh weights of calli varied significantly due to different concentrations and combinations of PGR at all observing dates. The minimum

fresh weights of calli (0.3730, 0.971 and 1.268 g) were produced in control (hormone free medium) at 15, 30 and 45 DAC, respectively. Kayum (2004) observed the best callus formation of tomato with the same concentrations and combinations of PGRs. These findings also support the results of Harish *et al.* (2010) while working with leaf disc, stem and hypocotyl of six tomato cultivars (Sindhu, Shalimar, CO_3, PKM, Vaishnavi and Ruchikar) with 0.5 mg/L NAA + 2 mg/L BAP.

4.3. Dry weight of callus

The dry weights of calli varied significantly due to different concentrations and combinations of PGRs. The stem explants cultured on the MS medium containing 3 mg/L BAP + 0.25 mg/L NAA produced the maximum (.2270 g) dry weight of callus. Capote *et al.*,(2000) reported similar results while working with leaf tissue and stem segments of different cultivars with BAP + NAA combinations of PGRs.

4.4. Changes of color in explants

After inoculation of explants to culture media, the stem segments showed light yellow appearance at the first sight and gradually became grey and then yellow. The color changes were observed gradually with the advancement of culture period. The results appeared that the color change of inoculated explants also showed clear variation due to different PGRs treatments. Harish *et al.*, (2010) also observed the color change of tomato explants while they worked with tomato for regeneration. The findings of his results support the present experiment.

4.5. Fresh weight of callus with shoots

The highest fresh weight of calli with shoots was 2.173 g obtained from stem explants at 45 DAC at the hormonal concentration of 2 mg/L BAP.

There was significant difference among the different concentrations and combinations of PGRs in MS medium in respect of fresh weight of callus with shoots at all sampling dates. The minimum fresh weight of calli with shoots was 1.257 g produced from control at 45 DAC. Ugandhar *et al.*, (2012) reported similar results in MS medium supplemented with 2 mg/L BAP.

4.6. Number of shoots

The maximum number of shoot was 3.0 produced by stem explants at 45 DAC at 2.0 mg/L BAP. In the present work, the number of shoots gradually increased with the advancement of culture duration in all hormonal treatments. The increasing of BAP concentration up to 2 mg/L caused the number of shoots to continue developing, but it fell down in presence of BAP (3 mg/L) that indicates the toxic effect of growth regulators due to their accumulation.

These results are also similar with the findings of Mohamed *et al.*, (2010) who had reported the highest number of shoots in MS medium supplemented with BAP (2 mg/L) and no adventitious shoots was noticed in the control (hormone free medium).

4.7. Length of the plantlets

The tallest plantlet was found as 4.064 cm from stem explants at 45 DAC at 0.25 mg/L IAA. The smallest plantlets were recorded at control (hormone free medium).

4.8. Number of leaves/plantlet

The highest number (5.0) of leaves per explant was produced from stem explants at 45 DAC on the half strength medium supplemented with 0.25 mg/L IAA and the lowest number of leaves was 2.5 for the hormone free medium (control) at 45 DAC.

4.9. Number of roots/plantlet

The highest number of roots 21.00was produced from stem explants at 45 DAC, on ½ MS medium supplemented with 0.5 mg/L IAA. Stem explants showed the most important organogenesis capacity in comparison to cotyledon explants (Majoul *et al*., 2007).The lowest number of roots was observed on ½ MS medium supplemented with 0.1 mg/L IAA. No root formation was observed in control treatment. Liu *et al.*, (2003) reported that tomato initiated high rooting at the same concentration and produced thick and strong roots. Similarly the highest number of roots/shoot (22.1) was observed on ½ MS medium supplemented with

IAA 0.5 mg/L (Osman *et al.,* 2010).Devi *et al*., (2008) also reported that the best rooting in tomato was obtained on ½ MS medium.

4.10. Length of roots

The results revealed that the explants differed significantly in respect of root length. Stem explants produced the longest root (7.676 cm) at 45 DAC on 0.5 mg/L in ½ MS medium. The shortest root (5.001 cm) was observed on ½ MS medium supplemented with 0.1 mg/L IAA. No root was formed in control. This result dissimilar to the findings of Ishag *et al.,* (2009), Osman *et al*., (2010) and Parmar *et al.* (2012) who had also observed the longest roots on ½ MS medium supplemented with IAA at 0.5 mg/L.

4.11. Fresh weight of plantlets

The highest fresh weight of plantlet was 0.6630 g produced from stem explants at 45 DAC on 0.25 mg/L IAA in ½ MS medium. The fresh weights of plantlets were significantly influenced by the application of IAA. Oppositely, the lowest weight 0.2480 g was produced in control at 45 DAC.

4.12. Survival rate of regenerated plants

The regenerated plantlets were transferred into pots, which were filled with soil, sand and cowdung (1: 2: 1). The percentage of survival rate of regenerated plants from stem was 70.

5. Conclusions

Results of these experiments show the influence and importance of growth regulators on the number of shoots and roots regenerated from tomato stem explants. The in-vitro morph genic responses of in-vitro cultured plant tissues are therefore affected by the different components of the culture media, especially by concentration of growth hormones. These responses are also dependent on cultivar and explants types.

Conflict of interest

No one to declare.

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