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In vitro Propagation from Shoot Tip and Nodal Segment in Summer Tomato (*Lycopersicon esculentum* Mill.)

N.A. Banu[1*], S. Islam[2], M.A. Islam[1] and M.K. Alam[1]

[1]Department of Biotechnology and Genetic Engineering, Faculty of Applied Science and Technology, Islamic University, Kushtia-7003, Bangladesh
[2]Tissue Culture Section, BCSIR Laboratories, Dhanmondi, Dhaka-1205, Bangladesh

*****Corresponding author e-mail: nab_bt_iu@yahoo.com

Abstract

An effort has been made to establish a simple and efficient tissue culture protocol for rapid micropropagation of three commercially important year-round hybrid tomato cultivars. Shoot tips and nodal segments were used as explants and were subsequently cultured on MS medium supplemented with different concentrations and combinations of growth regulators. Substantial variations for propagation were observed among the treatments and varieties. By using shoot tip explant, highest mean number of shoots per explant was obtained on MS medium with 1.0 mg/l BAP + 1.0 mg/l Kn in every variety. Longest shoot and the highest average number of nodes/shoot were recorded on MS medium with 0.5 mg/l BAP in BH-3 and MH and with 0.5 mg/l Kn in BH-4. Combined effects of BAP, Kn, IAA and GA_3 on shoot multiplication and proliferation from nodal segment explants was also studied using different concentrations but no remarkable advance responses were found over shoot tip culture. From these experiments, highest mean number of shoot/nodal explant was obtained 2.10 in BH-3 on MS medium with 1.0 mg/l BAP + 0.5 mg/l Kn + 0.25 mg/l IAA + 0.5 mg/l GA_3, and 6.84 cm longest shoot was recorded in MH on MS medium with 1.0 mg/l BAP + 0.5 mg/l Kn + 0.5 mg/l IAA + 0.1 mg/l GA_3. Therefore, shoot tip was better than nodal segment explant in terms of shoot multiplication and proliferation. In addition, shoot elongation study was conducted on the duration of culture period from nodal segment explant. Highest 11.87 cm shoot length and 6.0 average number nodes/explant were obtained on MS medium with 0.5 mg/l Kn + GA_3 in BH-3 after 9 weeks culture. *In vitro* root induction was performed on ½ strength MS medium fortified with auxins, and 0.5 mg/l NAA + 0.5 mg/l IAA was found to be best for root initiation. Over 90% plantlets survived *in vivo*.

Keywords: Regeneration, shoot tip, nodal culture, summer tomato, *Lycopersicon esculentum*.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most common, highly nutritive, and commercially important vegetable crop belonging to the Solanaceae family. It is a good source of vitamins, minerals and other biologically active compounds, such as lycopene, a powerful antioxidant. Lycopene has been reported to protect our body from free radicals (Gerster, 1997), and thereby, decreases the risk of chronic diseases including cancer and cardiovascular diseases (Rao and Rao, 2007). It is also commercially exploited for products like jam, jelly, salad, pickles etc. Furthermore, tomato is often used as a genetic model for crop improvement (Namitha and Nagi, 2013). Due to its versatile uses, fresh supply throughout the year has rapidly been increased over the past 25 years (Bhatia *et al*., 2004). Native to South America, it was perhaps introduced into Indian sub-continent by the Portuguese in the 17th century (Das, 1993).

Establishment of an efficient tissue culture protocol is essential for the collection and storage of germplasm, rapid multiplication for commercial exploitation as well as genetic improvement of plants (Devi *et al*., 2008; Sheeja *et al*., 2004). *In vitro* regeneration in different genotypes and cultivars of tomato has been reported from meristems (Mirghis *et al*., 1995), leaf (Behki *et al*., 1976; Kartha *et al*., 1976; Padmanabhan *et al*., 1974), stems, anthers (Zamir *et al*., 1970), hypocotyls (Ohki *et al*., 1978), cotyledons (Hamza and Chupeau, 1993), suspension cells (Meredith, 1979), protoplasts (Morgan and Cocking, 1982), and nodal bud (Ugandhar *et al*., 2012).

Tomato is usually grown in the winter season in Bangladesh at a temperature ranging from 18 to 27^0C, and the optimum temperature for pollination is around 21^0C. Therefore, a fresh supply is not possible throughout the year. Due to high nutritional and commercial value, the demand for fresh supply is increasing day by day throughout the year. Tomato cultivation in the winter is favorable for good climate but in the summer is mostly affected by high temperature, heavy rainfall, wind storm, and susceptibility to diseases and insect pests. Fruit setting needs night temperature between 15 and 20^0C which does not occur anywhere in Bangladesh during May to September (Charles and Harris, 1972). However, it has the large potentiality to be grown in summer as tomato is worldwide grown in a wide range of environmental conditions including tropical, sub-tropical and temperate areas (Bahati *et al*., 2004). In this context, Bangladesh Agriculture Research Institute (BARI) has developed some year-round hybrid varieties and now there are other few number of year-round hybrid varieties available in the market. In the case of hybrid varieties, isolated parents are maintained every time through strict isolation and consequently, it requires a considerable amount of technical and management skills that limit its usefulness.

The present study tried to circumvent the above-mentioned problems by applying tissue culture technique for propagation of summer tomato. The objective of the present study was to establish efficient tissue culture protocol or rapid micropropagation in summer tomato to meet the increased demand for tomato in Bangladesh. In fine, the clonal propagation and regenerative potentiality and the effect of different plant growth regulators on these genotypes through shoot tip and nodal segment culture under *in vitro* condition were studied and is reported in this paper.

MATERIALS AND METHODS

Three varieties of year-round hybrid tomato (*Lycopersicon esculentum* Mill.), namely BH-3 (BARI Hybrid-3), BH-4 (BARI Hybrid-4) collected from BARI (Bangladesh Agricultural Research Institute) and MH (Minto Hybrid) collected from Laal Teer Seed Limited, were used in the present study. For the preparation of explant, seeds were washed in running tap water with the addition of detergent to remove surface-adhered particles followed by a through wash by sterile distilled water. Seeds were surface sterilized in front of laminar airflow cabinet with 0.1% mercuric chloride for 5 minutes and then rinsed with sterile distilled water for 5-6 times. Surface sterilized seeds were inoculated in a sterile conical flask containing moistened cotton and water with only agar media for germination. Shoot tips from seedlings and nodal segments from *in vitro* grown stem were used as explants.

MS medium supplemented with varying concentrations and combinations of BAP and Kn were used for *in vitro* morphogenic study from shoot tip explants. Similarly, combined effects of BAP, Kn, IAA and GA_3 in shoot multiplication and proliferation from nodal segment culture, were observed on MS medium. Also, shoot elongation study was conducted on the duration of culture period from nodal segment culture at varying concentrations and combinations of BAP, Kn, IAA, and GA_3. About 3-4 cm long shoots were separated and used for rooting. Half-strength of MS medium supplemented with different concentrations and combinations of IAA and NAA were used for *in vitro* root induction. Controls were maintained on hormone-free MS medium. In every experiment, 3% sucrose was added to the medium and the final pH was adjusted to 5.8 by the addition of 1N NaOH/1N HCl dropwise before autoclaving (at 121^0C, 15 psi for 20 minutes). Cultures were maintained under a regime of 16 h photoperiod at 25 ± 2^0C. Every experiment was conducted in triplicate. Well-rooted *in vitro* regenerated plantlets were transferred into the soil after hardening. Survived plantlets were transferred to the field and observed till flowering and fruiting.

RESULTS AND DISCUSSION

Response from shoot tip explant

Shoot multiplication and proliferation from shoot tip culture were observed at different concentrations of BAP and Kn, singly and in combination. Morphogenic responses varied between treatments and varieties (Table-

1). Control plants (on growth regulators-free MS medium) always produced a single shoot. Regarding shoot multiplication, from raw data, some individuals were observed to produce 2 to 6 shoots, while some produced single shoot within a treatment.

Table. 1. Effect of different concentrations and combinations of BAP and Kn with MS medium on shoot multiplication and proliferation from shoot tip culture in summer tomato.

Tomato cultivars	Concentrations of growth regulators (mg/l)		Number of shoot/explant Mean ±SD	Length of longest shoot (cm) Mean ± SD	Number of node/shoot Mean ± SD
	BAP	Kn			
BH-3	0.5	-	1.27±0.345	7.06±0.109	4.27±0.449
	1.0	-	1.90±0.549	5.07±0.435	2.90±0.104
	2.0	-	1.91±0.164	4.54±0.362	2.33±0.092
	-	0.5	1.00±0.762	6.28±0.554	3.75±0.274
	-	1.0	2.08±0.543	6.00±0.715	3.41±0.800
	-	2.0	1.21±0660	4.22±0.263	2.14±0.221
	1.00	1.00	2.28±0.264	3.84±1.014	2.12±0.702
	Control		1.00±0000	3.10±0.813	1.27±0.208
BH-4	0.5	-	1.00±0000	6.75±0.904	3.33±1.063
	1.0	-	2.07±0.792	5.34±0.209	3.09±0.389
	2.0	-	1.76±0.223	3.54±0.711	1.53±0.609
	-	0.5	1.00±0000	6.91±0.509	3.63±0.528
	-	1.0	1.00±0000	6.47±0.221	3.33±0.448
	-	2.0	1.36±0.593	4.74±0.098	2.63±0.338
	1.00	1.00	2.12±0.921	4.17±0.639	2.36±0.199
	Control		1.00±0000	2.95±0.009	1.30±0.412
MH	0.5	-	1.00±0000	7.02±0.264	3.50±0.281
	1.0	-	2.08±0.182	5.31±0.551	2.72±0.900
	2.0	-	1.90±0.382	3.40±0.089	1.54±0.121
	-	0.5	1.00±0000	6.47±0.710	3.18±0.555
	-	1.0	1.00±0000	6.52±0.339	3.20±0.038
	-	2.0	1.81±0.910	4.64±0.229	2.18±0.805
	1.00	1.00	2.15±0.893	4.22±0.103	1.81±0.601
	Control		1.00±0000	3.53±0.309	1.60±0.390

When BAP and Kn were used in combination (1.0 mg/l BAP + 1.0 mg/l Kn), the highest mean number of shoots per explant was recorded 2.28 in BH-3 followed by 2.15 in MH and 2.12 in BH-4 respectively. By subject to control culture, the present study suggests that BAP and Kn can play role toward shoot multiplication (Figure 1a-1d). Ishag *et al*. (2009) conducted a similar experiment with 0.5 to 5.0 mg/l BAP, Kin and 2ip used singly and in combination of NAA at 0.5-1.0 mg/l, and obtained best responses (2.0 shoots/explants) in 4.0 mg/l Kin. Contradictory to the present investigation, almost similar number of shoots per explant was obtained with 1.0 mg/l BAP and Kn in both BH-3 and MH varieties. Otroshy *et al*. (2013) also found BAP as an effective growth regulator for best shoot regeneration from leaf, hypocotyls, and cotyledons at 2.0 mg/l concentration.

Shoot length was found better at low doses of BAP and Kn for every variety and that was 0.5 mg/l. In fact, with the increase in hormone doses, shoot length as well as the number of nodes per shoot decreased gradually. Of the three cultivars, the longest shoot length was recorded 7.06 cm in BH-3 with 0.5 mg/l BAP on MS medium followed by 6.91 cm in BH-4 at 0.5 mg/l Kn and 7.02 cm in MH at 0.5 mg/l BAP. In relation to shoot length, 0.5 mg/l BAP or Kn was also found to be effective for a highest average number of nodes per shoot. The frequency of adventitious shoot regeneration has been attributed to both types and concentration of growth regulators and the types of explants by various authors like Gubis *et al*., 2004 and Plana *et al*., 2005. Cytokinin is known to play a critical role in shoot multiplication (Khalafalla and Abdellatef, 2008).

Response from nodal explant

To observe the combined effects of different growth regulators on shoot multiplication and proliferation from nodal segment explants were cultured on MS medium supplemented with various concentrations and combinations of BAP, Kn, IAA, and GA_3 (Table 2). Highest mean number of shoot/explant was found 2.10 in BH-3 when treated with 1.0 mg/l BAP + 0.5 mg/l Kn + 0.25 mg/l IAA + 0.5 mg/l GA_3 followed by 2.09 in BH-4 with 1.0 mg/l BAP + 0.5 mg/l Kn + 0.5 mg/l IAA and 1.83 in MH with 1.0 mg/l BAP + 0.5 mg/l Kn + 0.25 mg/l IAA + 0.5 mg/l GA_3. From these observations it was found that there were no mentionable advances on the average number of shoot per explants from earlier experiments using shoot tip explant. The longest shoot was found in all three cultivars when treated with BAP 1.0 mg/l, Kn 0.5 mg/l, IAA 0.5 mg/l, and GA_3 0.1 mg/l combinedly. In this case, the longest shoot was recorded 6.84 cm in MH followed by 6.59 cm in BH-4 and 5.82 cm in BH-3. Regarding shoot length, no mentionable advanced responses were observed from this combination treatment over shoot tip explant culture.

Larger number of shoots/explant was not obtained in the above experiments from shoot tip and nodal segment culture, attention was diverted to shoot elongation study, by using different growth regulators. The length was

measured after six weeks and nine weeks in all three cultivars (Table 3). In the case of shoot elongation study, when used in low doses (i.e. 0.5 mg/l), Kn and GA_3 in combination, was found most effective in most of the

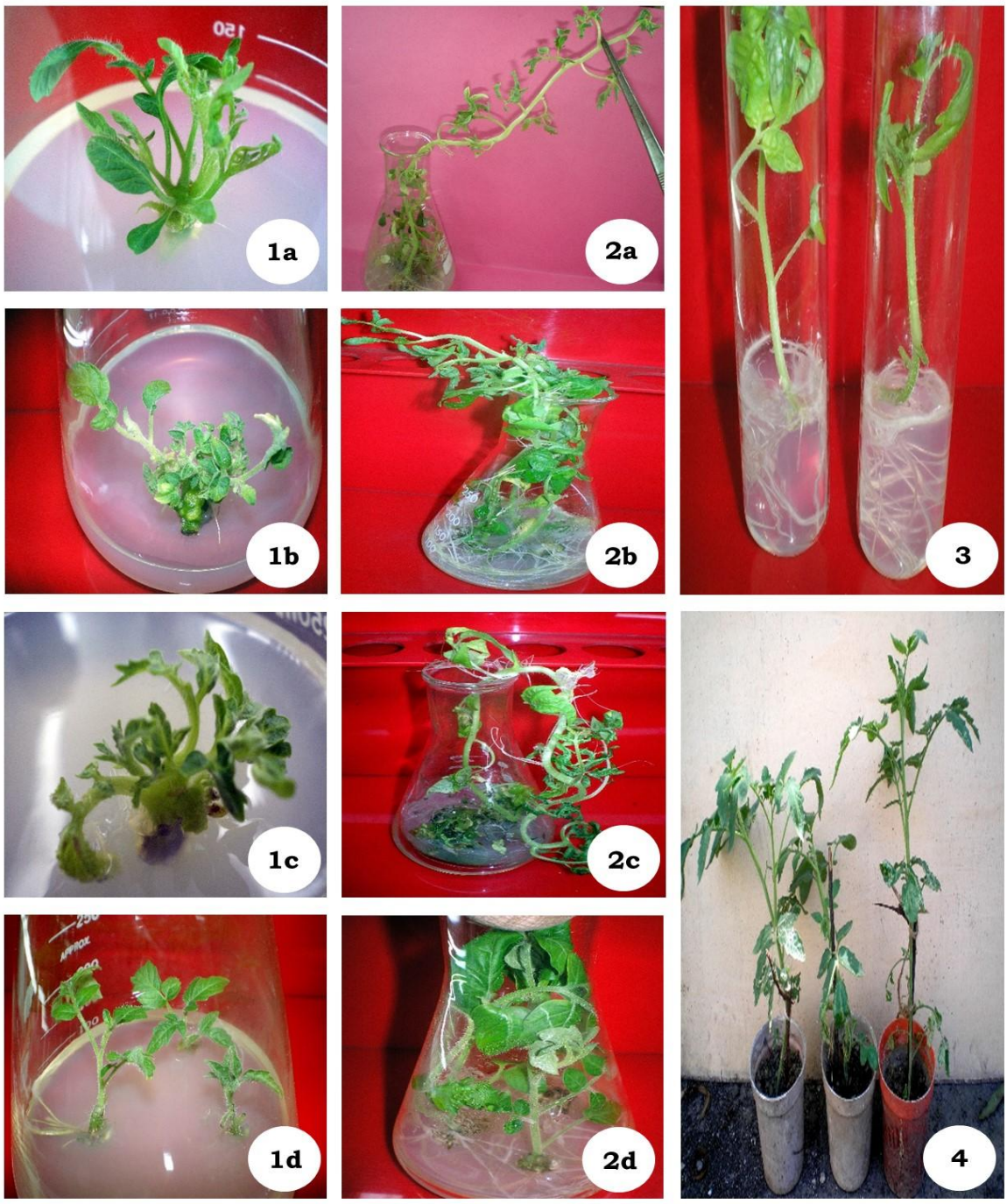

Figure 1-4: *In vitro* shoot multiplication, elongation and regeneration in summer tomato. **1a-1d.** Multiple shoot formation after 5 weeks of culture in (1a) BARI hybrid-3 (1b) BARI hybrid-4, (1c) MH hybrid on MS medium supplemented with 1.0mg/l BAP + 1.0mg/l Kn and single shoot in (1d) control on hormone-free MS medium. **2a-2d.** Elongation of shoots in (1a) BARI hybrid-3 and (2b) BARI hybrid-4 supplemented with 0.5mg/l Kn + 0.5mg/l GA_3, (2c) Minto hybrid supplemented with 0.5mg/l BAP + 0.5mg/l GA_3 on MS medium and (2d) control on hormone-free MS medium after nine weeks of culture. **3.** Root induction on ½ MS medium with 0.5mg/l NAA + 0.5mg/l IAA after five weeks of culture. **5.** Acclimatized plantlets.

varieties. After 6 weeks, longest shoot was found 6.07 cm in BH-4 at 0.5 mg/l concentrations of BAP + GA_3, and similar to this, the 5.75 cm longest shoot was obtained from BH-3 on 0.5 mg/l Kn + GA_3 supplemented MS medium. However, after 9 weeks, BH-3 produced 11.87 cm longest shoot at the same treatment. On the other hand, MH produced 10.31 cm longest shoot at 0.5 mg/l Kn + 0.5 mg/l GA_3 concentration followed by 10.13 cm longest shoot in BH-4 at 0.5 mg/l BAP + 0.5 mg/l GA_3 concentration after 9 weeks of culture (Figure 2a-2d). Maximum average number of nodes per shoot was observed 6.0 in BH-3 followed by 5.12 in BH-4 in combined treatment with Kn and GA_3 at 0.5 mg/l concentration each. It was observed that there was an almost proportional relationship between shoot length and the number of nodes per shoot after 9 weeks of culture. In terms of shoot elongation, BH-3 were more responsive than the other two varieties. By subject to control culture, it was found that use of growth regulators and the duration of culture period have positive effects on shoot elongation from nodal segment explant.

Table. 2. Combined effects of BAP, Kn, IAA and GA_3 with MS medium on shoot multiplication and proliferation from nodal segment explant in summer tomato.

Tomato cultivars	Concentrations of growth regulators (mg/l)				Number of shoot/explant Mean ± SD	Length of the longest shoot (cm) Mean ± SD
	BAP	Kn	IAA	GA_3		
BH-3	0.1	0.5	0.5	-	1.46±0.409	3.04±0.900
	1.0	0.5	0.25	0.5	2.10±0.228	4.96±0.751
	1.0	0.5	0.5	0.1	1.35±0.544	5.82±0.398
	0.5	0.5	0.25	0.5	1.00±0000	1.46±0.302
	Control				1.00±0000	2.55±0.119
BH-4	0.1	0.5	0.5	-	2.09±0.609	3.05±0.316
	1.0	0.5	0.25	0.5	1.45±0.109	5.99±0.492
	1.0	0.5	0.5	0.1	1.60±0.719	6.59±0.419
	0.5	0.5	0.25	0.5	1.00±0000	1.37±0.302
	Control				1.00±0000	2.98±0.225
MH	0.1	0.5	0.5	-	1.66±0.881	3.72±0.447
	1.0	0.5	0.25	0.5	1.83±0.219	5.80±0.331
	1.0	0.5	0.5	0.1	1.75±0.331	6.84±0.482
	0.5	0.5	0.25	0.5	1.00±0000	1.57±0.221
	Control				1.00±0000	2.55±0.602

Regenerated shoots were transferred into auxins-added rooting media for adventitious rooting to raise full-fledged plantlets. *In vitro* root induction was performed on half-strength MS medium with different concentrations of NAA and IAA (Table 4). In all treatments, 100 percent rooting was observed (Figure 3). However, days took to root initiation and number of roots per shoot varied considerably. Rooting time was reduced to 6-10 days in all three varieties when cultured on ½ strength-MS medium supplemented with 0.5 mg/l NAA + 0.5 mg/l IAA. Single use of IAA at 0.5 mg/l concentration resulted in a longer time (10-15 days depending on varieties) for root initiation; while at 1.0 mg/l concentration, root initiation was observed after 7-12 days depending on varieties. The present study suggests that moderate dose (1.0 mg/l) of IAA alone and combined use of low doses (0.5 mg/l) of IAA and NAA have the potential to reduce rooting time.

Table. 3. Effect of duration of culture period (6 weeks and 9 weeks) on shoot elongation from *in vitro* cultured nodal segment explant on MS medium with different growth regulators in summer tomato.

Tomato cultivars	Growth regulators concentration (mg/l)				Length (cm) of the longest shoot from nodal culture Mean ± SD		Number of nodes/shoot after 9 weeks Mean ± SD
	BAP	Kn	IAA	GA_3	After 6 weeks	After 9 weeks	
BH-3	0.5	-	-	-	4.90±1.022	8.10±0.776	4.54±0.719
	1.0	-	0.25	-	3.93±0.391	6.93±0.309	3.50±0.337
	0.5	-	-	0.5	4.90±0.261	9.26±0.220	4.10±0.183
	-	0.5	-	0.5	5.75±0.739	11.87±0.789	6.00±0.227
	Control				1.79±0.229	6.20±0.712	2.37±0.602
BH-4	0.5	-	-	-	5.09±0.622	9.76±0.267	4.53±0.618
	1.0	-	0.25	-	5.56±0.098	9.70±0.291	4.66±0.387
	0.5	-	-	0.5	6.07±0.388	10.04±0.339	4.72±0.287
	-	0.5	-	0.5	4.86±0.821	10.31±0.876	5.12±0.419
	Control				2.50±0.911	6.03±0.419	2.50±0.902
MH	0.5	-	-	-	4.99±0.391	8.48±0.390	4.30±0.826
	1.0	-	0.25	-	4.85±0.449	9. 82±0.813	4.60±0.389
	0.5	-	-	0.5	5.30±0.611	10.31±0.291	4.55±0.819
	-	0.5	-	0.5	4.24±0.312	9.83±0.326	4.70±0.290
	Control				2.76±0.425	6.36±0.612	2.66±0.629

In terms of highest average number of root per shoot, variations were found in the three varieties. In the present study, the highest number of roots/shoot was recorded 7.27 in MH variety at 1.0 mg/l IAA concentration

followed by 7.25 in BH-4 at 0.5 mg/l NAA + 0.5 mg/l IAA concentration and 6.62 in BH-3 at 1.0 mg/l IAA concentration.

The beneficial effect of using half-strength MS medium for rooting of *in vitro* grown shoots has already been reported in tomato. Devi *et al.* (2008) suggested that the best rooting was found on half MS medium supplemented with 0.2 mg/l IBA. Promotive effects of auxins were found in the present study which agreed with the study of De Klerk *et al*. (1999) and Ishage *et al*. (2009). Das (2011) conducted a similar experiment from cotyledonary leaf explants at various concentrations of IAA and IBA, and in his study BH-3 variety responded to root initiation within 7-8 days.

Table. 4. Effect of NAA and IAA supplemented with half-strength of MS medium on root induction of *in vitro* grown shootlets.

Tomato cultivars	Growth regulators concentration (mg/l)		Days taken to root initiation	% of rooted shoot	Number of root/shoot Mean ± SD
	NAA	IAA			
BH-3	-	0.5	10-15	100	5.69±0.691
	-	1.00	7-10	100	6.62±0.338
	0.5	0.5	6-10	100	4.86±0.538
	Control		15-20	84.62	3.11±0.612
BH-4	-	0.5	10-15	100	6.17±0.712
	-	1.00	7-12	100	7.15±0.782
	0.5	0.5	6-10	100	7.25±0.187
	Control		15-20	81.39	2.68±0.528
MH	-	0.5	10-12	100	7.25±0.523
	-	1.00	7-12	100	7.27±0.763
	0.5	0.5	6-10	100	7.18±0.678
	Control		15-20	87.09	1.94±1.341

In control culture, maximum 87% rooting was observed in MH variety. Root induction in control plants on hormone free half-strength MS medium indicates that genotypes of tomato possessed sufficient levels of endogeneous auxins. This means the exogenous application of auxin was not required for *in vitro* root induction (Devi *et al*., 2008). However, compared to control plantlets, the exogenous application of auxins (NAA and/or IAA) has been found effective in the formation of increased root number per shoot and percentage of rooted shoot and.to sufficiently reduce root initiation time in every treatment

In the present investigation, regenerated plantlets of tomato were transferred in plastic pots containing garden soil and compost-sand mixed soil after hardening and were subsequently acclimatized to *in vivo* condition (Figure 4). It has been observed that 90% plantlets survived *in vivo*. Ahmed *et al.* (2000) observed similar results in tomato plantlets.

A number of factors, acting individually or synergistically, have been reported to govern *in vitro* regeneration in tomato, including the type of nutrient media, concentrations and combinations of growth regulators, light and temperature regimes in incubation room, genotypic variations and the explants being used (Sheeja *et al*., 2004 and Bhatia *et al*., 2004). In this present study, varietal differences present in almost every experiment conducted. Varietal differences and genotypic variations in plantlets regeneration were also reported by Hammat *et al*. (1980) and Lutova and Zabelina *et al*. (1988). In the present study, it was evident that growth regulators played role on shoot multiplication and proliferation as well as root induction, and it was experimented by subject to control culture. It was also found that shoot tip was better explant than nodal segment. Highest average number of shoots per explants was limited up to 2.28 among the three cultivars. Responses were found different among varieties.

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