

Research Article

## Optimization of *in vitro* rooting protocol for tomato (*Lycopersicon esculentum* mill.) varieties

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### Abstract

*In vitro* rooting of micropropagated shoots were carried out with the aim of evaluating the root induction responses of two tomatoes (*Lycopersicon esculentum* MILL) varieties (Gelilema and Chali) using Indole -3- butyric acid (IBA). Seven levels of IBA (0.0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg L<sup>-1</sup>) were used in a completely randomized design (CRD) in factorial combinations (seven level of IBA\*two varieties) with three replications. After the plantlets were kept in the rooting media for three weeks, data on rooting percentage, number of roots/shoots and root length in cm were collected. The analysis of variance showed that the interaction of IBA\*Var was highly significantly different for rooting percentage, a number of roots/shoot and root length at  $p < 0.01$ . The highest rooting percentage (100.00±0.00), number of roots/shoot (14.20±0.35) and root length (10.7±0.29) were received from Chali on free Murashige and Skoog medium (MS). At the same time, the lowest percentage of rooting (11.11±0.00), number of roots/shoot (0.887±0.19) and root length (1.00±0.00 cm) were obtained from Gelilema on MS+1.5mg/l IBA. For acclimatization, the *in vitro* rooted shoots were transplanted into plastic pots containing a mixture of oven sterilized soil and sand at a ratio of 2:1. After three weeks, a survival rate of 67.7% for Chali and 58.1% for Gelilema was obtained. From the above result, it can be concluded that free MS medium was the best for *in vitro* rooting of the two tomato varieties. The optimized protocol will be useful for rapid *in vitro* multiplication of the two tomato varieties.

**Keywords:** Chali, Gelilema, IBA, *In vitro* rooting, Tomato

### INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables in the world. It is a dicotyledonous plant that belongs to the family of *Solanaceae* and genus *Lycopersicon* (Kalloo, 1991). It is a diploid with  $2n=2x=24$  chromosome. Nowadays, it grows almost in every country of the world either in the field, greenhouses or net houses (Jehan and Hassanein, 2013). The five leading tomato producing countries in the world are China, India, United States, Turkey and Egypt. The total area under tomato cultivation in the world, Africa and Ethiopia is about, 4.78 mln ha, 1.27 mln ha and 6299 ha with an average yield of

37.09, 15.59 and 4.5 ton/ha respectively (FAO STAT, 2016). In Ethiopia, tomato has been cultivated for economic importances like a source of income, creating employment opportunity and access to farmers to participate in the market (Eyob *et al.*, 2014). It also used as a sample for the study of genomics, proteomics and metabolomics (Jehan and Hassanein, 2013). Even if tomato has numerous benefits, its production is not equivalent to its area of coverage. This is due to the influence of several biotic and abiotic factors like diseases, pests, environmental stress, post-harvest losses and propagation method (Mukta, 2014; Datta, 2015). The use of a hybrid variety is a great option to increase yield for most of the vegetable crops, includ-

ing tomato. But the mentioned factors also cause the decline of hybrid seed recovery from the field. Tomato is one of the vegetable crops which have been grown from hybrid seeds that are obtained by crossing two or more genetically different parents in every cropping season (Gao, 2017). Hybrid tomato varieties continue to dominate high input agricultural systems. This increase in demand for hybrid seeds stresses commercial hybrid seed production abilities. Because most of the hybrid seeds of tomatoes are produced by hand emasculating, hand pollination and hybridization, which are labour-intensive (Cheema and Dhaliwal, 2005; Sudha *et al.*, 2006). Additionally, seeds saved after F1 hybrids produce plants that are inferior and not uniform because, seeds do not remain genetically true to type (Opeña *et al.*, 2001a). Such the improvement of a plant through conventional breeding method is slow, time-consuming and need more space (Moghaieb *et al.*, 1999).

Plant tissue culture is one of the important tools of biotechnology, which has been used for increasing the productivity of a given crop by supplying improved planting materials within a short period of time and a limited space (Mohamed *et al.*, 2010). In tomato, it has been used for selection of cell lines for biotic and abiotic stresses (Rahman and Kaul, 1989), development of haploids (Shtereva *et al.*, 1998), production of somatic hybrids (Wijbrandi *et al.*, 1988), mass propagation (Izadpanah and Khosh-Khui, 1992) and development of transgenic tomato (Kiran, 2007).

Different plant growth regulators (PGRs) have been used by different researchers for tomato *in vitro* rooting. According to Bahurupe *et al.* (2013), tomatoes do not require any exogenous growth regulators for root induction. Similarly, Ashakiran *et al.* (2011) found that MS medium devoid of exogenous auxin produced adventitious roots in two tomato varieties. However, Mamidala and Nanna (2011) reported that root formation of tomatoes had been achieved with auxin (Indole-3-Acetic Acid (IAA), Naphthalene Acetic Acid (NAA) or Indole-3 Butyric Acid (IBA) alone with a concentration of 0.1-1 mg/l. The establishment of one universal protocol for *in vitro* rooting of all the varieties is impossible (Gerszberg *et al.*, 2015). Because morphogenic responses of cultured tomato plant tissues are affected by the genotype and explant (size, age and orientation) (Bhatia, 2003). Therefore, this research work was initiated with the objective to find out the optimum concentration of IBA for root formation of two tomato varieties (Gelilema and Chali) of *L. esculentum*.

## MATERIALS AND METHODS

Two-hybrid tomato (*L. esculentum*) varieties named Chali and Gelilema were used as an experimental ma-

terial, which was obtained from Melkassa Agricultural Research Center (MARC). The varieties were selected based on their best performances. They are the newly introduced, and being widely distributed varieties to farmers.

Murashige and Skoog (MS) media supplemented with various plant growth regulators were used. Stock solutions of the macro salts, micro salts, vitamins, iron source and plant growth regulators (1mg: 1ml) were prepared and stored at 4°C in the refrigerator. Plant growth regulator, auxin (IBA) were dissolved using a drop of ethanol before making up the final volume with distilled water. Iron EDTA (Ethylene Di Amine Tetra Acetic Acid) stock solution was covered with aluminum foil. Culture medium was prepared from all stock solutions (macro, micro, iron and vitamins). The medium was solidified with 0.8% (w/v) agar and 3% sucrose was added as an energy supply. The pH was adjusted to 5.8 using 1N NaOH or HCl prior to the addition of agar. Growth regulators were added according to the concentration required. Then 50ml media were dispensed into washed and sterilized culture jars, then plugged and labelled properly. Then the medium was steam sterilized using an autoclave chamber at a temperature of 121°C and a pressure of 105 KPa for 15 min. Finally, the autoclaved media were taken out of the autoclaving chamber and put on the shelf for 4 days until used.

After the prepared culture media stayed for 4 days, the *in vitro* grown shoots that had ~3 cm length were excised and cultured on free MS medium for two weeks to avoid the carryover effect. Then, the shoots were transferred on MS medium supplemented with IBA at 0.0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/l. The experiment was laid out using CRD in factorial combinations (seven levels of IBA\*two varieties) with three replications. Three shoots/jar were used. After the plantlets were kept in the rooting media for three weeks, data on rooting percentage, number of roots/shoots and root length in cm were collected (Namitha and Negi, 2013, Sherkar and Chavan, 2014). For the collected data analysis of variance (ANOVA) was performed using SAS software Packages (version 9.3). A least significant difference (LSD) was used for the comparison of significant differences between means at P<0.01.

## RESULTS AND DISCUSSION

The ANOVA obtained from rooting experiment indicated that the interaction effect of variety and IBA were highly significant for rooting percentage, the number of roots/explant and root length of the two tomato varieties at P<0.01.

The highest rooting percentage (100.00±0.00) was obtained from an IBA level ranging from 0.0-0.75mg/l

IBA in both of the varieties of tomato (*L. esculentum*) used for the experiment (Table 1; Figure 1). Whereas, the lowest percentage of rooting (11±0.00) were obtained from Gelilema variety grown on MS medium supplied with 1.5 mg/l IBA while for Chali the lowest rooting percentage was (55.6±19.25) which was from the same level of IBA as Gelilema's lowest. This result indicates that each genotype responded differently, which may be due to differences in their endogenous auxin amount. A similar result was reported by Singh *et al.* (2006) who reported that different genotypes require different concentrations of auxin and their response is dependent on the amount of their endogenous auxin concentration for sugarcane varieties. Similar to our rooting percentage, Osman *et al.* (2009) found 100% rooting in both full and half-strength MS medium without growth regulators over different levels of NAA, IAA, or IBA for *in vitro* rooting of Omdurman tomato cultivar.

The increase in the concentration of IBA from 0 mg/l to 1.5 mg/l resulted in the decrease of rooting percentage. This is similar with the finding of Sakthivel and Manigandan (2011) who suggested that better rooting parameters were obtained from a media supplied with low level of IBA. In their experiment, they obtained 100% rooting in 0.5 mg/l IBA and 0.00% rooting at 2 mg/l IBA. The root length was also better in lower IBA levels. In another rooting experiment, Ishag *et al.* (2009) reported the same result. They recorded 100%

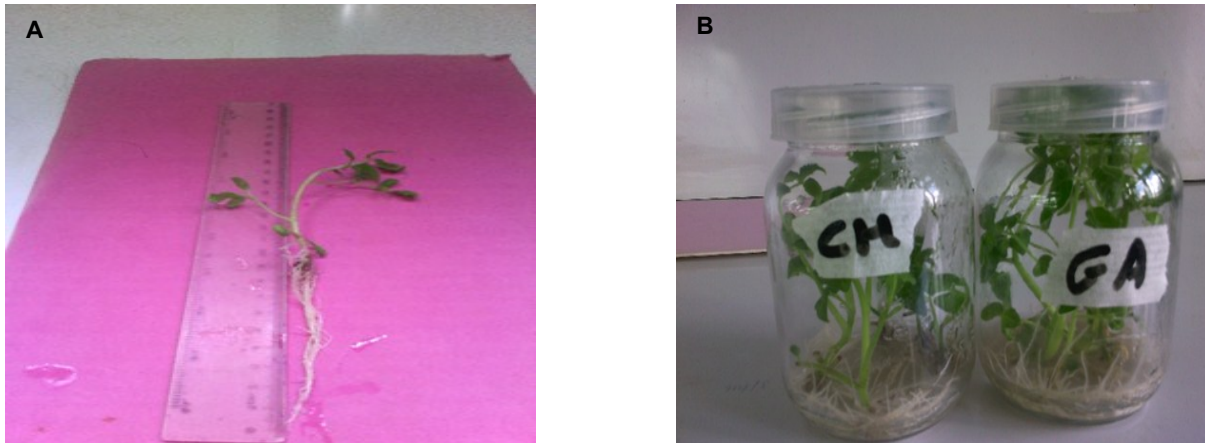
rooting in MS medium without IBA. Low concentration of IBA promotes root induction and elongation than higher concentration that inhibited rooting in both varieties. However, Bhatia (2003) reported that tomato rooting response was dependent on the auxin type used. The author said that tomato cultivar Red Coat response increased with an increase in IAA concentration whereas, with NAA, the response decreased as the concentrations increased. Pampanna (2009) reported a contradictory result to the present work. In an experiment conducted using cotyledon and hypocotyl explants derived from tomato cultivar VYBHAV, the researcher found that the rooting percentage of both explants increased as the concentration of IBA increased from the control to 1.5 mg/l. From MS medium free of IBA the author found 29 and 35% rooting respectively while at 1mg/l IBA 96 and 98% rooting was reported. This may also be due to the dependence of plants on the level of their endogenous auxin to give response for exogenous auxin. In another research, Sherkar and Chavan (2014) obtained 100% rooting on MS medium augmented with IBA concentrations ranging from control-3 mg/l.

During rooting of the tomato varieties used as an experimental material, there is no significant difference in rooting percentage in IBA levels 0-0.75 mg/l (Table 1). Mensuali-Sodi *et al.* (1995) and Devi *et al.* (2008) also reported the presence of exogenous auxin enables tomato to give roots without requiring

**Table 1.** Effect of IBA on *n vitro* rooting of tomato varieties (Gelilema and Chali).

Varieties	IBA in mg/l	%R Mean±SD	NR Means±SD	RL Means ±SD
Gelilema	0.00	100.00 <sup>a</sup> ±0.00	14.17 <sup>a</sup> ±0.29	7.58 <sup>c</sup> ±0.144
	0.25	100.00 <sup>a</sup> ±0.00	11.00 <sup>cb</sup> ±0.00	6.67 <sup>d</sup> ±0.058
	0.50	100.00 <sup>a</sup> ±0.00	9.533 <sup>d</sup> ±0.39	4.33 <sup>f</sup> ±0.144
	0.75	100.00 <sup>a</sup> ±0.00	4.00 <sup>g</sup> ±0.00	4.g±0.144
	1.00	66.67 <sup>b</sup> ±0.00	1.77 <sup>i</sup> ±0.19	3.92 <sup>g</sup> ±0.144
	1.25	33.33 <sup>c</sup> ±0.00	1.440 <sup>ji</sup> ±0.38	3.33 <sup>h</sup> ±0.144
	1.50	11.11 <sup>d</sup> ±0.00	0.887 <sup>j</sup> ±0.19	1.00 <sup>i</sup> ±0.00
	0.00	100.00 <sup>a</sup> ±0.00	14.20 <sup>a</sup> ±0.35	10.7 <sup>a</sup> ±0.29
Chali	0.25	100.00 <sup>a</sup> ±0.00	11.33 <sup>b</sup> ±0.58	8.00 <sup>b</sup> ±0.00
	0.50	100.00 <sup>a</sup> ±0.00	10.33 <sup>cd</sup> ±0.58	6.33 <sup>d</sup> ±0.144
	0.75	100.00 <sup>a</sup> ±0.00	7.67 <sup>e</sup> ±0.58	5.63 <sup>e</sup> ±0.351
	1.00	88.98 <sup>a</sup> ±19.25	6.00 <sup>f</sup> ±1.00	5.64 <sup>e</sup> ±0.271
	1.25	66.67 <sup>b</sup> ±0.00	3.67 <sup>g</sup> ±0.58	5.57 <sup>e</sup> ±0.12
	1.50	55.67 <sup>b</sup> ±19.25	2.67 <sup>h</sup> ±0.57	4.33 <sup>f</sup> ±0.577
	<b>CV</b>	11.11	6.826614	4.218004
	<b>LSD</b>	14.9	0.804	0.3885

Note: Values are given as Mean ± SD. Means with different letters within the same column (s) are significantly different from each other at P≤0.01. Where: %R=rooting percentage, NR= average number of roots and RL=average root length in cm.



**Fig. 1.** *In vitro* rooting of Two Tomato varieties (A. Root length measurement, B. Chali and Gelilema Varieties of Tomato on IBA free MS medium).



**Fig. 2.** Acclimatized plantlets of *Gelilema* and *Chali* varieties of tomato after 15 days in a lath house.

any exogenous plant growth regulators. This may, due to the presence of high endogenous auxin concentration in tomato plant. Likewise, Kartha *et al.* (1975) found that the presence of endogenous auxin in the regenerated shoots of tomato cultivar Starfire facilitates an exogenous-auxin-independent root formation and production of complete plants. The present result is contradictory to the report of (Rao *et al.* 2005; Dan *et al.*, 2006). Rao *et al.* (2005) reported that culturing on MS medium containing auxin resulted in a large proportion of rooted micro-shoots and early rooting than cultivation on an auxin-free medium. Dan *et al.* (2006) also conducted an experiment using cotyledon explants derived from tomato Micro-Tom cultivar and concluded that MS medium supplemented with 1mg/l IBA gave a good response to rooting over the control and 2 mg/l.

In the case of a number of roots/explants Chali gave the highest ( $14.20 \pm 0.3464$ ) roots per shoot with an average root length of  $10.7 \pm 0.29$  cm on free MS medium (Table 1; Figure 1). On the same media, Gelilema resulted in  $14.17 \pm 0.29$  roots/shoot having an average root length of  $7.58 \pm 0.14$  cm. There was no significant difference in the number of roots per shoot in the two varieties cultured on the free MS media. A similar re-

sult was obtained by Amitav (2011) who reported that tomato varieties Maple and BARI-3 produced the same number of roots on MS+0.5 IBA. But as that of our root length result, there was a significant difference in the two varieties. The minimum numbers of roots/shoot and average root length were recorded from MS medium supplemented with 1.5 mg/l IBA in both varieties. Overall the lowest value of a number of roots/shoot ( $0.887 \pm 0.196$ ) with an average root length of  $1.00 \pm 0.00$  cm was recorded from Gelilema variety on MS+1.5 mg/l IBA. On the same media, Chali produced  $2.67 \pm 0.57$  numbers of roots per shoot having an average root length of  $4.33 \pm 0.577$  cm.

In both of the varieties used the number of roots per shoot and root length per shoot were decreased as the IBA level increased until 1.5 mg/l. This may occur due to the inhibitory effect of higher auxin concentration. Similarly, Sedaghat and Rahemil (2012) found that higher concentrations of auxin provoke ethylene production and inhibit root growth. Likewise, Sakthivel and Manigandan (2011) found that the increase in the level of IBA from 0.5mg/l to 1.5 resulted in the decrease of both the number of roots and root length mg/l in PKM1 tomato variety. The present result is also in conformity with Ishag *et al.* (2009) who found that the number of

roots/shoot and root length of plantlets that originated from shoot tip explant of tomato cultivar Omdurman decreased as the level of IBA increased from 0 to 0.5 mg/l. On the other hand, Banu *et al.* (2017) said that exogenous application of auxin has been found effective in the formation of increased root number per shoot and percentage of rooted shoot compared to control plantlets in three tomato varieties namely H-3 (BARI Hybrid-3), BH-4 (BARI Hybrid-4) collected from BARI (Bangladesh Agricultural Research Institute) and MH (Minto Hybrid). In another research, Mukta (2014) also found the contradictory result to the present who reported that when the concentration of exogeneous growth regulators particularly IAA increased, the highest number of roots (15-30) was formed but required highest day for root initiation and all the roots were short (average length 1.5-3 cm). This may due to the dependence of the cultured plants on the type of auxin used. Similarly, Bhatia (2003) found that the *in vitro* response of cultured tomato is dependent on the auxin type used for rooting.

In another work, Pampanna (2009) reported a contradictory number of roots per plant and shoot length to the present result by which the highest number of roots per plantlet and root length were found from MS medium supplemented with the highest level of IBA in both explants. On the control, a root number of 9.6 having a length of 1.96 cm in cotyledon and 9.8 with 2.16 cm in hypocotyl were reported by the author. Both numbers of roots and root length increased as the level of IBA increased from the control to 1 mg/l in both the explants and concluded that root length, number of roots per plantlet and nature of roots were better at 1mg/l IBA.

All the values rooting %, number of roots per shoot and root length in cm obtained from the present rooting experiment were in line with Otroshya *et al.* (2013) who found that all the parameters were better on MS medium free of IBA for *in vitro* rooting of Cerasiforme varieties of tomato. The researchers recorded 100% rooting with 15.38 numbers of roots having a root length of 16.6 cm on free MS medium. This may be due to the existence of sufficient endogenous level of auxin in tomato (Devi *et al.*, 2008) in tomato. Similarly, Himabindu (2008) said that the response of a plant species to an exogenous growth regulator depends on the endogenous level of that growth regulator in that species.

**Acclimatization:** For acclimatization, plantlets with well-developed root and leaf were removed from rooting medium and washed thoroughly to remove adhering gel. Then transplanted into plastic pots containing a mixture of oven sterilized soil and sand at a ratio of 2:1 and covered with a white plastic to maintain high humidity and kept under washing room conditions for 7

days. Then the plastic covers were removed and transferred to lath house and placed under shade until growth was observed. After 15 days, the survival rate was recorded. 31 *in vitro* rooted plantlets from each variety were used for acclimatization experiment. Out of the 31 plantlets acclimatized a retrieval rate of 67.7% and 58.1% for Chali and Gelilema respectively was obtained after 15 days (Figure 2). Some plantlets failed to survive in the *ex-vitro* environment after transferred to lath house. This may due to the change in the environmental condition. Because, during *in vitro* culture, plantlets grow in closed containers under controlled humidity, light, nutrient and aseptic conditions. A contradictory result was reported by Namitha and Negi (2013) who found that survival of 70-80% from *in vitro* grown plantlets of tomato cultivar Arka Ahuti.

## Conclusion

Rooting of *in vitro* propagated micro-shoots is one of the important stages of micropropagation that affects the survival rate of plantlets under *ex-vitro* environment. *In vitro* propagation is affected by a number of factors, one of them is genotype of the given explant. Thereby, it is mandatory to optimize an *in vitro* rooting protocol for micro-shoots obtained through micropropagation. In the present study, an efficient *in vitro* rooting protocol was optimized for two tomato (Gelilema and Chali) varieties. In conclusion, MS medium free of PGRs resulted in the superiority of the collected parameters for *in vitro* rooting of plantlets over MS medium supplied with IBA. Thus, this genotype-specific protocol could be useful for *in vitro* rooting of the two varieties of tomato (Gelilema and Chali) in the future. Further studies using other types and combination of PGRs are suggested. The acclimatization to the external environment using other mixtures for getting a higher rate of survival also deserves attention.

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