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

Endalkachew Baye*
Department of Plant Sciences, College of Agriculture and Natural Resource, Salale University, P.O BOX 245, Fitch, Ethiopia

Temesgen Matewos
Department of Horticulture and Plant Sciences, Jimma University, P.O BOX 138, Jimma, Ethiopia

Derbew Belew
Department of Horticulture and Plant Sciences, Jimma University, P.O BOX 138, Jimma, Ethiopia

*Corresponding author. Email: bayeendalkachew4@gmail.com

How to Cite

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⁻¹) 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 emasculation, 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 material, 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 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 Coats 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 grow roots without requiring

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

<table>
<thead>
<tr>
<th>Varieties</th>
<th>IBA in mg/l</th>
<th>%R Mean±SD</th>
<th>NR Mean±SD</th>
<th>RL Means ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelilema</td>
<td>0.00</td>
<td>100.00±0.00</td>
<td>14.17±0.29</td>
<td>7.58±0.144</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>100.00±0.00</td>
<td>11.00±0.00</td>
<td>6.67±0.058</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>100.00±0.00</td>
<td>9.53±0.39</td>
<td>4.33±0.144</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>100.00±0.00</td>
<td>4.00±0.00</td>
<td>4.9±0.144</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>66.67±0.00</td>
<td>1.77±0.19</td>
<td>3.92±0.144</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>33.33±0.00</td>
<td>1.44±0.38</td>
<td>3.33±0.144</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>11.11±0.00</td>
<td>0.88±0.19</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>100.00±0.00</td>
<td>14.20±0.35</td>
<td>10.7±0.29</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>100.00±0.00</td>
<td>11.33±0.58</td>
<td>8.00±0.00</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>100.00±0.00</td>
<td>10.33±0.58</td>
<td>6.33±0.144</td>
</tr>
<tr>
<td>Chali</td>
<td>0.75</td>
<td>100.00±0.00</td>
<td>7.67±0.58</td>
<td>5.63±0.351</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>88.98±19.25</td>
<td>6.00±1.00</td>
<td>5.64±0.271</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>66.67±0.00</td>
<td>3.67±0.58</td>
<td>5.57±0.12</td>
</tr>
<tr>
<td></td>
<td>1.50</td>
<td>55.67±19.25</td>
<td>2.67±0.57</td>
<td>4.33±0.577</td>
</tr>
<tr>
<td></td>
<td>CV</td>
<td>11.11</td>
<td>6.826614</td>
<td>4.218004</td>
</tr>
<tr>
<td></td>
<td>LSD</td>
<td>14.9</td>
<td>0.804</td>
<td>0.3885</td>
</tr>
</tbody>
</table>

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.
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±0.3464) roots per shoot with an average root length of 10.7±0.29 cm on free MS medium (Table 1; Figure 1). On the same media, Gelilema resulted in 14.17±0.29 roots/shoot having an average root length of 7.58±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 result 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. On the same media, Chali produced 2.67±0.57 numbers of roots per shoot having an average root length of 4.33±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 Gelliema 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 (Gelliema 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 (Gelliema 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.

**ACKNOWLEDGEMENTS**

We would like to express our thanks to the Ethiopian Ministry of Education for funding the research and Jimma University College of Agriculture and Veterinary Medicine, Ethiopia for providing the required facilities of Plant Tissue culture laboratory.

**REFERENCES**

Leaf Culture of Tomato

5

Physiol. Bd.

Leaf Culture of Tomato (L. esculentum Mill.) Plant Environ. Dev. 6(1):31-38.


Datta, A., 2015. Transgenic tomato (S. lycopersicum Mill.) regeneration by comparing different transformation techniques. 135p. A Dissertation Submitted to BRAC University in Partial Fulfillments of the Requirements for the Master of Science in Biotechnology, BRAC University, Bangladesh.


Himabindu, K.B., 2008. Standardization of Agrobacterium mediated transformation protocol in tomato (Solanum Lycopersicon l. Cv. Pkm-1). (Doctoral Dissertation, Acharya Ng Ranga Agricultural University, Rajendrana- gar, Hyderabad.).


Sedaghat S. and Rahemi, M., 2012. Root Regeneration in


