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Micropropagation of Selected Hybrid Tomato (*Solanum lycopersicum* L.) Varieties Using Shoot Tip Culture

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Authors' contributions

This work was carried out in collaboration among all authors. Author KSA helped in project conceptualization, investigation, project execution and wrote the manuscript. Authors TMM and DBY helped in project conceptualization, supervision, guidance and resources provision. Author BMG did project supervision and resources provision. Author JB analyzed and wrote the manuscript, did data compilation, provided technical support and communications. All authors read and approved the final manuscript.

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world. The use of hybrid varieties is a great option to increase its production but using F2 seed leads to lack of uniformity as a result of segregation. Therefore mass propagation using tissue culture could help to solve these problems. The objective of this study was to develop an optimum micropropagation protocol for Valouro, Uwezo and Shelter hybrid tomato varieties using shoot tip culture. Three successive experiments: shoot initiation; shoot multiplication and root inductions were conducted. Different concentrations of BAP (0.0, 0.25, 0.5, 0.75, 1.0 and 1.25 mg/l) and (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3 mg/l) were used for shoot initiation and shoot multiplication, respectively. While different concentrations of IBA (0.0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/l) were used for root induction. The three separate experiments were laid out as factorial arranged in completely randomized design. The result of shoot initiation showed that the interaction of BAP*Varieties highly significant ($P<0.01$) effect. In the multiplication experiment, all parameters showed highly significant difference ($P<0.01$). In rooting experiment the interaction effect of IBA*Variety showed highly significant difference for the studied parameters. In conclusion MS medium without growth regulators was the optimum for shoot initiation. For shoot multiplication experiment 2mg/l, 0.5mg/l and 1.5mg/l of BAP was the optimum concentration for number of shoots per explant, shoot length, and leaf number respectively. For in vitro rooting MS medium containing 0.5 mg/l IBA was optimum for all varieties.

Keywords: BAP; IBA; micropropagation; tomato.

1. INTRODUCTION

Growing all over the world, tomatoes (*Solanum lycopersicum* L.) are one of the most significant vegetable crops [1]. Based on Mamidala and Nanna's 2011 research, it is regarded as the second most popular and highly nutritious vegetable crop, right behind potatoes. It has 24 chromosomes and is diploid (2n=2x=24) [2].

Tomato originated in the western coastal plain of South America in the area extending from Ecuador to Chile (Jenkins 1948). 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 million ha, 1.27 million ha and 5,235.19 ha with an average yield of 37.09, 15.59 and 5.31 t ha^{-1} respectively FAO STAT [3] CSA. (2018).

The crop provides important nutrients like lycopene, betacarotene, flavonoids and vitamins [4]. Medicinally, it is used as mild aperients, blood purifier, cholagogues, digestives and production of oral vaccines [5]. A group of biochemical in red tomatoes is found to have an antioxidant property which reduces several cancers and heart diseases (Giovannucci, 1999). Tomato is a model crop for functional genomics, proteomics and metabolomics to improve abiotic and biotic stress tolerance [6].

According to Gemechis and Emana [7] smallholder farmers, commercial state farms, and private farms cultivate tomatoes as one of the most significant and extensively farmed vegetable crops in Ethiopia, both throughout the rainy and dry seasons. According to Gemechis et al. [7] the average yield of tomato in Ethiopia is low, ranging from 6.5-24 metric ton/ha. The lack of adaptive hybrid varieties, low-quality seeds, disease and insect pests, significant post-harvest loss, and inadequate marketing mechanisms are the main obstacles to tomato production in Ethiopia [8].

Although using hybrid varieties is an excellent way to boost tomato yields, there are biotic and abiotic factors that lead to a loss in tomato yields as well as a decrease in the recovery of hybrid seeds from the field. The primary technique in tomato breeding, according to Cheema and Dhaliwal [9] is manual emasculation and hand pollination to create F1 seeds, which are hybrid tomato seeds. This process takes a long time and requires a lot of specialized labour. As a result of segregation, utilizing F2 seed results in a lack of genetically true to type [10].

Furthermore, as irrigation infrastructure has expanded in Ethiopia in recent years, there has been a rise in demand for vegetable seeds in that country. However, hybrid seeds are imported from other nations and exchanged and grown in Ethiopia [11]. The cost of hybrid seeds has increased due to all of the above listed issues; according to Bhatia [12] it can reach as high as $104/g. As a result, tissue culture propagation

may offer the greatest substitute for more conventional tomato propagation techniques.

One of the key biotechnology techniques is plant tissue culture, which enables the rapid generation of disease-free planting materials and a huge number of genetically identical plants on a big scale [13]. Numerous biotechnology techniques have been developed to improve tomato crops over the past 20 years [14]. Tomatoes have been used in several biotechnological applications of in vitro culture. Applications included genetic transformation [15] pest and disease tolerance [16] and increased production. Tissue culture is a better method of producing plants than traditional propagation techniques since it produces plants free of viruses and diseases.

Plants under in vitro conditions may exhibit significant variation in terms of shoot regeneration, shoot proliferation, and root induction despite the numerous studies on tomato micropropagation using shoot tip culture. These variables include genotype, explant type, composition of the culture media, presence of plant growth regulators (PGRs), and culture conditions Bhatia [17].

A micropropagation technique should be in place for each and every one of the more than 3000 tomato species that exist worldwide. Therefore, it is challenging to design a single, universal procedure for the in vitro growth of all tomato varieties due to the variations in each variety's morphogenic response [4]. Consequently, the goal of this research was to close this gap by creating the best possible methodology for the use of shoot tip culture in the micropropagation of particular hybrid tomato varieties.

General objective of the work was to develop of an optimum protocol for micropropagation of selected hybrid tomato varieties using shoot tip culture.

Specific objectives were to establish the optimal BAP concentration for shoot initiation, shoot multiplication and to determine the optimum concentration of IBA for root induction.

2. MATERIALS AND METHODS

2.1 Plant Materials

Three hybrid tomato varieties named Valouro, Uwezo and Shelter were developed by Rijk Zwaan and used as experimental materials. Valouro is a fresh market type, with indeterminate growth habit, which is short compact and has a strong stem, round fruit shape and fruit weight of 180-200g. It is an early maturing with a period of 90 days. While Uwezo is both processing and fresh market type, having a strong stem with indeterminate growth habit, an oval fruit shape and fruit weight up to 120g. Its maturity period is 70-80 days. Shelter is both fresh market and processing type with determinate growth habit and fruit weight of 90 g and its maturity period is 96 days (MoANR, 2016).

2.2 Seed Sterilization and Explant Preparation

To get rid of any dust, the seeds were rinsed under running water for fifteen minutes. Following washing, the seeds were submerged in 70% ethanol for three minutes. They were then rinsed three times in a laminar air flow hood with sterilized distilled water for five minutes each. Following that, seeds were infected for 20 minutes using 20% commercial sofi bleach (1% active chlorine) [18]. After that, distilled water that had been sterilized was used three times to rinse the cleaned seeds, each time for five minutes. After all, the seeds were put into sterile Petri dishes for germination, with the interior surface covered in cotton soaked in sterile water. In a Petri plate, the seeds were cultivated until two fully formed leaves emerged [19].

Finally, shoot tips of 2 cm length were excised and used as an explant.

2.3 Media Preparation

The growing conditions were treated with different plant growth regulators (Murashige and Skoog, 1962). Stock solutions were made by extracting x50/1000 macronutrients, x100/1000 micronutrients, x50/500 iron EDTA, x100/500 vitamins, and x50/500 calcium chloride. To keep out light, aluminium foil was placed over the iron EDTA (ethylene diamine tetra acetic acid) stock solution. Weighing the powder in a ratio of one milligram to one millilitre produced the growth regulators [20].

Plant growth regulators, auxin (IBA) and cytokinin (BAP) was dissolved using a drop of ethanol and NaOH respectively. After that, distilled water was added to get the total volume

down to 100 ml. finally, the solution was kept for later use at 4^{o}C in a refrigerator.

From the MS stock solutions, 20 ml/l of macro, 5 ml/l of micro, 10 ml/l of iron EDTA, 5 ml/l of vitamins, and 10 ml/l of calcium chloride were used to make the culture medium. The fluid was then supplemented with 3% sucrose as an energy source. Before adding agar, the pH of the medium was brought to 5.8 with NaOH or HCl. Then, 0.7% (w/v) agar was added to solidify the medium. Growth regulators were added in accordance with the necessary concentration. Subsequently, 50 millilitres of medium were transferred into sterilized and cleaned culture jars, sealed, and appropriately labelled. After that, the medium was steam sterilized for 15 minutes at 121°C and 105 KPa of pressure in an autoclave room [21,22].

2.4 Culture Conditions

The cultures were maintained in controlled growth room set at average of 25±2^{0}C temperature, 60 to 70% of relative humidity (RH) and 200lux of light intensity under 16h light and 8h dark period.

2.5 Treatments and Experimental Design

Experiment 1: Optimizing different concentrations of BAP for shoot initiation

In this experiment, shoot tip explants of two centimeter length, which were excised from the *In vitro* grown seedlings, were cultured on MS medium supplemented with different concentrations of BAP (0.0, 0.25, 0.5, 0.75, 1.0 and 1.25 mg/l) for shoot initiation. The experiment was laid out using Completely Randomized Design (CRD) in factorial combination (six level of BAP* three varieties) with five replications. Five explants per jar were cultured in each replication. Days to shoot initiation and percentage of initiation were recorded. Days of shoot initiation was recorded as average number of days required to the seedling to develop new shoot while percentage of initiation was the number of plants showing initiation divided by the total cultured plants after fifteen days. A total of 25 plants per treatment were used and data was collected from all plants.

Experiment 2: Optimizing different concentration of BAP for shoot multiplication

To eliminate growth hormone carryover effects during the shoot multiplication experiment, the initiated shoots were removed from the culture medium and cultivated on hormone-free MS media. Shoots were then grown on fresh MS medium containing BAP at concentrations of 0.0, 0.5, 1, 1.5, 2.0, 2.5, and 3.0 mg/l to promote shoot growth. Four explants were cultivated per jar and each replication. The experiment was designed using CRD in factorial combination (seven levels of BAP* three varieties) with four replications. Sub-culturing was performed twice, at fifteen days intervals. A total of 16 plants per treatment were used, and data were obtained from each one. The number of shoots/explant, leaves/shoot, and average shoot length were all recorded.

Experiment 3: Optimizing different concentrations of IBA for in vitro rooting

To eliminate carryover effects, well-developed shoots were removed from the culture tubes and cultured on hormone-free MS media for one week. The shoots were then moved to half strength MS media supplemented with IBA at concentrations of 0.0, 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 mg/l to induce root growth. Each treatment involved the cultivation of four explants per jar. The experiment was designed using CRD in factorial combinations (seven levels of IBA* three tomato varieties) and three replications. A total of 12 plants per treatment were used, and data were obtained from each plant. This experiment collected data on days to rooting, rooting percentage, number of roots, and root length.

2.6 Acclimatization

53 in vitro rooted plantlets from each cultivar were used for acclimatization. Well-regenerated plantlets, including shoots, roots, and leaves, were gently removed from the culture jars, and the roots were rinsed in sterile water to remove any remnants of agar. The plants were then transplanted into plastic pots containing various ratios of oven sterilized top soil, sand, and compost, such as 1:2:1, 2:1:1, and 2:1:0. The pots were then covered with white plastic with a small hole and housed under a lathhouse for fifteen days. The plastic covers were then removed and the plants were brought to a greenhouse, where they were watered every morning until completely developed. After 30 days, data on survival rate (%) was collected.

2.7 Data Analysis

The data was analysed using analysis of variance (ANOVA) and the least significant

difference test (LSD) at the P≤0.01 probability level, using SAS software (version 9.3).

The ANOVA model for the analyses using CRD was as follows:

In CRD $Yijk = \mu + Ti + YK + (TY)\ jk + Eijk$

Where

μ = over all mean effect
Ti = the effect of treatments at the i^{th} level
YK= the effect of treatments at the k^{th} level
(TY) jk = effect of treatment combinations at the j^{th} and k^{th} levels and
Eijk = a random error compared for whole factors

3. RESULTS AND DISCUSSION

3.1 Effect of BAP on Shoot Initiation

The analysis of variance (ANOVA) revealed that the interaction effects of variety and BAP were highly significant for days till shoot initiation but not for percentage of initiation. Different BAP concentrations significantly altered the proportion of start (p≤0.01) (Appendix Table 1).

The earliest shoot initiation occurred in 5 days for the Valouro variety, 6.4 days for the Uwezo variety, and 5.2 days for the Shelter variety, all grown on MS medium without growth regulators. Shoot initiation occurred late (12.2 days for Valouro, 13.6 days for Uwezo, and 15 days for Shelter variety) in MS medium supplemented with 1.25 mg/l BAP. Days of shoot initiation increased from 5 to 15 days as BAP concentrations rose from 0.0 to 1.25 mg/l (Table 1).

In this investigation, the hormone-free MS medium yielded the greatest shoot initiation rate (92%). The MS medium supplemented with 1.25 mg/l BAP showed the lowest proportion of initiation (60%) (Fig. 2). As a result, hormone-free MS medium was found to be the most effective for shoot initiation. In this investigation, the percentage of shoot initiation reduced as the BAP concentration increased. This could be attributed to the production of calluses at a greater level of BAP. This is primarily because at greater BAP levels, explants developed excessive callus and failed to improve the efficiency of shoot multiplication.

As a result, hormone-free medium is the best option for achieving the highest percentage of shoot initiation while also reducing the expense of PGRs. In this investigation, the percentage of shoot initiation reduced as the concentration of BAP increased. The efficacy of tomato regeneration response has been determined to be primarily dependent on genotype, explants, and the plant growth regulator utilized in the culture media.

3.2 Effect of BAP on Shoot Multiplication

The analysis of variance (ANOVA) for shoot multiplication revealed a very significant interaction between Varieties and BAP (P≤0.01) on number of shoots/explant, number of leaves/shot, and shoot length (Appendix Table 2).

On MS medium with 2 mg/l BAP, the Valouro and Uwezo varieties produced the most shoots per explant (5±0.08 and 4.3±0.08, respectively). Shelter variety produced the highest number of shoots per explant (4±0.07) on MS medium with 1.5 mg/l BAP (Table 2). MS medium supplemented with 2 mg/l BAP was optimal for the Valouro and Uwezo kinds, whereas 1.5 mg/l BAP was ideal for the Shelter variety. The Valouro and Uwezo cultivars produced varied shoot numbers at the same BAP concentration. This could be attributed to genetic heterogeneity in genotypes for in vitro response.

Tomato regeneration response to plant growth regulators (PGRs) has proven very genotype specific, so the type and concentration that works best for one genotype may not work for another. In this experiment, the number of shoots grew with increasing BAP concentration in all kinds until the optimum was reached, but then declined after 2.5 mg/l BAP. This could be owing to greater levels of cytokinin, which lower apical dominance, encourage shoot development, and release lateral buds. Furthermore, the toxic effect of growth regulators caused by accumulation has an impact on plant growth performance. During the current experiment, explants grown at greater BAP concentrations (after 2.5 mg/l) created excessive callus and failed to improve shoot multiplication efficiency.

MS medium supplemented with 0.5mg/l BAP resulted in maximum shoot length (5.8±0.08 cm, 5.3±0.11 cm, and 5±0.36 cm), while MS medium supplemented with 3 mg/l BAP resulted in minimum shoot length (2.69 ±0.1 cm, 2.81 ±0.17 cm, and 2.29±0.08 cm) in Valouro, Uwezo, and Shelter varieties. Lower BAP concentrations

result in longer shoot lengths. This is because the use of BAP increases apical dominance while decreasing shoot length. In the current experiment, increasing the level of BAP above 0.5 mg/l resulted in a decrease in shoot length.

Table 1. Effect of different concentration of BAP on shoot initiation

	Days to shoot initiation (Mean ± SD)		
BAP (mg/l)	**Valouro**	**Uwezo**	**Shelter**
0.0	5.00 ± 1.22^{l}	6.40 ± 1.43^{kj}	5.20 ± 0.46^{l}
0.25	7.00 ± 0.50^{ij}	7.20 ± 0.44^{ih}	6.00 ± 0.25^{k}
0.5	7.80 ± 0.83^{gh}	7.80 ± 0.57^{gh}	8.20 ± 0.27^{gf}
0.75	8.80 ± 0.27^{f}	9.60 ± 0.22^{e}	9.90 ± 0.54^{e}
1.0	11.00 ± 0.35^{d}	10.00 ± 0.35^{e}	12.20 ± 0.44^{c}
1.25	12.20 ± 0.27^{c}	13.60 ± 0.41^{b}	15.00 ± 0.70^{a}
LSD	0.78	0.78	0.78
CV%	6.80	6.80	6.80

Note; the values assigned by the same letter are not significantly different (p ≤ 0.01)

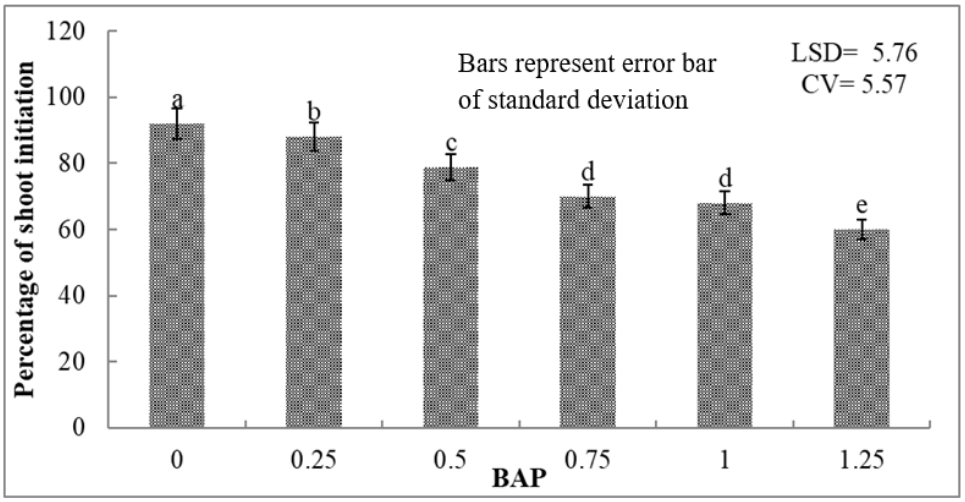

Fig. 1. Effect of different concentrations of BAP on percentage of shoot initiation
Note: Values assigned by the same letters are not significantly different (p ≤ 0.01)

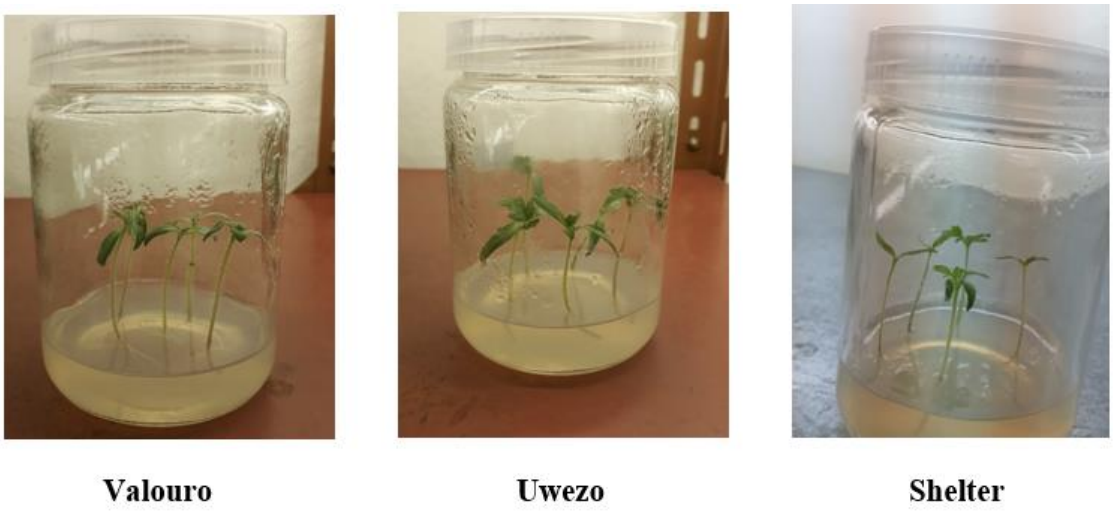

Fig. 2. Shoot initiation in the three tomato varieties on hormone free MS medium

Table 2. Effect of different concentration of BAP on shoot multiplication of tomato varieties

Variety	BAP (mg/l)	NS (mean ±SD)	NL (mean ± SD)	SL(cm) (mean ± SD)
Valouro	0.0	1.30±0.08^{l}	2.00±0.16^{kj}	3.64±0.04^{l}
	0.5	2.75±0.12^{g}	2.60±0.08^{h}	5.80±0.08^{a}
	1.0	3.25±0.05^{e}	2.85±0.12^{g}	5.55±0.12^{b}
	1.5	3.67±0.06^{d}	4.00±0.2^{a}	5.10±0.11^{dc}
	2.0	5.00±0.14^{a}	3.02±0.05^{ef}	4.80±0.08^{fe}
	2.5	3.00±0.16^{f}	2.50±0.07^{ih}	4.05±0.10^{k}
	3.0	1.87±0.14^{k}	1.8±0.05^{l}	2.69±0.10^{o}
Uwezo	0.0	0.80±0.08^{m}	2.00±0.16^{kj}	3.30±0.04^{m}
	0.5	2.43±0.12^{ih}	2.86±0.008^{g}	5.30±0.11^{c}
	1.0	2.87±0.14^{gf}	3.15±0.12^{ed}	4.68±0.16^{fg}
	1.5	3.50±0.08^{d}	3.60±0.04^{b}	4.42±0.30^{hi}
	2.0	4.30±0.13^{b}	3.20±0.05^{d}	4.10±0.31^{jk}
	2.5	2.56±0.12^{h}	2.50±0.04^{ih}	3.67±0.07^{l}
	3.o	1.81±0.08^{k}	1.91±0.11^{kl}	2.81±0.17^{o}
Shelter	0.0	0.50±0.20^{n}	2.06±0.10^{j}	3.04±0.04^{n}
	0.5	2.25±0.12^{j}	2.09±0.06^{j}	5.00±0.36^{de}
	1.0	2.31±0.23^{ij}	2.57±0.09^{ih}	4.57±0.05^{hg}
	1.5	4.00±0.07^{c}	3.40±0.08^{c}	4.31±0.07^{ji}
	2.0	2.93±0.12^{f}	2.90±0.12^{gf}	4.00±0.16^{k}
	2.5	1.72±0.09^{k}	2.45±0.12^{i}	3.50±0.07^{ml}
	3.0	0.93±0.05^{m}	1.45±0.04^{m}	2.29±0.08^{p}
LSD		0.22	0.14	0.18
CV%		4.94	4.06	3.79

Note; means with the same letter in the same column are not significantly different, where NS= number of shoots, NL = number of leaves and SL= shoot length.

Fig. 3. *In vitro* shoot multiplication of Valouro, Uwezo and shelter tomato varieties after four weeks respectively

MS medium supplemented with 1.5 mg/l BAP resulted in the highest number of leaves/shoot (4±0.2 from Valouro, 3.6±0.04 from Uwezo, and 3.4±0.08 from Shelter variety), while MS medium supplemented with 3 mg/l BAP resulted in the lowest number of leaves/shoot (1.8±0.05, 1.91±0.11, and 1.45±0.04, respectively). This is because using BAP at the optimal level promotes cell division and increases the number of leaves.

3.3 Effect of IBA on *In Vitro* Rooting

The ANOVA demonstrated that the interaction between variety and IBA significantly ($P<0.01$) influenced days to rooting, number of

roots/plantlet, and root length. The percentage of roots was significantly different (P≤0.05) (Appendix Table 3).

Early root induction was measured on MS medium without hormones and was 5 days for Valouro and Shelter cultivars and 7.67 days for Uwezo (Table 3). Late root induction (12.67 days for Valouro, 16 days for Uwezo, and 11 days for Shelter) was observed on half strength MS medium supplemented with 1.5 mg/l IBA. Early root induction was found in the control treatment, however with greater concentrations of IBA, roots were developed later. This could be owing to the negative effect of auxins at higher concentrations on root development. The influence of ethylene on root growth is primarily mediated via the regulation of auxin production and transport-dependent local auxin distribution. Ethylene promotes auxin production and basipetal auxin transport toward the elongation zone, activating a local auxin response that inhibits cell elongation. Ethylene reduces primary root growth by controlling cell proliferation and elongation in the elongation zone.

On MS medium with 0.5 mg/l IBA, the Valouro, Uwezo, and Shelter varieties produced the most roots (15.36±0.35, 13.06±0.83, and 18.26±0.3, respectively). Valouro, Uwezo, and Shelter types had the fewest roots (3.3±0.2, 2.03±0.15, and 4.33±0.35) when grown in half strength MS medium with 1.5 mg/l IBA. The number of roots dropped as the concentration of IBA exceeded 0.5 mg/l. This could be attributed to an increase in the formation of the growth retardant chemical ethylene at high concentrations of auxins such as IBA.

Half strength MS medium containing 0.5 mg/l IBA resulted in maximum root lengths of 5.92 ±0.22 cm for Valouro, 5.5 ± 0.2 cm for Uwezo, and 6.55 ± 0.01 cm for Shelter variety. IBA doses of 0.0-0.5 mg/l resulted in the maximum rooting percentage (100.00±0.00) across all three kinds (Table 2). Therefore, utilizing IBA-free MS medium may be superior for minimizing the cost of PGRs. The Valouro, Uwezo, and Shelter cultivars had the lowest rooting percentages (56.67±3.51, 46.67±2.08, and 60.00±2) when grown on half strength MS medium with 1.5 mg/l IBA.

In the present finding, the minimum percentage of rooting was obtained at higher concentrations of IBA. This variation may be due to the different genotypes requiring different concentrations of auxin and their responses are dependent on the amount of their endogenous auxin concentration.

3.4 Acclimatization

The survival percentages of fifty-three acclimatized plantlets were 81.13%, 73.58%, and 69.8% for Valouro, Uwezo, and Shelter types at a 1:2:1 ratio, respectively (Fig. 5). The survival rate derived from a 2:1:1 ratio was 67.92%, 58.49%, and 62.26 for the Valouro Uwezo and Shelter types, respectively. Some plantlets did not survive after being moved to the lath house. This could be due to a shift in the environmental conditions, which may have influenced the plant's growth performance.

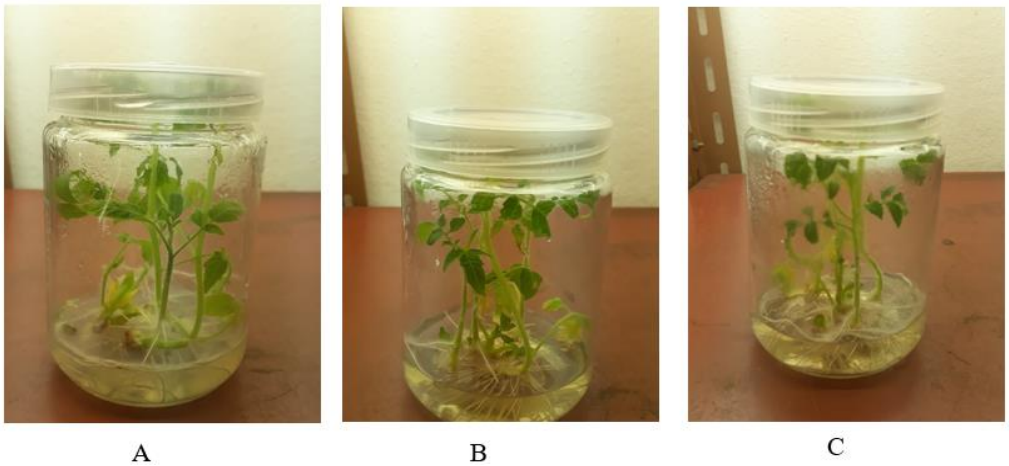

Fig. 4. *In vitro* rooting of the three tomato varieties

Table 3. Effect of different concentration of IBA on in vitro rooting of three tomato varieties

Variety	IBA (mg/l)	DR (Means± SD)	NR (Means± SD)	RL (cm) (Mean ±SD)	R% (mean ±SD)
Valouro	0.0	5.00±1.00^{m}	9.10±0.36^{gh}	3.58± 0.03^{ji}	100.00±0.0^{a}
	0.25	7.00±1.00^{kl}	12.86±0.15^{d}	3.86± 0.05^{hg}	100.00±0.0^{a}
	0.5	8.33±0.57^{hij}	15.36±0.35^{b}	5.92± 0.22^{b}	100.00±0.0^{a}
	0.75	9.66±0.57^{egf}	9.67±0.30^{gf}	4.30± 0.20^{f}	93.3±2.51^{b}
	1.0	10.33±0.57^{ed}	7.46±0.15^{i}	3.47± 0.01^{jk}	86.67±4.16^{c}
	1.25	11.00±1.00^{d}	5.00±0.20^{k}	2.70±0.08^{n}	76.67±3.21^{e}
	1.5	12.67±0.57^{c}	3.30±0.20^{m}	1.82±0.01^{q}	56.67±3.51^{g}
Uwezo	0.0	7.67±0.57^{kji}	6.00±0.20^{j}	2.53±0.02^{o}	100.00±0.0^{a}
	0.25	8.67±0.57^{hgi}	10.00±1.00^{f}	3.32±0.03^{lk}	100.00±0.0^{a}
	0.5	9.67±0.57^{egf}	13.06±0.83^{d}	5.50±0.20^{c}	100.00±0.0^{a}
	0.75	10.00±0.72^{edf}	8.83±0.20^{h}	3.73±0.05^{hi}	93.3 ±4.04^{b}
	1.0	11.00±1.00^{d}	6.50±0.10^{j}	3.20±0.08^{l}	85.3±3.51^{cd}
	1.25	14.33±0.57^{b}	3.16±0.05^{m}	2.13±0.11^{p}	67.67±11.67^{f}
	1.5	16.00±1.00^{a}	2.03±0.15^{n}	1.50±0.08^{r}	46.67±2.08^{h}
Shelter	0.0	5.00±1.00^{m}	12.03±0.35^{e}	4.40±0.08^{f}	100.00±0.0^{a}
	0.25	6.00±1.00^{ml}	14.5±0.43^{c}	5.20±0.10^{d}	100.00±0.0^{a}
	0.5	7.33±0.57^{kj}	18.26±0.30^{a}	6.55±0.01^{a}	100.00±0.0^{a}
	0.75	8.00±0.50^{hkji}	13.49±0.49^{d}	4.80±0.10^{e}	93.3±3.21^{b}
	1.0	9.00±0.43^{hgf}	10.10±0.10^{f}	3.93±0.02^{g}	88.67±4.5^{cb}
	1.25	10.00±0.87^{edf}	7.50±0.10^{i}	3.41±0.02^{jk}	80.0±4.58^{ed}
	1.5	11.00±0.20^{d}	4.33±0.35^{l}	2.98±0.02^{m}	60.0±2.0^{g}
LSD		1.23	0.63	0.16	5.92
CV%		8.07	4.06	2.69	4.19

Note: means with the same letter in the same column are not significantly different, where DR= days to rooting, NR= number of root, RL= root length and R% rooting percentage.

Table 1. Survival percentage of acclimatized plantlets on different soil composition

Varieties	1:2:1	2:1:1	2:1:0
Valouro	81.13%	67.92%	54.71%
Uwezo	73:58%	58.49%	49.05%
Shelter	69.8%	62.26%	56.6%

Fig. 5. Acclimatized plantlets of three tomato varieties

4. CONCLUSIONS

This paper presents an essential in vitro micropropagation strategy for three hybrid tomato varieties. For the Valouro and Owezo types, the MS medium boosted with 2 mg/l BAP produced the most shoots (5 and 4.3, respectively). While shelter cultivars produced the greatest number of shoots (4), they were regenerated on MS medium enriched with 1.5mg/l BAP.

In conclusion, MS medium without growth regulators was optimum for shoot initiation. For For the Valouro and Uwezo cultivars, MS medium supplemented with 2 mg/l BAP was optimal for shoot multiplication. For the Shelter variety, MS medium supplemented with 1.5 mg/l BAP was ideal for shoot multiplication. For in vitro rooting, half strength MS medium with 0.5 mg/l IBA was shown to be the best for all three varieties.

DISCLAIMER

This manuscript is taken from thesis 'Micropropagation of Selected Hybrid Tomato (*Solanum lycopersicum* L.) Varieties Using Shoot Tip Culture' submitted to Jimma University as part of Masters course at the department of Horticulture and Plant Science. http://repository.ju.edu.et//handle/123456789/5709

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Appendix Table 1. ANOVA table for the shoot initiation

Source	DF	Days to Shoot initiation	Percentage of initiation
		MS	MS
BAP	5	131.691***	2293.377***
Variety	2	4.658***	2.411NS
BAP*Variety	10	3.605***	3.051NS
Error	68	0.379***	18.047***
CV (%)		6.80	5.57

*Note ***= highly significant at P ≤ 0.0001, P= Probabilities Value at P≤ 0.01, ns = non-significant, MS = Mean square, DF = Degree of freedom, CV = Coefficient of Variation.*

Appendix Table 2. ANOVA table for shoot multiplication

Source	DF	Number of shoot/explant	Number of leaf/shoot	shoot length
		MS	MS	MS
BAP	6	15.327***	5.055***	11.145***
Variety	2	5.536***	0.841***	3.594***
BAP*Variety	12	0.618***	0.133***	0.111***
Error	60	0.016***	0.011***	0.024***
CV (%)		4.94	4.06	3.79

*Note ***= highly significant at P ≤ 0.0001, P= Probabilities Value at P≤0.01, MS=Mean square, DF=Degree of freedom, CV=Coefficient of Variation*

Appendix Table 3. ANOVA for *in vitro* rooting

Source	DF	Days to Rooting	Number of root	Root length	Percentage of rooting
		MS	MS	MS	MS
IBA	6	58.174***	159.359***	13.898***	2634.513***
Variety	2	48.396***	101.157***	9.515***	94.968**
IBA*Variety	12	1.619**	1.534***	0.180***	30.005*
Error	40	0.577***	0.139***	0.010***	13.315***
CV (%)		8.07	4.06	2.69	4.19

*Note ***= highly significant at P ≤ 0.0001, ** =highly significant at p≤ 0.01, *= significant at p≤ 0.05 and NS = non-significant where, P= Probabilities Value at P ≤ 0.05, MS = Mean square, DF = Degree of freedom, CV = Coefficient of Variation.*

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