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Effect of 6-Benzyl Amino Purine on *In Vitro* Multiplication of Tomato (*Lycopersicon esculentum* Mill.) Varieties using Shoot Explant

Endalkachew Baye[1*], Temesgen Matewos[2] and Derbew Belew[2]

[1]Department of Plant Sciences, Salale University, P.O Box 245, Fitch, Ethiopia

[2]Department of Horticulture and Plant Sciences, Jimma University, P.O Box 138, Jimma, Ethiopia

[*]**Corresponding author:** Endalkachew Baye, Department of Plant Sciences, Salale University, P.O Box 245, Fitch, Ethiopia, E-mail: bayeendalkachew4@gmail.com

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Abstract

The production of tomato using improved varieties is necessary to increase its production. However, the improved varieties could be from selection or hybrid seeds. Distribution of hybrid seeds through conventional breeding is slow, time-consuming; need more space, labor-intensive and transmission of pathogens may also occur. Mass propagation using tissue culture may help to solve these problems. Hence, the present study was initiated to optimize an *in vitro* multiplication protocol for two Ethiopian tomato varieties using shoot tip culture. For shoot initiation BAP at 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L concentrations were. The initiated shoots were cultured in MS having BAP at a concentration of 0.0, 0.75, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L for multiplication. The experiments were laid out in CRD. The ANOVA showed that the interaction of BAP*Var was not significant for percentage and days of initiation at $p<0.01$. Both the earliest (5.6 days) and the highest percentage of initiation (98%) were obtained on free MS medium. While the minimum percentage of initiation (64%) and late initiation (12 days) was on 2.5 mg/L BAP. It also revealed that the interaction of BAP*Var on the number of shoots/explants was highly significant, but not for shoot length and number of leaves. Chali gave the highest number of shoots/explant (3.00 ± 0.00) at MS +2.5 mg/L. For Gelilema it was 2.78 ± 0.19 shoots/explant on MS +2 mg/L. The highest shoot length (5.32 cm) and a number of leaves (4.33) was recorded on MS +0.75 mg/L. whereas, the lowest shoot length (3.31 cm) and leaf numbers (2.48) was obtained on MS +3 mg/L. It can be concluded that the free MS medium was the best for shoot initiation. But, for shoot multiplication, MS +2 mg/L for Gelilema and MS +2.5 for Chali were preferred. The optimized protocol will be useful for the rapid *in vitro* multiplication of the two varieties. However, further studies are suggested for protocol improvement using other PGRs, combinations and explants.

Keywords: BAP; Chali; Gelilema; *in vitro* multiplication

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 [1]. It is a diploid with a 2n=2x=24 chromosome. It originated in the western coastal plain South America in the area extending from Ecuador to Chile. Mexico was the first domesticator of tomato [2]. Nowadays, it grows almost in every country of the world either in the field, greenhouses or net houses [3]. 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 [4]. In Ethiopia tomato has been cultivated for economic importances like the source of income, creating employment opportunities and access to farmers to participate in the market [5]. It also used as a sample for the study of genomics, proteomics and metabolomics [3].

Even if the 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 [6,7]. The use of a hybrid variety is a great option to increase yield for most of the vegetable crops including 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 [8]. 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 labor-intensive [9,10]. Additionally, seeds saved after F1 hybrids produce plants that are inferior and not uniform because seeds do not remain

genetically true to type [11]. Such the improvement of a plant through conventional breeding method is slow, time-consuming and need more space [12]. Plus, the vegetative propagation method that uses nodal and apical micro-cuttings from seedlings of tomato produces only one shoot/node [13]. Additionally, most of the vegetable seeds traded and grown in Ethiopia are imported from other countries [14]. Therefore, integrating hybrid seed production with plant tissue culture is important.

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 [15]. In tomato, it has been used for selection of cell lines for biotic and abiotic stresses [16], development of haploids [17], and production of somatic hybrids [18], mass propagation [19] and development of transgenic tomato [20].

Numerous studies from different tissues and organs of tomato using Murashige and Skoog (MS) medium supplemented with a variety of growth regulators along with different explants from different cultivars of tomato have been conducted. Several researchers have reported the induction of shoots from various explants and varieties of tomato like; stem and cotyledonary node explants from Tomato cultivars MHTM and Shalimar [21], hypocotyl and leaf disc explant from tomato Var. Moneymaker [22] and shoot apex, nodal and root segments from Moneymaker [23]. For direct shoot formation shoot tip was found to be the best explant while for shoot formation via callogenesis hypocotyl was preferred [24]. However, the choice of the right explant is dependent on the genotype [25,26]. Different Plant Growth Regulators (PGRs) have been used by different researchers for tomato *in vitro* culture [15]. Reported that BAP was a more suitable growth hormone for maximum shoot bud differentiation and multiple shoot induction compared to Kinetin (Kin). The author also reported that MS supplied with 2 mg/l 6-Benzyl Amino Purine (BAP) gave maximum values for growth parameters (shoot number and length). Datta [7] also reported that MS medium supplied with 2 mg/l BAP was the best medium for multiple shoot formation.

Although, *in vitro* culture of tomato was successfully performed for a variety of cultivars, studies that can extend the amenability of genotypes are needed. Additionally, the establishment of one universal protocol for *in vitro* growth of all the varieties is impossible [27]. Morphogenic responses of cultured tomato plant tissues are affected by the genotype and explant (size, age, and orientation) [28]. Thereby, the techniques established for globally known cultivars are not efficient for all genotypes. Thus, the optimization of an efficient *in vitro* propagation protocol for selected tomato varieties using tissue culture could help to solve those problems. Therefore, this research work was initiated with the following objectives:

- To determine the optimum concentration of BAP for shoot initiation
- To determine the optimum concentration of BAP for shoot multiplication

Materials and Methods

Plant materials

Two-hybrid tomato varieties named Chali and Gelilema were used as experimental material, which were 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. Chali is a processing type, with a determinate growth habit, which is short compact and has a strong stem, round fruit shape, fruit weight of 94 gm and all fruits occur at the same time. It is an early maturing with a period of 75 days and a potential yield of 49.2 ton/ha. While Gelilema is both processing and fresh market type, having a strong stem with determinate growth habit, an oval fruit shape and fruit weight of 90 gm. Its maturity period is 88 days. It has a potential yield of 50 ton/ha [29].

Media preparation

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 (1 mg: 1ml) were prepared and stored at 4^{o}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. The 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 50 ml media were dispensed into washed and sterilized culture jars, then plugged and labeled properly. Then the medium was steam sterilized using an autoclave chamber at a temperature of 121^{o}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

Treatments and experimental design

Effect of BAP on shoot initiation: In this experiment, shoot tip explants of 2 cm length, which were prepared from the *in vitro* grown seedlings, were cultured on MS medium supplemented with BAP having various concentrations (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) for shoot initiation. The experiment was laid out using Completely Randomized Design (CRD) in factorial combination (six-level of BAP*two varieties) with five replications. Five explants/jars were used. From this experiment date and percentage of initiation were recorded. Date of initiation was the average number of days in counting from culturing to the date that the plants/jar, took to initiate was recorded in daily follow up while the percentage of initiation was recorded after three weeks and its value is obtained by dividing the number of plants resulted in initiation

to the total number of plants cultured and multiplied by 100 [30].

Effect of BAP on shoot multiplication: Prior to shoot multiplication experiment, the initiated shoots were taken out from the culture medium and cultured on hormone-free MS medium for two weeks to avoid carry-over effects of growth hormones. Then shoot, explants of about 3 cm length were sub-cultured on fresh MS medium+BAP at 0.0, 0.75, 1.5, 2.0, 2.5, and 3.0 mg/l levels for the purpose of shoot multiplication. The experiment was laid out using CRD in factorial combination (six-level of BAP*two varieties) with three replications. Three shoots were used per jar. After 55 days, the number of shoots/explants, number of leaves and average shoot length were recorded [31].

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 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.

Data analysis: 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 $p<0.0$.

Results and Discussion

Effect of BAP on shoot initiation of two tomato varieties

The analysis of variance (ANOVA) showed that the interaction effects of variety and BAP were not significant for the percentage of initiation and days to shoot initiation. It also indicated that the effect of BAP concentrations was highly significantly different for both percentages of initiation and days to initiate at $p<0.01$.

The highest percentage of initiation (98%) was recorded from MS medium without BAP **(Table 1 and Figure 1)**. Whereas, the minimum percentage of initiation (62%) was recorded on MS medium +2.5 mg/l BAP. This may due to the involvement of endogenous factors in the regulation of plant growth. Similarly, Steward and Rao [32] found that growth regulation is the interaction of numerous factors and most of the factors are present in the tissue and invisible to experimenters. George and Sherrington [33] also reported that growth and morphogenesis *in vitro* are regulated by the interaction and balance between growth regulators augmented in the medium and the growth substances produced endogenously by cultured cell/tissues. The initiation percentage decreased with an increase in BAP. This may due to the formation of callus at higher level BAP. A similar result was reported by Soressi et al. [13], who found that the regeneration percentage of both shoot tip and cotyledonary explants of tomato cultivar Omdurman decreased and resulted in callus as the level of BAP increased above 1 mg/l. Bahurupe et al. [30] also reported that shoot tip explant that derived from tomato variety Dhanashree resulted in callus when cultured on MS medium supplied with 2 mg/l BAP mg/l and observed that callus induction was increased with increase in the concentration of BAP. Likewise, Mohamed et al. [15] found that cotyledon and hypocotyl explants of tomato cultivars (Pearl and Beril) cultured on MS +4 mg/l BAP resulted in callus and regeneration was not obtained. Ashakiran et al. [21] also reported the negative impact of increased supplementation of BAP on regeneration.

These results were contradictory with the result of Chaudhry et al. [22], who reported that there is no regeneration response in explants of leaf disc, cotyledonary and their respective callus derived from tomato cultivar Moneymaker when cultured on BAP free MS medium. This may due dependence of the degree of *in vitro* regeneration on the explant used. Bhatia [28] also found that the presence of cytokinin was essential for regeneration, as no regeneration occurred for either cotyledon or leaf and only limited response for hypocotyl explants derived from tomato cultivar Red coat in the absence of cytokinin. The researcher also reported that the regeneration response of tomato is dependent on both genotype and explant type. Another inconsistent result also reported by Otroshya et al. [34], who found that a higher *in vitro* regeneration percentage of cherry tomato cultivar was obtained from MS medium supplied with BAP and no response was observed in the control. The authors also reported that the regeneration response increased with the increase in BAP. Bahurupe et al. [30] also reported the opposite result to the present work who found that shoot initiation from callus originated from shoot tip explant of tomato variety Dhanashree takes up to 17 days when inoculated on MS medium fortified with lower BAP concentration.

Datta [7] also conducted a tissue culture experiment on five different cultivars of tomato (BARI 2, BARI 3, BINA 2, BINA 3 and Bahar) and recorded an initiation date of 15-18 and regeneration percentage of 96%-98% when cultured on 2 mg/l BAP. Himabindu [35] also suggested that MS medium supplied with BAP 1.5 and Kin 1.0 mg/l gave the best regeneration response in terms of days to initiation (12.3 days) over other treatments used. Consistently to ours, Chowdhury and Islam [36] reported that significant differences for regeneration percentages and time taken to regenerate were observed for different treatments. But the BAP concentration by which maximum values obtained for the above measurements (MS +2 mg/l BAP) was not consistent with ours. However, Jabeen et al. [37] reported that the regeneration capacity was strongly influenced by the explant type. The authors said that shoot tip was the best explant source for direct shoot formation by which 80% shoot primordial were regenerated while hypocotyl was the best explant source for shoot formation through callogenesis that resulted in 64.5% shoot primordial.

There was a significant difference in the percentage of initiation among the BAP concentrations (control, 0.5, 1.5 2.0

and 2.5 mg/l) **(Table 1)**. The percentage of initiation decreased when the concentration of BAP increased. Osman et al. [38] also conducted an experiment using shoot tip and cotyledonary node explants of Omdurman cultivar and got the same result. At a higher concentration of cytokinin, the regeneration percentage and shoot regeneration frequencies were decreased as reported by the authors. Plana et al. [39] also used hypocotyl explant of tomato cv. 90% of regeneration was recorded in the absence of PGRs. Therefore, the hormone-free medium could be taken as the best option when we want to get the maximum percentage of initiation. Mukta [6] also reported that the regeneration percentage of tomato cultivars (BARI 2, BARI 9 and Bahar) decreased from 96% to 19% as the concentration of BAP increased from 1 mg/l to 7 mg/l. Contrary to this, Abu-El-Heba et al. [40] suggested that the presence of the cytokinin BA and zeatin in the culture medium had a stimulating effect on cell differentiation in tomato hypocotyl explant. Nogueira et al. [41] also reported that high regeneration frequency (92%) on cotyledonary explants of the tomato genotype Santa Clara were obtained on MS medium supplemented with cytokinin. In shoot tip explant derived from tomato variety Pusa Ruby, Kiran [20] obtained a contradictory result to ours. Maximum values (100%) initiation was obtained on MS medium fortified with a higher level of BAP (1, 1.5 and 2 mg/l) and lower percentage (80%) on the control as reported by the author. In another research, Rashid and Bal [42] observed that the optimal medium for plant regeneration was MS supplemented with 0.5 mg/l BAP in two tomato genotypes.

Early initiation (5.6 days) was recorded on free MS medium while the late day for initiation (12 days) was from MS medium supplemented with 2.5 mg/l BAP **(Table 1)**. In conformity to our result, Shah et al. [43] reported that higher regeneration frequency was recorded on free MS/N6 media fortified with only carbon sources (sucrose and sorbitol) from three tomato cultivars. There is a significant difference in days to initiation among BAP concentrations of 0.0, 0.5, 1.5 and 2.5 mg/l. This may due to the dependence of the frequency of regeneration on the concentrations and growth regulators used. Similarly, Plastira and Perdikaris [44] found that direct regeneration of tomato varies with concentrations and combinations of hormones, light genotype and explants used. Liza et al. [45] also reported that the morphogenesis response is highly dependent on plant growth regulators used in the media. The days of shoot initiation increased from 5.6 to 12 with an increase in the BAP concentrations from 0.0 to 2.5 mg/l. a similar result was reported by Mukta [6] who reported that days of shoot initiation of cotyledonary leaf explants derived from tomato cultivars of BARI 9 and Bahar increased as the BAP concentration increased.

Table 1: Effects of BAP on shoot initiation.

BAP mg/l	Mean of DI	Mean of IN%
0.0	5.60^{d}	98.00^{a}
0.5	8.80^{c}	82.00^{b}
1.0	9.20^{c}	78.00^{cb}
1.5	9.90^{b}	74.00^{cd}
2.0	10.40^{b}	68.00^{ed}
2.5	12.00^{a}	62.00^{e}
CV	6.71	10.81
LSD	0.56	7.16

Note: Means superscripted with different letters within the same column are significantly different at $p \leq 0.01$. Where: DI=Days to Initiation;IN%= percentage of shoot initiation. The letters a,b,c, indicate significant difference between two means.
Meaning mean values assigned with similar letters are not significantly different while means assigned with different letters are significantly different each other.

Effect of BAP on shoot multiplication of two tomato varieties

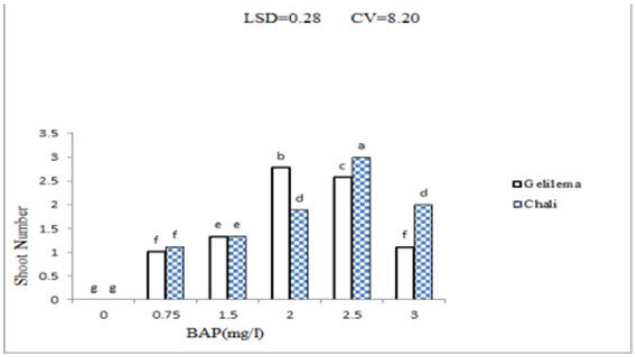
The ANOVA for shoot multiplication revealed that the interaction effect of variety and BAP was highly significant for the number of shoots/explant but not significant for shoot length and the number of leaves ($p<0.01$; Appendix 3). The effect of BAP on shoot multiplication showed a highly significant difference in all the parameters recorded. Varieties show a highly significantly different for shoot length in cm and number of leaves/shoots. In MS media free of BAP any extra shoots were not developed except the primary shoot. The highest number of shoots/explant (3.00 ± 0.00) was recorded from the Chali variety on MS supplemented with 2.5 mg/l BAP **(Figure 2)**. Whereas, Galilema's maximum was (2.78 ± 0.19) shoots/explant on MS +2 mg/l BAP. The Lowest numbers of shoots per explant (0.00 ± 0.00) for both varieties were obtained from MS medium without BAP. This might be due to the difference in genotype. A similar result also reported by Venkatachalam et al. [46] who found that genotype affects both the frequency of shoot organogenesis and shoot bud multiplication of cultured organs. Likewise, Hattab et al. [47] used shoot tip explant derived from two tomato cultivar super Regina and flacon and obtained a significantly different shoot number/explant in the two cultivars. The authors reported 2.7 and 2.08 shoots/explant from super Regina and flacon respectively. Mohamed et al. [15] reported a similar result to ours.

Figure 1: Effect of BAP on shoot initiation of tomato varieties (Gelilema and Chali).

The difference in the formation of shoots of two tomato cultivars (Pearl and Beril) cultured on MS medium supplied with BAP was reported by the authors. Medium augmented with 2 mg/l was the best in the number of shots and shoot length in both cultivars and explants. In another work, Jamous and Abu-qaoud [48] used two tomato cultivars Baladi and 593 and cultured on MS medium supplemented with BAP and NAA and found the different number of shoots/explant. From Baladi cultivar cultured on BAP alone the authors recorded 7.6 shoots/explants while 593 cultured on the same media gave 5.8 shoots/explant. A similar result also reported by Cruz-Mendivil et al. [49]. The researchers reported that Micro-Tom cultivar on 2 mg/l BAP resulted in 2.8 shoots per explant. The result obtained from Gelilema variety is in agreement with Singh et al. [50] who reported that the number of shoots/explant was enhanced with the addition of 2.0 mg/l BAP. Mukta [6] also reported different shoot numbers/explants from cotyledonary leaf explants derived from three tomato cultivars which decrease as the concentration of BAP increased.

In another experiment, Kiran [20] used a shoot tip explant of tomato Var. Pusa Ruby and found that MS+BAP (0.1-5.0 mg/l) resulted in shoot formation with different degrees of response depending on the concentration used. The author recorded the highest number of multiple shoots 5.6 and 5.2 shoots/explant on MS +1.5 mg/l and 1.0 mg/lBAP respectively. However, the number of shoots/explant reported by the author decreased with the increase in the BAP concentration in the medium (BAP 2 mg/l with 3.15 shoots/explant). The number of shoots/explants increases as the level of BAP increased in both varieties used until it reached its optimum **(Figure 2)**. This may due to the fact that cytokinin at an elevated level reduced apical dominance and ectopic shoot formation and released lateral buds [51,52]. In another finding, Hussey [53] found that BAP induces shoot multiplication by means of suppressing apical dominance which can be used in mass plant propagation by *in vitro* techniques. In line with the present result, Bahurupe et al. [30] found that the numbers of shoots/explant were increased with the increase in the concentration of BAP until it reached its optimum. The researchers also compared shoot tip and hypocotyl explant and concluded that shoot tip explant was superior in terms of days to callus initiation, percent callus induction, days to shoot initiation, percent of shoot multiplication and the number of multiple shoots formed/explant. Likewise, Bhatia [28] reported that the shoot response of Red coat tomato cultivar increased, as BAP concentration increased, but no further change occurred at higher concentrations. The researcher also said that the optimal concentration of plant growth regulators for shoot induction varied with the type of explant and PGRs used. Mamidala and Nanna [54] reported another conformity result in the present work. The authors found that the numbers of shoots produced/explant were dependent on the concentrations, type of PGRs used and their combination and said that the number of shoots produced increased until the PGRs reached its optimum and then declined. From leaf explants derived from tomato cultivar Micro-MsK cultured on MS +2 mg/l BAP 2.2 shoots, MS +2 mg/l BAP +0.1 NAA 3.2 and MS +2 mg/l BAP +0.1 IAA 4.1 shoots were the values reported by the authors.

As the concentration of BAP surpassed its optimum the number of shoots per explant tends to decreased **(Figure 2)**. This may because at higher cytokinin level explants produced excessive callus and failed to improve the efficiency of shoot multiplication. Similar to our result, Sakthivel and Manigandan [55] found that the mean number of shoots decreased with an increase in the concentrations of BAP above its optimum. The author's said that a maximum number of shoots (11.11) was obtained from MS +1.5 mg/l. Jafari et al. [56] also found that higher concentrations of cytokinin have an adverse effect on the multiplication rate and morphology of the culture. The number of shoots produced/explant in tomato are dependent on the genotype, explant type and growth regulator used [30,57]. This may due to, the interaction of genotype and explant type for shoot regeneration rate and the number of shoots produced/explant. Mamidala and Nanna [57] used five tomato varieties (PKM-1, White Cherry, Money Maker, Micro-Tom and Micro-Msk) and cultured on MS medium augmented with different levels of BAP and reported that the varieties respond differently in growth hormones and explants used.



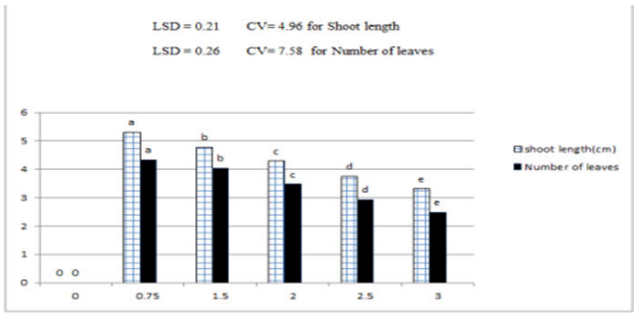
Note: Values assigned by the same letters are not significantly different at P≤0.01.

Figure 2: Effect of BAP on shoot multiplication of tomato varieties (Gelilema and chali).

The highest shoot length (5.31) was recorded on MS medium +0.75 mg/l BAP **(Figure 3)**. This may due to the endogenous factors for growth regulation. A similar report was reported by George and Sherrington [33] who found that the influence of naturally occurring auxin within cultured tissues is capable of controlling various distinctive processes such as cell growth and elongation. The lowest shoot length (3.31 cm) was obtained from MS medium supplied with 3 mg/l BAP. The increase in the level of BAP from the control to 3 mg/l resulted in a decrease of shoot length from 5.3 to 3.31 cm. This may due to the role of BAP to enhance shoot formation and release lateral buds and has lower impacts on shoot length. A similar result was obtained by Bhatia [28] who reported that shoot length was greater on lower concentrations of cytokinin and decreased as the cytokinin concentration increased for tomato cultivar Red coat. Han and Stephens [58] also found that shoot elongation was reduced as the cytokinin concentration increased. Himabindu [35] used cotyledon explants derived from tomato cv. PKM-1 and cultured on MS medium supplemented with different levels of BAP and found the highest shoot length (3.43 cm) on MS medium fortified by the lowest BAP concentration (0.5 mg/l). Mukta [6] also got a shoot height of 3-4 cm on lower BAP. In other shoot tip culture experiments, Kiran [20] reported that the length of a shoot is higher at lower (0.1 and 0.2 mg/l BAP) compared to a higher concentration. Likewise, Yousry [59] used a shoot tip explant derived from husk tomato variety Balady and reported similar results. On MS medium fortified with different concentrations of BAP, shoot length and leaf number were higher at lower BAP. The author also said that those values decreased as the BAP concentration increased. On MS+0.25 BAP the author got a shoot length of 4.68 cm having 5.50 leaves. The decrease in the mean values of the studied characters with increasing BAP could be attributed to the accumulation of higher levels of cytokinin which exerts adverse effects on growth performance [60].

Contradictorily to the present result, Otroshya et al. [34] found that the best shoot length (4.5 and 4.05 cm) for hypocotyl and leaf explants derived from tomato Cherry cultivar was obtained from MS medium fortified by BAP (3 and 2 mg/l) respectively. The author also reported that the shoot length of tissue cultured cells is dependent on the explant and concentration of PGRs used. In an experiment conducted using hypocotyl, cotyledon and leaf explants of tomato cultivar Arka Ahuti cultured on the same media the length of shoots was different for all the explants used reported by Namith and Negi [61]. In another research, Sherkar and Chavan found that better length of plantlets (4.7 cm) was obtained on MS with 0.1 BAP [62].

The maximum numbers of leaves per shoot (4.33) were produced from MS medium +0.75 mg/l BAP **(Figure 3)**. While the minimum number of leaves/shoot (2.48) were recorded on MS medium +3 mg/l BAP. A similar result was reported by Sherkar and Chavan [62] who found that higher number of leaves/plantlets (5) was recorded on MS supplied with 0.1 BAP +2.0 mg/l IAA. Contradictorily, Ferdous et al. [63] reported that higher number leaves (3.5) per explant were obtained from MS medium supplied with a higher level of BAP (5 mg/l). In the present experiment, the number of leaves/explants also decreased as the level of BAP increased. Schmiilling [64] also reported the opposite result to ours, who said that cytokinin is a stringent requirement for leaf formation **(Figure 4).**



Note: Means assigned by the same letters are not significantly different at $p \leq 0.01$.

Figure 3: Effect of BAP on shoot length and number of leaves.

Figure 4: *In vitro* shoot multiplication of Chali and Gelilema on MS +2.5 mg/l BAP and MS +2.0 mg/l BAP after 55 days respectively.

Rooting and acclimatization

The *in vitro* multiplied shoots were cultured on MS having different concentrations of IBA for rooting. Finally, plantlets with well-developed roots and leaves were removed from rooting medium and transplanted into plastic pots containing a mixture of oven sterilized soil and sand at a ratio of 2:1 and covered with white plastic to maintain high humidity for acclimatizing. 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. A retrieval rate of 67.7% and 58.1% for Chali and Gelilema respectively was obtained **(Figure 5)**. Some plantlets failed to survive in the *ex-vitro* environment after transferred to the 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 [61] who found that survival of 70%-80% from *in vitro* grown plantlets of tomato cultivar Arka Ahuti.

Figure 5: Acclimatized plantlets of Gelilema and Chali varieties of tomato. After 15 days in a lath house.

Conclusion

The improvement of a plant using conventional methods is slow, time, consuming and need more space. The integration of plant tissue culture with conventional breeding will solve those problems. So far numerous studies have been conducted using MS medium supplied by different PGRs at varying concentrations along with different explants from different cultivars of tomato. But, using one universal protocol for *in vitro* growth of different varieties is impossible because the *in vitro* response is dependent on the genotype, explant and PGRs type and concentration used. Therefore, this research work was initiated for optimizing a variety-specific micropropagation protocol for two Ethiopian tomato varieties (Chali and Gelilema) using shoot tip tissue culture. In conclusion, MS medium free of BAP resulted in the superiority of the collected parameters in the initiation of shoots over MS medium supplied with BAP. But, MS medium augmented with 2 and 2.5 mg/l were the better concentrations for multiplication of Gelilema and Chali respectively. Thus, this genotype-specific protocol could be useful for micropropagation of the two varieties in the future.

Future Line of Work

Further studies using other types and combinations of PGRs and other explants are suggested for increasing the efficiency of multiplication and choosing the best explant and PGRs.

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