MICROPROPAGATION OF SELECTED HYBRID TOMATO *(Solanum lycopersicum* L.) VARIETIES USING SHOOT TIP CULTURE

M.Sc. THESIS

KALKIDAN SHIFERAW ABEBE

FEBRUARY, 2020

JIMMA, ETHIOPIA

MICROPROPAGATION OF SELECTED HYBRID TOMATO *(Solanum lycopersicum* L.) VARIETIES USING SHOOT TIP CULTURE

By

Kalkidan Shiferaw Abebe

M.Sc. Thesis

Submitted to the School of Graduate Studies, Jimma University College of Agriculture and Veterinary Medicine, Department of Horticulture and Plant Sciences in partial fulfillment of the requirements for the degree of Master of Science in Plant Biotechnology

Major advisor: Temesgen Matiwos (Assi. Professor)

Co- advisor: Derbew Belew (Professor)

FEBRUARY, 2020

JIMMA, ETHIOPIA

DEDICATION

I dedicate this thesis to my beloved husband Mr. Besufekad Enideg and all my parents especially my mother Etenat Teshome, who supported me throughout my life.

STATEMENT OF THE AUTHOR

I, Kalkidan Shiferaw Abebe, hereby declare that this thesis is my original work and all sources of materials used for writing this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. Degree at Jimma University College of Agriculture and Veterinary Medicine and is deposited in the University's Library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Brief quotations from this thesis are allowable without prior and or special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotations from or reproduction of this manuscript in whole or in part may be granted by the head of graduate studies when in his/her judgment the proposed use of the material is for scholarly interest. In all other instances, however, permission must be obtained from the author.

Name: Kalkidan Shiferaw

Signature: _____

Place: Jimma University

Date of submission_____

BIOGRAPHIC SKETCH

The author Kalkidan Shiferaw Abebe was born on December 26, 1995 in Wolkite town SNNPR Regional State. She attended her elementary education at Hudadad Primary School and high school education and secondary school education at Yaberus Senior Secondary and Preparatory School. She joined Gambella University in 2013 and graduated with B.Sc. Degree in Plant Science in July 2015. After her graduation, she was employed in Gambella University as graduate assistant for one year and joined Jimma University, College of Agriculture and Veterinary Medicine on September 2017 to pursue her study leading to the Degree of Master of Science in Plant Biotechnology.

ACKNOWLEDGEMENTS

Above all I would like to thank the almighty God and his blessed mother St' Mary for helping me from the start to the end of this work and throughout my life.

I would like to express my heartfelt and deepest thanks, appreciation and gratitude to my major advisor Mr. Temesgen Matiwos (Assistant Professor) for his excellent guidance, critical reading and editing of both my proposal and final thesis report. I am immensely grateful to my co-advisor Professor Derbew Belew for his decisive comments during proposal writing, critical reading and editing of my final thesis report and for his support to obtain the Tomato seeds. I am also thankful to Mr Amare Gebeyehu for his support to obtain hybrid Tomato seeds.

My deepest gratitude goes to the Ethiopian Ministry of Education for offering the financial support to pursue my study. I also extend my gratitude to Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM). I am also thankful to all the staff members of Horticulture and Plant Sciences Department. I thank Jimma Agricultural Research Center for providing me the Plant Tissue Culture Laboratory and Greenhouse. My gratitude also goes to Mr Berihu Mengs, coordinator of Plant Tissue Culture Laboratory, and all staff members of Biotechnology for their positive approaches. Melkassa Agricultural Research Center (MARC) is also highly acknowledged for providing me the hybrid Tomato seeds.

My sincere gratitude goes to my beloved husband Mr Besufekad Enideg and all my parents who have been supporting me during my study and for giving me an endless love throughout my life

I am also thankful to my friends Mewuledeg Zebro and Yonas Getachew for their immense help and intangible encouragement during those times. Finally, I thank all individuals who were beside me during both the course work and research undertaking.

ABBREVIATIONS AND ACRONYMS

2ip	2- Isopentyl Adenine
6-BAP	6-Benzyle Amino Purine
ANOVA	Analysis of Variance
B-5	Gamborg Medium
CRD	Completely Randomized Design
CSA	Central Statistical Agency
IAA	Indole Acetic Acid
IBA	Indole-3- Butyric Acid
Kin	Kinetin
MARC	Melkassa Agricultural Research Center
MS	Murashige and Skoog
NAA	Naphthalene Acetic Acid
PGRs	Plant Growth Regulators
SAS	Statistical Analysis System
TDZ	Thiadiazuron
ZT	Zeatin

TABLES OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF TABLES IN APPENDICES

Page

ABSTRACT

Tomato (Solanum lycopersicum L.*) is one of the most important vegetable crops in the world. Its production is low because of many production constraints like biotic and abiotic stresses. The use of hybrid varieties is a great option to increase its production. The hybrid seeds of tomato are being produced by hand emasculation and hand pollination to get F1 seeds, which is the major method used in tomato breeding. However this method is slow, time consuming, labor intensive and need more space. Using F2 seed also 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 optimize 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 at Jimma Agricultural Research Center plant tissue culture laboratory. 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 significantly ($P<0.01$) influenced days to shoot initiation. The early days for shoot initiation took 5, 6.4 and 5.2 days for Valouro, Uwezo, and Shelter varieties, respectively on hormone free MS medium. Highest percentage of shoot initiation (92%) was also recorded from hormone free MS medium for all the three varieties. The lowest percentage of shoot initiation was recorded from higher concentration of BAP (1.25 mg/l). Hormone free MS medium was optimum for days of shoot initiation and percentage of shoot initiation. In multiplication experiment, all parameters: number of shoots per explant, average shoot length and number of leaves per shoot, showed highly significant difference ($P<0.01$). The maximum numbers of shoots (5, 4.3 and 4) were recorded from MS medium augmented with 2mg/l BAP for Valouro and Uwezo varieties and 1.5mg/l for Shelter variety. While the lowest shoot numbers was obtained from hormone free MS medium. The highest shoot length (5.8, 5.3 and 5 cm) was obtained from 0.5 mg/l BAP, whereas the lowest shoot length (2.69, 2.81 and 3.04 cm) was recorded from 3 mg/l BAP. The maximum number of leaves/shoot was recorded on MS medium supplemented with 1.5 mg/l BAP for Valouro (4 leaves), Uwezo (3.6 leaves) and Shelter (3.4 leaves) varieties. In rooting experiment the interaction effect of IBA*Variety showed highly significant difference for days to rooting, number of roots and root length ($P<0.01$). Rooting percentage also showed a significant ($P<0.05$) difference. Early root induction (5 days for Valouro and Shelter and 7.67 days for Uwezo) was recorded on hormone free media. Maximum number of roots (15.36, 13.06 and 18.26), highest root length (5.92cm, 5.5cm and 6.55cm) were observed at 0.5 mg/l IBA for Valouro, Uwezo and Shelter varieties. Maximum percentage of rooting (100%) was recorded from IBA concentration ranging from 0.0-0.5 mg/l but the minimum was observed from higher level of IBA. Well rooted shoots were transferred to green-house and the survival percentage were 81.13%, 73.58% and 69.8% for Valouro, Uwezo and Shelter varieties, respectively. In conclusion, hormone free MS medium is found to be optimum for shoot initiation in all the three varieties. For shoot multiplication 2.0 mg/l BAP was optimum for Valouro and Uwezo varieties and 1.5 mg/l BAP for Shelter variety. In rooting experiment, half strength MS medium augmented with 0.5 mg/l IBA was optimum for number of roots and root length while for days to rooting and percentage of rooting half strength MS medium without IBA was optimum in all the three varieties. Since this study focused only on the effect of BAP hormone alone for shoot multiplication, further research is suggested to be done on multiplication performance by using other combination of hormones and by taking other explant sources.*

Key words: BAP, IBA, Micropropagation, Uwezo, Shelter, Valouro

1. INTRODUCTION

Tomato *(Solanum lycopersicum* L.) is one of the most important vegetable crops grown throughout the world (Devi *et al*., 2008). It is considered as the second most popular and highly nutritive vegetable crop after potato (Mamidala and Nanna 2011). It is a dicotyledonous plant that belongs to the family *Solanaceae* and genus *Solanum* (Kalloo, 1991). Tomato is a model species for introduction of agronomically important genes into dicotyledonous crop plants (Wing *et al.*, 1994). It is a diploid with 2n=2x=24 chromosomes (Banks, 1984). Tomato is a perennial plant naturally, due to its economic and commercial importance; it is cultivated as an annual crop (Kalloo, 2012).

Tomato originated in the western coastal plain of South America in the area extending from Ecuador to Chile (Jenkins, 1948.) Nowadays, it grows almost in every country of the world (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 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, 2016; CSA, 2018).

Tomato is an important crop worldwide for fresh market or processing. It provides important nutrients like lycopene, betacarotene, flavonoids and vitamins (Gerszberg *et al.,* 2015). Medicinally, it is used as mild aperients, blood purifier, cholagogues, digestives and production of oral vaccines (Indrani *et al.,* 2013). In addition, tomato is rich in vitamins A and C which is a remedy to night blindness, energy values, iron and dietary fiber (Goode, 1989). Also tomato is known as a health stimulating vegetable because of the antioxidant properties of its main compounds. A group of biochemical in red tomatoes is found to have an antioxidant property which reduces several cancers and heart diseases (Rick, 1980; Giovannucci, 1999). It has also great potential for transgenic applications; creation of a new anthocyanin enriched food for cancer prevention and as model for functional genomics, proteomics and metabolomics to improve abiotic and biotic stress tolerance (Khuong *et al.*, 2013).

In Ethiopia tomato is one of the most important and widely grown vegetable crops, both during the rainy and dry seasons for its fruit by smallholder farmers, commercial state and

private farms (Gemechis and Emana, 2012). The total production of tomato in the country has shown a marked increase since it became the most profitable crop providing a higher income to small scale farmers compared to other vegetable crops. However, average yield of tomato in Ethiopia is low, ranging from 6.5-24 metric ton/ha (Gemechis *et al*., 2012). The major constraints of tomato production in Ethiopia are shortage of adaptable hybrid varieties, poor quality seeds, disease and insect pests, high post-harvest loss and poor marketing systems are prominent factors to affect the productivity of tomato (Debela *et al.*, 2016). The use of hybrid variety is a great option to increase the yield of tomato. But biotic and abiotic factors cause decline in yield of tomato as well as decrease hybrid seed recovery from the field. The hybrid seeds of tomato are being produced by hand emasculation and hand pollination to get F1 seeds, is the major method used in tomato breeding (Cheema and Dhaliwal 2005). This method is time consuming and need large number of skilled labor. Consequently, using F2 seed leads to lack of genetically true to type as a result of segregation (Georgiev, 1991). Additionally, the demand for vegetable seeds in Ethiopia is increasing with the expansion of irrigation infrastructures in recent years. But, the hybrid seeds traded and grown in Ethiopia are imported from other countries (Kahsay, 2016). All the above mentioned factors contribute to increased cost of hybrid seeds, which can be as high as $104/g (Bhatia, 2003). Therefore, propagation by tissue culture technique could provide a best alternative to the traditional methods of tomato propagation.

Plant tissue culture is one of important technique of biotechnology because it permits the production of large number of genetically identical plants on a massive scale and disease free planting materials in short period of time (Tileye *et al.,* 2005). In addition, rapid multiplication rate of plants that are difficult to propagate conventionally can be easily achieved through *in vitro* culture (Ribeiro and Carneiro, 1989). During the last two decades many biotechnological approaches were focused on tomato crop improvement (Mandal and Sheeja, 2003). Several researchers have reported about adventitious regeneration in tomato deal with induction of shoots on hypocotyls, apical meristem, cotyledons, stems petioles, leaves, anthers and inflorescences explants (Moghaleb *et al*., 1999). 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, 1989) and development of

transgenic tomato (Kiran, 2007). Different biotechnological applications of *in vitro* culture have been used in tomato. The applications aimed at improving production and tolerating pest and diseases (Moghaieb *et al.*, 2004), as well as genetic transformation (Ling *et al,* 1998). Plant production via tissue culture is advantageous over traditional propagation methods because it leads to the production of disease and virus free plants.

Even though there are many research reported on tomato micropropagation using shoot tip culture, plants could display great variation under *in vitro* conditions in terms of shoot regeneration, shoot proliferation and root induction because of several factors such as genotype, explant type, culture media composition, plant growth regulators (PGRs) and culture condition (Bhatia, 2003). There are more than 3000 species of tomato in the world and each and every variety should have a micropropagation protocol. So, the establishment of one universal protocol for *in vitro* growth of all tomato varieties is difficult because the morphogenic response of tomato varieties is different (Gerszberg and Hnatuszko-Konka, 2015). Therefore this research aimed at addressing this gap and was conducted with the following objectives:

General objective:

- To develop an optimum protocol for micropropagation of selected hybrid tomato varieties using shoot tip culture.

Specific objectives:

- To determine the optimum concentration of BAP for shoot initiation.
- To determine the optimum concentration of BAP for shoot multiplication.
- To determine the optimum concentration of IBA for root induction.
- To investigate the survival percentage of acclimatized plantlets on different soil composition

2. LITERATURE REVIEW

2.1. Description and Importance of Tomato

Tomato belongs to the Solanaceae family, which contains more than 3,000 species, including plants of economic importance such as potatoes, eggplants, tobacco, petunias and peppers (Bai and Lindhout 2007). Linnaeus placed the tomato in the *Solanum* genus (alongside with potato) under the specific name *Solanum lycopersicum*. Tomato is herbaceous plant with a hairy, vine, weak and trailing stems that often require staking. It can grow up to 3 meters above the surface of the ground. The leaves of tomato are 10 to 30 cm long and unevenly imparipinnate compound with variously indented or lobed margins (Tindall, 1983). Both the stems and the leaves are slightly rough and fuzzy. The inflorescence of tomato contains small yellow flowers, each with five pointed lobes on the corolla. The tomato fruit is a fleshy berry, green when unripe and becomes deep red and shiny when ripe. The cultivars differ in fruit size, shape and color.

Tomato cultivars can also be distinguished on the basis of the indeterminate or determinate growing habit. Cultivars for processing purposes are determinate growing and plants have a compact growth habit with grouped fruits ripening at a single moment, which are suitable for a mechanical harvest. In addition, fruits for processing should have certain characteristics that are related to processing quality, such as high viscosity, dry extract, pH value and high value of total soluble acids. Indeterminate growth habit is typical of fresh market cultivars in a greenhouse. Important characteristics for fresh market cultivars are, for example, long shelf-life, external quality of fruits (like shape and color) and internal quality fruits (like flavor, sweetness, and juiciness) (Fentik, 2017).

Tomato is one of the most common, highly nutritive, and commercially important horticultural crop worldwide (Dorais *et al.*, 2001). It is a good source of vitamins, minerals and other biologically active compounds, such as lycopene, a powerful antioxidant. Lycopene has been reported to protect our body from free radicals (Gerster, 1997). The most important antioxidants in tomatoes are carotenes (Clinton, 1998). Tomato contains approximately 20 to 50 mg of lycopene/100 g of fruit weight (Kalloo, 1991) and its content varies significantly

depending on ripening, variety and environment (Brandt *et al*., 2006). Tomato has been widely used not only as food, but also as research material (Kimura and Sinha, 2008).

2.2. Origin and Distribution of Tomato

Tomato is originally from Central and South America (Jenkins, 1948) and was brought to Europe by Christopher Columbus on his second voyage in 1498. The crop is probably from Peru Ecuador area and it was distributed to many parts of tropical America as a weed, but become domesticated first in Mexico (Tindall, 1993). Tomato arrived in Africa through Portuguese traders and was brought across the continent from Egypt and then Sudan (Tindall, 1986). The wild cherry tomato species transported to Mexico, where it has been grown and consumed (Sink and Reynolds, 1986). It was accessed to Europe by the Spanish conquistadors in the 16th century and later distributed to southern and eastern Asia, Africa and the Middle East. Nowadays, it grows widely in China, USA, Turkey, Russia, Egypt, India, Spain, Mexico and many other countries of the world. It is growing in tropical, sub-tropical and temperate areas of the world (Atherton and Rudich, 1986).

The introduction of cultivated tomato into Ethiopian agriculture dates back to the period between 1935 and 1940. The Ethiopian Institute of Agricultural Research (EIAR) was established in 1966 during which tomato was recognized as a commodity crop. The first record of commercial tomato cultivation is from 1980 with a production area of 80 ha in the upper Awash by Merti Agro-industry for both domestic as well as export markets. The total area increased to 833 ha by the year 1993 and later on the cultivation spread towards other parts of the country. Since 1994 up to present, tomato acreage increased to 5338 ha with a total production of 55,635 MT (CSA, 2011). Currently tomato is one of the regional export crops of the country.

2.3. Production Status of Tomato in Ethiopia

Tomato accounts for about 10% of the total cost of production depending on the enterprise. Two major processing methods of tomato which yield a variety of products are the drying (dehydration) and wet milling. Tomato is one of the major vegetable crops produced in Ethiopia, being highly perishable in nature; it has a limited shelf life. It creates glut during it

short production season and become very scarce and expensive during it off season, its short life and inadequate processing and preservation leads to loss of revenue to the farmers (Adegbola *et al*., 2012). Ethiopia has a comparative advantage in a number of horticultural commodities due to its favorable climate, proximity to European and Middle Eastern markets and cheap labor. However, the production of horticultural crops is much less developed than the production of Food grains in the country. On average more than 2,399,566 tons of vegetables and fruits are produced by public and private commercial farms, this is estimated to be less than 2% of the total crop production (Abdu, 2016). In Ethiopia, the demand of commercial hybrid vegetables seed has been rapidly increased. After adaptation and verification, more than 90 hybrid vegetable have been approved and registered for production in Ethiopia. Tomato takes the highest share of commercial vegetables. About 20 commercial hybrid tomato varieties have verified and under production in Ethiopia (MARDPR, 2016). Smallholders have grown tomato for long time for their livelihood needs since the start of its commercialization. However, the average yield of tomato in Ethiopia is low, 8 ton ha-1 compared with world average yields of 34 ton ha-1 (FAOSTAT, 2012). This may be related to limited access and use of improved commercial tomato varieties and poor production management.

The total production of tomato in Ethiopia has shown a marked increase, indicating that it has become the most profitable crop providing a higher income to smallholder farmers compared to other vegetable crops. However, average yield of tomato in Ethiopia is low, ranging from 6.5-24 metric ton/ha (Gemechis *et al*., 2012) as compared with average yields of 51, 41, 36 and 34 metric ton/ha in America, Europe, Asia and the entire world, respectively (FAOSTAT, 2010). Currently tomato is planted in 5,235.19 ha of land in Ethiopia and produced 27,774.54 ton; with the productivity of about 5.31 t ha^{-1} (CSA, 2018). The highest production of tomato in Ethiopia was observed in 2016. However, production of tomato was decreased unexpectedly in 2017 and 2018 (Figure. 1).

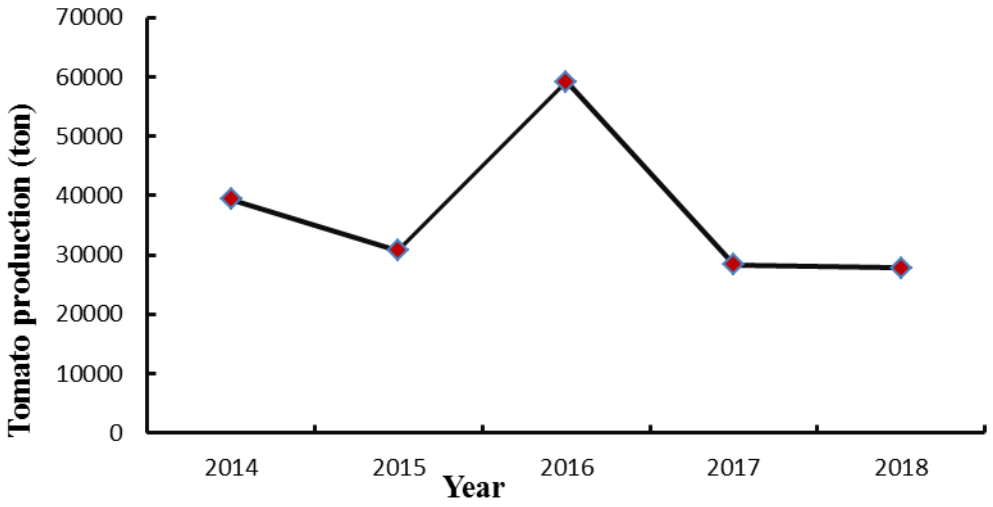


Figure 1. Tomato production trend in Ethiopia from 2014-2018 (CSA, 2014-2018).

2.4. Conventional Propagation of Tomato

Tomato can be propagated through sexual and asexual. Sexual means of propagation is plants propagated through seeds which are obtained by the combination of pollen and egg which come from the two parents. Asexual means of propagation are plants propagated through grafting and cutting (Kubota *et al*., 2001; Bhatia, 2003; Lee and Oda, 2003).

Conventionally, tomatoes are propagated through seeds. Conventional breeding techniques were attempted to develop agronomically important properties of tomato, but it was not successful. Because conventional breeding technique needs longer time, extending from seven to eight years, this includes crossing and selection of desirable traits. Vegetative methods of propagation are not well developed in tomato for commercial purposes (Kubota *et al*., 2001). However, vegetative propagation of tomato using single node cuttings as propagules have been used for commercial production of genetically superior cultivars having heterozygous genotypes (Soressi *et al*., 2007). However, the difficulty of environmental control in the greenhouse often results in underproduction of transplants is major problem in productivity of tomato.

Traditional improvement methods are time consuming and troublesome due to the time of breeding, and there is a problem with the choice of criteria appropriate for breeding purposes.

Conventional vegetative multiplication of tomato has been several negative impacts, including transmission of diseases and lack of genetic uniformity (Georgiev, 1991).

2.5. *In vitro* Propagation of Tomato

Plant tissue culture is the growing of plant cells, tissues or organs isolated from the mother plant on synthetic media under aseptic and controlled environmental condition. It is an important technique in basic and applied biological sciences as well as in commercial applications (Sarker *et al*., 2015). The most popular use of plant tissue culture is micropropagation, which is an alternative way for vegetative propagation of plants. It has been used for producing a large number of plantlets from a small piece of mother plant within a short period of time and a limited space (Kumar and Reddy, 2011). It becomes an important part of commercial propagation of plants because of its advantages as a multiplication system for many plant species (Debergh, 1987). Thus, the establishment of simple and efficient regeneration systems is a fundamental prerequisite of taking advantage of cell and tissue culture for genetic improvement (genetically transformed plants for commercial applications). The *in vitro* culture of the tomato has been successfully used in different biotechnological application including the clonal propagation of high value commercial cultivars, virus-free plants, and genetic transformation (Hanus-Fajerska 2005; Li *et al*, 2011; Yarra *et al*, 2012; Namitha and Negi 2013).

Tomato shoot regeneration was first reported in cell cultures by Norton and Boll in 1954. Ulrich and Mackinney (1969) recognized in early studies that the genotypes respond differently to various levels of nutrients and growth stimulators or inhibitors in the medium. For tomato regeneration, a wide variety of plant growth regulators have been used with varying concentrations. Many cytokinin and auxin combinations could induce shoot proliferation in tomato from different source of explants. Jatoi *et al.* (2001) found that 6-benzylaminopurine (BAP) + indole-3-acetic acid (IAA) are the best for callus induction from shoot tips and also found that kinetin (Kin)+IAA are the best for regeneration of shoots from callus. Gubis *et al*. (2004) studied the effect of different growth regulators and plant regeneration of tomato explants. They found that the best regeneration medium was the

Murashige and Skoog (MS) medium supplemented with 1 mg/l of Zeatin and 0.1 mg/l IAA and 100% frequency of regeneration was observed when hypocotyl explants were used.

Chaudhry *et al*. (2004) reported that callus formation from hypocotyl is best when gotten from leaf discs in 2 mg/l IAA + 2 mg/l BAP or 2 mg/l naphthalene acetic acid (NAA) + 4 mg Kin and the maximum percentage of shoot formation on the MS medium supplemented with 2 mg/l IAA+5 mg/l BAP or 2 mg/l +4 mg/l Kin. These authors reported that hypocotyl is the best explant source for callus formation and regeneration.

Rashid and Bal (2010) studied the effect of hormones on direct shoot regeneration in hypocotyl explants of two genotypes of tomato (Punjab Upma and IPA-3), the explants were cultured on MS medium supplemented with 0.5 mg/l Kinetin and 0.5 mg/l BAP. Shoot regeneration percentage in 'Punjab Upma' and 'IPA-3' the percentage was recorded to be highest (86.02) and (82.57) respectively.

2.5.1. Effect of MS medium on *in vitro* propagation of tomato

The success of plant tissue culture as a means of plant propagation is greatly influenced by the nature of the culture medium used (George *et al*., 2008). Optimal growth and morphogenesis of tissues vary according to their nutritional requirements. Tissues from different parts of plants may also have different requirements (Murashige and Skoog, 1962). The principal components of most tissue culture media are inorganic nutrients (macro and micro), organic nutrients (vitamins and amino acids), carbon source, PGRs and gelling agents. Murashige and Skoog (MS) medium is the most preferable media for tomato regeneration, shoot proliferation and root induction. It consisting of macro salts, micro salts, vitamins and iron sources (Ellul *et al.*, 2003). The purpose of a culture medium is to provide optimum conditions for the growth of explants. These conditions are determined by physical factors like light and temperature and chemical factors such as PGRs, salt, sugars and vitamins. Each species, variety or even ex-plant from different parts of the same plant may have different requirement for optimum growth.

The sucrose concentration of 30 g/l was found to be optimal for tomato explants compared to 5, 10, or 20 g/l (Gubis *et al.* 2004). Hussey (1971) also reported the highest growth rate of tomato on sucrose concentrations ranging from 1-4%. In addition, there are other types of carbon sources, which have been used in tomato tissue cultures such as glucose, fructose, maltose, galactose, mannose, sorbitol and lactose which have an important role in supporting good growth (Fowler, 2000). El-Bakry (2002) used four different carbon sources (sucrose, fructose, maltose and glucose) and recorded a significantly different number of shoots from three tomato cultivars.

2.5.2. Effect of explant type and source on callus induction and regeneration

Different types of explants of tomato have been used by different researchers, which include hypocotyl, leaf, shoot tips, stem, cotyledon, pedicel and inflorescence. Though almost all the explants used are amenable to regeneration, cotyledon and hypocotyl explants are the most and widely used explants (Pampanna, 2009). Most genotypes of tomato respond differently to plant growth regulators (PGRs) during regeneration (Kurtz and Lineberger, 1983). Variations in quantity and type of PGRs influence both the percentage of explants responding, and the number of shoots produced by an explant (Plastira and Perdikaris, 1996).

Improvement of the adventitious shoot regeneration system using tissue culture methods for tomato plants is still important due to the diverse morphogenic potential of the different genotypes. Scientists have used different types of explant, but it should be emphasized that the type of explants determines not only the frequency of the explants' organogenesis but also determines the number of shoots produced per explant (Bahurpe *et al*, 2013; Jehan and Hassanein 2013). Namitha and Negi (2013) demonstrated that the efficiency of shoot regeneration ability followed the order hypocotyls > cotyledon > leaf. In earlier studies, Mamidala and Nanna (2011) reported that a cotyledon explants showed organogenesis superiority over hypocotyls and leaf explants.

According to Chaudry *et al*. (2010) the higher regeneration potential of hypocotyls was observed than of leaf explants. On the other hand, Harish *et al*. (2010) reported that shoot formation efficiency was greatest in the order: hypocotyls > leaf > stem. Moreover, they noticed significant differences in regeneration capacity between genotypes in terms of

regeneration process, duration as well as the number and size of the regenerated shoots. The difference in the regeneration capacity of different explants may be due to the level of endogenous hormones present in the explants used for the culture (Tileye *et al*., 2005).

Choice of appropriate explant is an important determinant in tissue culture responses along with the age of the seedling and nature of explants as they can strictly affect callus induction and regeneration (Takashina *et al.,* 1998). Hypocotyls and cotyledons of 10-12 day old tomato *in vitro* seedlings were used as explant sources. The younger plants in general responded better than the older ones which is evident from the use of six days old *in vitro* seedlings of tomato for callogenesis and organogenesis responses (Hu and Philips, 2001; Moghaieb *et al.*, 2004). In this experiment best callus induction response was observed for hypocotyl explant which is almost similar to many of the previous studies where hypocotyls explants have been reported to be superior to cotyledon explants in different tomato cultivars (Osman *et al.,* 2010; Yasmeen, 2009).

The age of explant influences the success of tissue culture as it is known to influence the regeneration capability of the explant. Previous studies demonstrated that explants of tomato obtained from 8 to 10 days old seedlings were superior for promoting shoot organogenesis (Hamza and Chupeau, 1993; Ling *et al*., 1998; Gubis *et al*., 2004; El-Heba *et al*., 2008). This indicates young and soft tissues are generally more amenable to *in vitro* culture compared to old and woody tissues. However, Dai *et al*. (1988) reported that the regenerative capacity of tomato increased with an increase in the age of the explant. They have investigated the effect of seedling age of hypocotyl and cotyledon explants on shoot and root regeneration of tomato. They found that the regeneration increased up to seedlings of age 30 days. However, regeneration capacity declined sharply at age of 40 days. They also stated that the explants from seedlings younger than 20 days initiated calluses, some of which developed shoots, while older plants produced principally adventitious shoots. Hamza and Chupeau (1993) obtained the best regeneration rate from cotyledon explants from 8–10 days old tomato.

Gubis *et al.* (2005) evaluated the effect of *in vitro* regeneration and plant growth of tomato from hypocotyl and cotyledon explants. They observed significant influence of explant type

on regeneration capacity. The most responsive explants were hypocotyls, with 90-92 percent regeneration and mean production of 0.18-0.38 shoots per explant.

Regeneration is also dependent on position from where explants from the seedlings were chosen. Costa *et al*. (2000a) reported that the position of the cotyledon segments (apical and basal) on *in vitro* regeneration in two tomato cultivars (IPA-5 and IPA-6). They found that the position of the explant did not result in any significant differences in average regeneration frequency and shoot number. In contrast, the position on the original seedling influenced the morphogenetic potential of the Aurea mutant. Nearly 50 percent of the apical segments regenerated shoots while regeneration from the middle and basal segments was negligible. None of the growth regulator supplements could compensate for the differences in the regeneration capacity of the hypocotyl segments.

2.5.3. *In vitro* shoot initiation of tomato

After shoot tips have been excised, explants are cultured *in vitro* for shoot initiation. Different types of media can be used for shoot initiation. The most appropriate medium for tomato micropropagation using shoot tip culture is the Murashige and Skoog medium (Murashige and Skoog, 1962).

Pampanna (2009) observed that among different growth regulator treatments tested, the significantly higher regeneration response of 95.0 percent was observed with the treatment consisting of MS + TDZ 1.0 mg/l + IAA 0.1 mg/l. This treatment was significantly superior over other treatments but for MS+TDZ 1.5 mg/l + IAA 0.1 mg/l (91.0%) and MS+TDZ 2.0 mg/l + IAA 0.1 mg/l (92.0%). Placing the explants on MS media without any growth regulators (control) resulted in no response indicating the importance of growth regulators in regeneration of tomato. MS medium supplemented with BAP 4.0 mg/l + IAA 0.1 mg/l resulted in 82 percent of regeneration response. Significant difference was observed among the two types of explants studied. Cotyledon explants resulted in significantly higher mean regeneration response of 71.0 percent as compared to hypocotyl explants (66.5%).

Cultured tomato shoot tip explant on MS medium supplemented with BAP (0.1-5.0 mg/l) and found that shoot regeneration was different depending on the concentration of the BAP used. The author reported that the regeneration frequency of shoot apices is more than 90 % in all the BAP concentrations tested with a maximum of 100 % in 1.0, 1.5, 2.0 mg/l BAP. The researcher also found that cotyledon explant cultured on MS medium supplemented with ZT and IAA resulted in highest (> 90%) regeneration response than Kin 50-60 % (Kiran, 2007). Bhatia *et al*. (2003) reported that the type of explant used determines both the proportion of explants which show organogenesis and the number of shoots produced/explant. Pampanna (2009) used two types of explants (hypocotyl and cotyledon) derived from tomato cultivar VYBHAV and got a different regeneration response. Devi *et al*. (2008) also reported that the shoot regeneration response of a given explant is genotype dependent.

Ishag *et al* (2009) reported that at higher concentration of the cytokinin the regeneration percentage and shoot regeneration frequencies decreased. This is mainly, because at higher cytokinin level explants produced excessive callus and failed to improve the efficiency of shoot multiplication. Callus growth on explant usually interferes with the propagation process. In order to evaluate the synergistic effect of BA and Kin with NAA for direct shoot regeneration, it was found that, the combination of NAA with BA or Kin negatively affected the multiplication rate of the tomato compared with cytokinin used singly. The inhibitory effect of auxin on multiple shoot induction has been demonstrated in numbers of plants (Thiem 2003).

2.5.4. *In vitro* shoot multiplication

Multiplication is the experimental stage in micropropagation in which initiated shoots have been cut into pieces and sub cultured in order to get more number of shoots. For multiplication masses of tissues are repeatedly sub-cultured under aseptic conditions onto fresh culturing media to encourage propagules proliferation. The culture can supply shoots for the subsequent propagation phases as well as materials that are required to maintain the stock (Reddy, 2011). Determination of the most optimal types and concentrations of PGRs together with other *in vitro* factors as constituents of a medium is one of the most important aspects of successful micropropagation (Ružić and Vujović, 2008). The most common additives for

multiplication media are cytokinins usually BAP and Kin alone or in combination with low amount of auxins like NAA, IBA and IAA. The concentration of cytokinin is known to be critical in multiple shoot induction (Abdellatef and Khalafalla, 2007). Typically, the same medium and environmental conditions are used for both shoot initiation and multiplication.

Jamous (2013) reported that the effect of different levels of NAA and Kinetin on the number of adventitious shoot produced of tomato Baladi landrace. Kinetin at different concentrations induced more shoots per explant; the highest shoot number (8.4) was achieved on medium containing 18.4 μM Kinetin only. More shoots were produced when higher levels of kinetin were used. However, when kinetin was combined with 2.7 μM NAA, the shoot number was significantly decreased (3.6).

According to Papry *et al*. (2016), the maximum number of shoot was 3.5 produced by leaf explants at 2.0 mg/l BAP. According to the authors the number of shoots gradually increased with the advancement of culture duration in all hormonal treatments. The increasing of BAP concentration up to 2 mg/l caused the number of shoots to continue developing, but it fell down in presence of BAP (3 mg/l) that indicates the toxic effect of growth regulators due to their accumulation. In addition to this, Janani *et al.* (2013), Shah *et al.* (2014) and Otroshy *et al.* (2013) found the highest shoot regeneration response from leaf explants. These results are also similar to the findings of Mohamed *et al.* (2010) who had reported the highest number of shoots in MS medium supplemented with BAP (2 mg/l) and no adventitious shoots was noticed in the control (hormone free medium).

Shoot tip of 8-10 days old explant of tomato variety Pusa Ruby and cultured on MS+BAP (0.1-5.0 mg/l) and resulted in shoot formation with different degree of response depending on the concentration. The highest number of multiple shoots (5.64 and 5.2 shoots/explant) was obtained on a medium containing 1.5 and 1.0 mg/l BAP respectively (Kiran 2007). However, the number of shoots decreased with the increase in the BAP concentration in the medium (BAP 2 mg/l with 3.15 shoots/explant). Izadpanah and Khoshkhoy (1989) also reported the formation of multiple shoots from *in vitro* shoot tip culture of three tomato varieties (Calj, Petomech and Red Cloud), on MS medium supplemented with 1.5 mg/l BAP and 3.0 mg/l Kin, 2.0 mg/l, BAP and 5 mg/l of kinetin orderly. Mamidala and Nanna (2011) also used three

explants (leaf, hypocotyl and cotyledon) derived from five tomato cultivars (PKM-1, Money Maker, Micro-Tom, Micro-Msk and White Cherry) and reported that MS medium supplied with 2 mg/l BAP was the best in the number of shoots per explant. These authors also reported that leaf explant and PKM-1 was better than other explants and cultivars respectively.

Banu *et al*. (2017) reported that BAP and Kinetin were used in combination (1.0 mg/l BAP + 1.0 mg/l Kinetin), the highest mean number of shoots per explant 2.28 in BH-3 was recorded followed by 2.15 in MH and 2.12 in BH-4 respectively. By subject to control culture, the author suggests that BAP and Kinetin can play a significant role toward shoot multiplication. Shoot length was found better at low doses of BAP and Kinetin for every variety and that was 0.5 mg/l. In fact, with the increase in hormone doses, shoot length decreased gradually. From the three cultivars, the longest shoot length (7.06 cm) was recorded in BH-3 with 0.5 mg/l BAP on MS medium followed by 6.91 cm in BH-4 at 0.5 mg/l KN and 7.02 cm in MH at 0.5 mg/l BAP.

Otroshy *et al*. (2013) the average number of shoots per explant was recorded after 8 weeks of cultivation. Highest number of shoots per explant for leaf and cotyledons was recorded in the presence of 2 mg/l BAP. Hypocotyls showed the highest number of shoots per explant on MS supplemented with 3 mg/l BAP. Mohamed *et al*. (2010) also reported the highest number of shoots on MS medium supplemented with 2 mg/l BAP. In addition, Devi *et al.* (2008) has also reported that the best shoot number per explant was obtained on MS medium supplemented with 3 mg/l BAP + 2.5 mg/l IAA. Similar results were also achieved from cotyledon explants on MS medium supplemented with 3.0–3.5 mg/l BAP in combination with IAA (0.1 mg/l) (Vikram *et al.*, 2011). On another research BAP (2 mg/l) in combination with 0.2 mg/l IAA for more number of multiple shoot formation (Gunay and Rao, 1980). Highest number of shoots per explant was 13.33, 12.25, and 7.94 for hypocotyls, leaves, and cotyledons, respectively.

2.5.5. *In vitro* rooting

Rooting is the final step of the regeneration protocol in plant tissue cultures. There are many factors affecting the rooting process (e.g. the physiological status of plantlets, medium composition, growth regulators). Mostly, MS or 1/2MS are used as a basal medium for rooting. Mensuali Sodi *et al*. (1995), Rashid and Bal (2010) and Bahurpe *et al*. (2013) suggested that for root induction, the tomato does not require any exogenous plant growth regulators. However, in most cases, root formation would be achieved with auxins (IAA, NAA or IBA) alone with concentrations ranging from 0.1 to 1 mg/l (Chaudry *et al*. 2010; Ashakiran *et al*. 2011; Mamidala and Nanna 2011; Zhang *et al*. 2012; Namitha and Negi 2013; Sherkar and Chavan 2014; Wayase and Shitole 2014). Abundant rooting is usually observed after 2 weeks.

According to Indrani *et al.* (2013) After 30 days, when the multiple shoots were observed, they were transferred to the root induction medium with different concentrations of IBA (0.5-1.0 mg/l). In this step, shoots were excised and transferred into root induction media. Best rooting was noted in 0.5 mg/l IBA. Chaudhury *et al.* (2010) reported that 0.1 mg/l IBA in combination with 0.0025 mg/l BAP promoted root induction. Highest rooting was also reported in IBA by Osman *et al*. (2010) and Mamidala and Nanna (2011) reported that adventitious shoots developed from different explants were excised (3 to 4 cm) and shifted on to root induction medium (MS + 0.1 mg/l NAA). Profuse rooting was observed within two weeks of incubation in all the cultures tested.

Regenerated shoots were shifted to media containing 2 mg/l IBA in both full strength and half strength MS media for rooting. Although rooting was observed in both cases, but longer and profuse rooting system was observed on ½ strength MS media containing 2 mg/l IBA which is in line with the previous studies (Sherkar and Chavan, 2014) obtained. Devi *et al*. (2008) reported that the best rooting was found to be in half-strength medium supplemented with 0.2mg/l IBA.

According to Papry *et al.* (2016) leaf explants produced the longest root (8.785 cm) on 0.5 mg/l in ½ MS medium. The shortest root (5.106 cm) was observed on ½ MS medium

supplemented with 0.1 mg/l IAA. No root was formed in hormone free media. On the other hand, the presence of endogenous auxin enables tomato to induce roots without the addition of any extra exogenous plant growth regulators (Rashid and Bal 2010 and Bahurupe *et al*., 2013). For root induction, tomato does not seem to require any exogenous plant growth regulators (Mensuali-Sodi *et a*l., 1995). Many researchers have speculated that tomato has a high level of endogenous auxin, based on the observations of shoot cultures producing roots without the addition of auxins in the medium.

3. MATERIALS AND METHODS

The experiment was conducted at Jimma Agricultural Research Center tissue culture laboratory, Jimma, Ethiopia.

3.1. Plant Materials

Three hybrid tomato varieties named Valouro, Uwezo and Shelter were developed by Rijk Zwaan and used as experimental materials. The plant materials were kindly obtained from Melkassa Agricultural Research Center.

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 180-200 g. 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 120 g. 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).

3.2. Seed Sterilization and Explant Preparation

The seeds were washed under running tap water for 15 minutes to remove dust particles. After washing, seeds were immersed in 70 % ethanol for 3 minutes and then rinsed three times with sterilized distilled water for 5 minutes each in laminar air flow hood. Then seeds were disinfected with 20% commercial sofi bleach (1 % active chlorine) for 20 minutes (Chaudhry *et al*., 2004). Then, the disinfected seeds were rinsed three times using sterilized distilled water for 5 minutes each. After all, the seeds were transferred to sterilized Petri dish (internal surface covered by cotton with sterilized water) for germination. The seeds were grown in a Petri dish until two fully developed leaves appeared. Finally, shoot tips of 2 cm length were excised and used as an explant.

3.3. Media Preparation

The growth media (Murashige and Skoog, 1962)) supplemented with various plant growth regulators were used. Stock solutions were prepared by taking from macro nutrients x50/1000, from micro nutrients x100/1000, from iron EDTA x50/500, from vitamins x100/500 and from calcium chloride x50/500 (Appendix 1). The stock solution of iron EDTA (Ethylene Di-Amine Tetra Acetic Acid) was covered with aluminum foil to protect light. The growth regulators were prepared by weighing the powder in 1 mg: 1 ml ratio. Plant growth regulators, auxin (IBA) was dissolved using a drop of ethanol and cytokinin (BAP) by NaOH. Then the total volume was adjusted to 100 ml by distilled water. Finally the solution was stored in a refrigerator at 4^{o}C for further use.

Culture medium was prepared by taking 20 ml/l from macro, 5 ml/l from micro, 10 ml/l from iron EDTA, and 5 ml/l from vitamins and 10 ml/l from calcium chloride from the MS stock solutions. Then 3% sucrose was added to the solution as an energy supply. The pH of the media was adjusted to 5.8 using NaOH or HCl prior to addition of agar and the medium was solidified using 0.7% (w/v) agar. Growth regulators were added according to the concentration required. Then 50 ml media was 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 minutes.

3.4. 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.

3.5. Treatments and Experimental Design

3.5.1. Experiment 1: Optimizing different concentrations of BAP for shoot initiation

In this experiment, shoot tip explants of 2 cm 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.

3.5.2. Experiment 2: Optimizing different concentration of BAP for shoot multiplication

Prior to shoot multiplication experiment, the initiated shoots were taken out from the culture medium and cultured on hormone-free MS medium to avoid carry over effects of growth hormones. Then shoots were cultured on fresh MS medium containing BAP at 0.0, 0.5, 1, 1.5, 2.0, 2.5, and 3.0 mg/l for shoot multiplication. Four explants were cultured per jar and each replication. The experiment was laid out using CRD in factorial combination (Seven level of BAP* three varieties) with four replications. Sub-culturing was done twice at fifteen days interval. A total of 16 plants per treatment were used and data were collected from all plants. Data on the number of shoots/explant, number of leaves/shoot and average shoot length were recorded.

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

Well-developed shoots were taken out from the culture tubes and again cultured on hormone free MS medium for one week to avoid carry over effect. Then, the shoots were transferred to half strength MS medium supplemented with IBA at 0.0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/l for root induction. Four explants per jar were cultured in each treatment. The experiment was laid out using CRD in factorial combinations (Seven level of IBA* three varieties) with three replications. A total of 12 plants per treatment were used and data were collected from all plants. From this experiment data were recorded on; days to rooting, rooting percentage, number of roots and root length.

3.5.4. Acclimatization

A total of 53 *in vitro* rooted plantlets from each variety were transferred to acclimatization. Well regenerated plantlets with shoots, roots and leaves were gently removed from the culture jars and the roots were washed in sterilized water to remove traces of agar. Then transplanted into plastic pots containing different mixture of oven sterilized top soil, sand and compost at a ratio of 1:2:1, 2:1:1 and 2:1:0 then the pots were covered by a white plastic with a small hole and kept under lathhouse for fifteen days. Then the plastic covers were removed and transferred to green-house and watered every day in the morning until fully developed. After 30 days, data on survival rate (%) was recorded.

3.6. Data Analysis

All data recorded were subjected to analysis of variance (ANOVA) and significant differences among treatments mean were determined by least significant difference test (LSD) at the $P \leq 0.01$ probability level using SAS software (version 9.3).

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

$$\text{In CRD } Yijk = \mu + Ti + YK + (TY)\,jk + Eijk$$

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

4. RESULTS AND DISSCUSSION

4.1. Effect of BAP on Shoot Initiation

The analysis of variance (ANOVA) showed that the interaction effects between variety and BAP were highly significant for days to shoot initiation, but was not significant for percentage of initiation. The different concentrations of BAP highly significantly influenced percentage of initiation at $p \leq 0.01$ (Appendix Table 2).

The earliest initiation of shoot took 5 days for Valouro variety, 6.4 days for Uwezo variety and 5.2 days for Shelter variety, which was obtained from MS medium without growth regulators. The late initiation of shoots (12.2 days for Valouro, 13.6 days for Uwezo and 15 days for Shelter varieties) was noted in MS medium supplemented with 1.25 mg/l BAP. Days of shoot initiation increased from 5 to 15 days with increase in BAP concentrations from 0.0 to 1.25 mg/l BAP (Table1). This may be due to the dependence of frequency of shoot regeneration on the concentrations and growth regulators used. George and Sherrington (1984) also reported that growth and morphogenesis in *in vitro* are regulated by the interaction and balance between PGRs augmented in the medium and the growth substances produced endogenously by cultured cells/tissues. From the present finding MS medium without growth regulators was optimum for shoot initiation. Similarly, Mukta (2014) found higher level of BAP took more days for shoot initiation for BARI 9 and Bahar tomato cultivars. This result is also supported by the finding of Liza *et al.* (2013) who reported increase in days of shoot initiation from 5.6 to 12 days with increase in the BAP concentrations from 0.0 to 2.5 mg/l.

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 ($p \leq 0.01$)

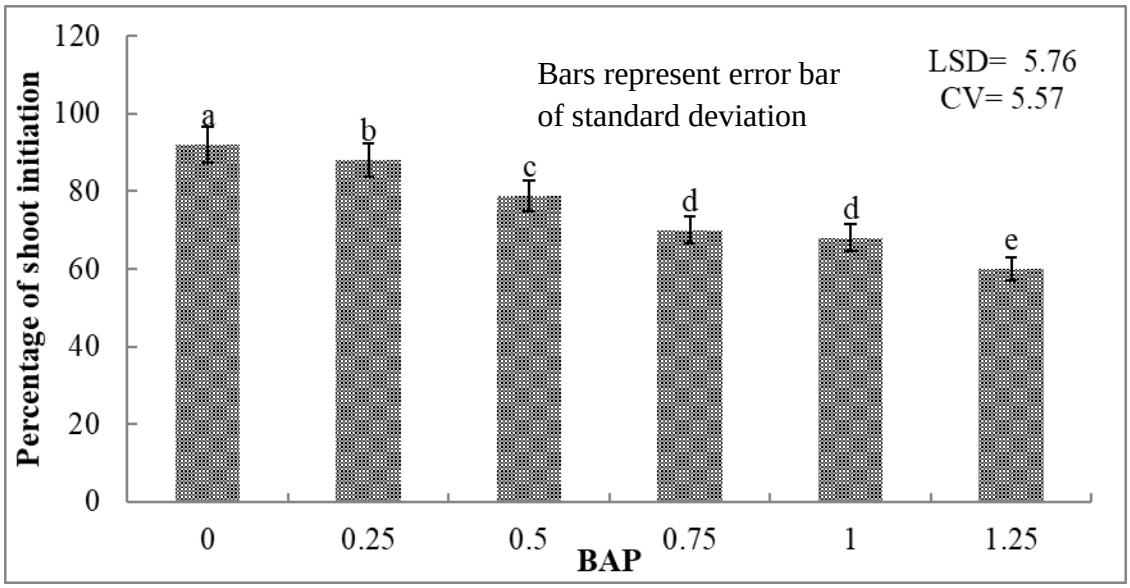
In this study the highest shoot initiation percentage (92%) was recorded from hormone free MS medium. The minimum percentage of initiation (60%) was recorded from MS medium supplemented with 1.25 mg/l BAP (Figure 2). From this result hormone free MS medium was optimum for percentage of shoot initiation. In the present study, the percentage of shoot initiation decreased with increase in the concentration of BAP. This might be due to the formation of callus at higher level of BAP. Similarly, Ishag *et al.* (2009) observed a decreased regeneration percentage and shoot regeneration frequencies at higher concentration of BAP. This is mainly, because at higher BAP level explants produced excessive callus and failed to improve the efficiency of shoot multiplication. Likewise, Bahurupe *et al*. (2013) found that shoot tip explant of tomato variety resulted in callus when cultured on MS medium supplied with 2 mg/l BAP and observed that callus induction was increased with increase in concentration of BAP.

Similarly, Palana *et al*. (2005) reprorted that highest regeneration percentage (90%) was achieved on hormone free MS medium. Therefore, hormone free medium could be taken as the best option when we want to get maximum percentage of shoot initiation and it is the best alternative to minimize the cost of PGRs. Mukta (2014) also reported that the shoot regeneration percentage of three tomato cultivars decreased from 96% to 19 % as the concentration of BAP increased from 1 mg/l to 7 mg/l. In another finding, Rashid and Bal

(2010) found the optimal medium for shoot regeneration to be MS medium supplemented with 0.5 mg/l BAP in the three tomato genotypes.

Result of the present study does not agree with that of Kiran (2007) who reported the maximum values (100%) of shoot initiation achieved from MS medium supplemented with higher level of BAP (1, 1.5 and 2 mg/l) and this author found lower shoot initiation percentage (80%) on hormone free MS medium. In another research, Pampanna (2009) observed significantly higher regeneration response of 95.0 percent from MS medium fortified with 1.0 mg/l TDZ + 0.1 mg/l IAA. Placing the explants on to MS media without any growth regulators (control) resulted in no response indicating the importance of growth regulators in regeneration of tomato. MS media supplemented with BAP 4.0 mg/l + IAA 0.1 mg/l resulted in 82 percent of regeneration response. Significant difference was observed among the two types of explants studied. Cotyledon explants resulted in significantly higher mean regeneration response of 71.0 percent as compared to hypocotyl explants (66.5%).

Liza *et al.* (2013) also found contradictory result from the present study. The authors reported that the effect of different growth regulators and plant regeneration of tomato explants, the best regeneration was found on MS medium supplemented with 2 mg/l of BAP and 1.5 mg/l NAA and (75-80) % frequency of regeneration was observed when cotyledon explants were used. In the present study the percentage of shoot initiation is decreased when the concentration of BAP increased. The success in tomato regeneration response has been found to depend largely on genotype, explants, and plant growth regulator used in culture medium (Bhatia *et al*., 2004).



Note: Values assigned by the same letters are not significantly different ($p \leq 0.01$)

Figure 2**.** Effect of different concentrations of BAP on percentage of shoot initiation

Valouro **Uwezo** **Shelter**

Figure 3. Shoot initiation in the three tomato varieties on hormone free MS medium

4.2. Effect of BAP on Shoot Multiplication

The analysis of variance (ANOVA) for shoot multiplication showed interaction between Varieties with BAP was highly significant ($P \leq 0.01$) on number of shoots/explant, number of leaves/shoot and shoot length (Appendix Table 3).

The highest numbers of shoots/explant (5±0.08 and 4.3±0.08) were recorded from Valouro and Uwezo variety, respectively on MS medium supplemented with 2 mg/l BAP. Whereas, maximum (4±0.07) shoots/explant was obtained from Shelter variety on MS medium augmented with 1.5 mg/l BAP (Table 2). MS medium supplemented with 2 mg/l BAP was optimum for Valouro and Uwezo varieties while for Shelter variety 1.5 mg/l BAP was optimum. Valouro and Uwezo varieties gave different shoot number in the same BAP concentration. This might be due to the genetic variation of genotypes towards *in vitro* response (Rahman *et al.,* 2005). Regeneration response of tomato to plant growth regulators (PGRs) has been highly genotype specific, and as such, the type and concentration suitable for one genotype may not be optimal for others (Frankenberger *et al*. 1981; Kurtz and Lineberger, 1983). In the present experiment the number of shoots increased with the increasing concentration of BAP in all varieties used until it reached to the optimum but decreased after 2.5 mg/l BAP. This might be due to at higher level of cytokinin reduced apical dominance, promote shoot formation and released lateral buds (Wickson and Thiemann, 1958; Kerstetter and Hake, 1997). Additionally the toxic effect of growth regulators due to their accumulation affects the growth performance of the plant (Papry *et al.,* 2016). During the present experiment, at higher concentration of BAP (after 2.5 mg/l) explants produced excessive callus and failed to improve the efficiency of shoot multiplication. According to Carimia *et al*. (2004) if added at high dosage (3 mg/l) to plants, BAP induces programmed cell death (PCD) by accelerating senescence. Similarly, Gubis *et al*. (2004) reported that the frequency of adventitious shoot regeneration differed depending on the type of explants and both the type and concentration of growth regulators added to the regeneration medium.

Similarly, Papry *et al.* (2016) reported that the increasing of BAP concentration up to 2 mg/l caused the number of shoots to continue developing, but it failed in the presence of 3mg/l BAP. Otroshy *et al.* (2013) also reported that the highest number of shoots recorded from hypocotyls and leaves explants in the presence of 2 mg/l BAP. These authors found the highest number of shoots/explant (12.25 and 7.94) from hypocotyls and leaf explants, respectively. Similarly, Hattab *et al*. (2015) used shoot tip explant derived from super Regina and Flacon tomato cultivars and reported a significant difference of shoot number/explant with the mean values of 2.7 and 2.08, respectively.

The present experiment is in agreement with that of Mohamed *et al*. (2010) who reported the highest number of shoots (3.43) on MS medium supplemented with 2 mg/l BAP. Likewise, Jehan and Hassanein (2013) reported the highest number of shoots/explant (3.67) on MS medium supplemented with 2 mg/l BAP. In another finding, Kiran (2007) reported that BAP at 1.5 and 1.0 mg/l gave the highest number of shoots per explants (5.6 and 5.2) from shoot tip culture, respectively. However, this author reported that the number of shoots/explant decreased with increase in the BAP concentration. In another study, Banu *et al*. (2017) found that BAP and Kn were used in combination (1.0 mg/l BAP + 1.0 mg/l Kn), the highest number of shoots per explant was recorded 2.28 in BH-3 followed by 2.15 in MH and 2.12 in BH-4. The present study is supported by Bhushan and Gupta (2010) who observed shoot buds initiated on cotyledonary explants on MS medium containing 2.0 mg/l BAP were sub cultured on medium supplemented with different concentrations of BAP for shoot multiplication. The results revealed that the best responses on MS medium supplemented with 2 mg/l BAP. The maximum number of shoots per explant was observed in hybrid TH802 (5.17) as compared to its parents, Accession-2 (5.05) and Haelani (4.22). Similarly, hybrid TH2312 showed maximum number of shoots per explant (5.11) as compared to its parents VFN8 (5.01) and Punjab chuhara (4.94).

Result of the present study contrasts with that of Devi *et al.* (2008) who obtained the highest shoot number per explant on the MS medium supplemented with 3 mg/l BAP+ 2.5 mg/l IAA. This may be because the combined application of both BAP and IAA might have effect on the shoot number of plants. In another experiment, Vikram *et al. (*2011) achieved best response for shoot multiplication from cotyledon explants on MS medium supplemented with 3.0-3.5 mg/l BAP in combination with 0.1mg/l IAA. Beside this the present result differed may be due to the type of explants and the interaction effect of growth regulators (BAP and IAA) added to the culture medium. Palana *et al*. (2005) also reported that tomato adventitious shoot capacity depend on explant source.

The maximum shoot length (5.8±0.08 cm, 5.3±0.11 cm and 5±0.36 cm) was recorded on MS medium supplemented with 0.5mg/l BAP, whereas the minimum shoot length (2.69 ±0.1 cm, 2.81 ±0.17 cm and 2.29±0.08 cm) was recorded on MS medium supplemented with 3 mg/l BAP in Valouro, Uwezo and Shelter varieties, respectively. Shoot length is better at lower

concentration of BAP. This is because the application of BAP to enhance shoots formation and increase leaf number and has lower impacts on shoot length. Similar result was also reported by Banu *et al.* (2017) who indicated that shoot length was better at lower concentration of BAP and KN for every variety and that was 0.5 mg/l. In fact, with the increase in hormone doses, shoot length decreased gradually. From the three cultivars, the longest shoot length (7.02) was recorded in BH-3 with 0.5 mg/l BAP on MS medium followed by 6.91 cm in BH-4 at 0.5 mg/l KN and in MH at 0.5 mg/l BAP. In the present experiment the increase in the level of BAP beyond 0.5 mg/l resulted in decrease in shoot length. This finding is in agreement with that of Bhatia (2003) who reported that shoot length was greater on lower concentrations of cytokinin and decreased as the cytokinin concentration increased for tomato cultivar Red coat.

Contrary to finding of the present study, Pampanna (2009) the length of shoot was influenced by different growth regulators. This author found that the highest shoot length (3.63 cm) was recorded on MS medium supplemented with higher level of BAP (4 mg/l). The investigator also reported that increasing the concentration of BAP resulted in increase in mean length of shoots. Increase in concentration of TDZ beyond 1.0 mg/l resulted in decrease in shoot length. The present result is different from this finding. This variation might be due to the genotypic variation in response to growth regulators and the difference in performance of different growth regulators towards the increase or decrease of shoot length *in vitro.* Additionally Otroshy *et al*. (2013) reported that the highest shoot length (4.5 and 4.05 cm) was obtained on MS medium augmented with 3 and 2 mg/l BAP respectively from hypocotyl and leaf explants of tomato cultivar. These authors also reported the increasing of BAP concentration up to 3 mg/l causes shoot length to continue developing, but it fell down in the presence of 4 mg/l BAP. Mohamed *et al.* (2010) also obtained the highest shoot length (1.625 and 1.15 cm) in both cultivars on MS medium supplemented with BAP at 2 mg/l for Pearl and Beril tomato varieties and there was no regeneration in control and 4 mg/l BAP supplement.

The maximum number of leaves/shoot (4±0.2 from Valouro, 3.6±0.04 from Uwezo and 3.4±0.08 from Shelter variety) were recorded from MS medium supplemented with 1.5 mg/l BAP while the minimum number of leaves/shoot (1.8±0.05, 1.91±0.11 and 1.45±0.04) were recorded from Valouro, Uwezo and Shelter varieties on MS medium supplemented with 3

mg/l BAP, respectively. This is because the application of BAP at optimum level increases cell division and increases the number of leaves. Similarly, Sherkar and Chavan (2014) found that highest number of leaves/shoot (5.0) was recorded on MS medium supplemented with 0.1 mg/l BAP +1.5 mg/l IAA and 0.1 mg/l BAP+2mg/l IAA. In another finding Papry *et al.* (2016) reported that the maximum number of leaves/plantlet was observed on lower concentration of IAA. The highest numbers of leaves (4.25, 4.5 and 5.5) were produced from leaf explants on the half strength MS medium supplemented with 0.25 mg/l IAA and the lowest number of leaves (2.3, 2.50 and 3.25) was on the hormone free 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.

2mg/l BAP 2mg/l BAP 1.5mg/l BAP

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

4.3. Effect of IBA on *In Vitro* Rooting

The ANOVA revealed that the interaction of variety with IBA highly significantly ($P<0.01$) influenced days to rooting, number of roots/plantlet and root length. Percentage of rooting showed a significant difference at $P\leq0.05$ (Appendix Table 4).

The early root induction was recorded on MS medium without hormone, which was 5 days for Valouro and Shelter varieties and 7.67 days for Uwezo variety (Table 3). The late root induction (12.67 days for Valouro, 16 days for Uwezo and 11 days for Shelter) was recorded on half strength MS medium supplemented with 1.5 mg/l IBA. Early root induction was observed in control treatment, but at higher concentration of IBA roots were formed later. This might be due to the adverse effect of auxins at higher concentration towards root formation. The effect of ethylene on root growth is largely mediated by the regulation of the auxin biosynthesis and transport dependent local auxin distribution. Ethylene stimulates auxin biosynthesis and basipetal auxin transport toward the elongation zone where it activates a local auxin response leading to inhibition of cell elongations (Ruzicka *et al*., 2007). Ethylene inhibits primary root growth by regulating cell proliferation and cell elongation in the elongation zone. Likewise, Sedaghat and Rahemil (2012) reported that higher concentration of auxins, promote ethylene production and inhibit root growth. The present result was in

agreement with that of Das (2011) reported minimum days (6-7) required for root initiation when the explants were treated with half strength MS medium supplemented with small amount of auxin (0.2 mg/l IAA). Likewise, Papry *et al.* (2016) reported that the higher concentration of IBA required more days to initiate the roots. Mukta (2014) also indicated half strength MS media supplemented with different concentrations of IAA (0.1, 0.2 and 0.5 mg/l) to produce roots. In all the three varieties, (BARI tomato 2, BARI tomato 9 and Bahar) early root induction were observed on half strength MS medium supplemented with 0.2 mg/l IAA, which were 3.60, 4.52 and 4.82 days for the three tomato varieties, respectively.

Contrary to finding of the present study, Pampanna (2009) obtained that MS media supplemented with IBA at different levels, resulted in early initiation of rooting in comparison with control treatment. Rooting was initiated early in about 6.3 days when the shootlets were placed on root induction medium consisting of MS + IBA at 1.0 mg/l and MS + IBA at 1.5 mg/l, whereas, MS + IBA at 0.5 mg/l has taken 7.5 days for the initiation of rooting. The initiation of rooting was (19.5 days) with control treatment (MS without IBA). The shootlets obtained either from cotyledon or hypocotyl explants did not differ significantly each other by taking 10.35 and 9.45 days, respectively for initiation of rooting.

The maximum number of roots (15.36±0.35, 13.06±0.83 and 18.26±0.3) was recorded on MS medium supplemented with 0.5 mg/l IBA for Valouro, Uwezo and Shelter variety, respectively. The minimum number of roots (3.3±0.2, 2.03±0.15 and 4.33±0.35) for Valouro, Uwezo and Shelter varieties, respectively, were obtained from half strength MS medium augmented with 1.5 mg/l IBA. The number of roots decreased when the concentration of IBA increased beyond 0.5 mg/l. This might be due to the increase in the level of production of the growth retardant compound ethylene at high concentration of auxins like IBA (Mensuali-Sodi *et al*., 1995). An increase in ethylene may modify the physiological and morphogenetic processes that influence the success of root regenerations (Sedaghat and Rahemil 2012). This finding is in agreement with that of Indrani *et al.* (2013) who observed that the maximum number of roots was recorded from MS medium supplemented with 0.5 mg/l IBA. Another finding also reported by Papry *et al.* (2016) who found that the highest numbers of roots (16, 21 and 25.25) were produced from leaf explants on half strength MS medium supplemented with 0.5 mg/l IAA but it required more days for root initiation. The lowest numbers of roots

were observed on half MS medium supplemented with 0.1 mg/l IAA and there was no root formation observed in hormone free media. Devi *et al*. (2008) also reported best rooting was noted in half-strength MS medium supplemented with 0.2 mg/l IBA.

This finding is in agreement with that of Alatar *et al.* (2017) who reported that *in vitro* regenerated shoots of tomato were excised and transferred to half-strength MS medium supplemented with IAA, IBA and NAA (0, 0.1, 0.5, 1.0 and 2.0 µM) for root induction. Among the different auxins used, IBA was found to be most efficient for *in vitro* root formation. Among the different IBA concentrations used, the highest number of roots (4.1±0.15) per shoots was recorded in half strength MS medium supplemented with 0.5 µM IBA. While on increasing, or decreasing the auxin concentration beyond the optimum level, there was significant reduction in rooting of micro shoots. Similarly, Indrani *et al*. (2013) found MS medium supplemented with IBA (0.5mg/l) to be the best for root induction of tomato.

The present results contrast with that of Pampanna (2009) who found the highest number of root/plantlet from MS medium supplemented with IBA at 1.0 mg/l (28.50 roots/plantlet), followed by IBA at 1.5 mg/l (27.50 roots/plantlet. This author obtained higher number of roots/plantlet from MS medium supplemented with the highest level of IBA. On the control treatment, a root number of 9.6 in cotyledon and 9.8 in hypocotyl were reported by the author. Number of roots increased as the level of IBA increased from 0.0-1.0 mg/l. Durrani *et al.* (2017) also found that regenerated shoots were shifted to media containing 2 mg/l IBA in both full strength and half MS media for rooting. Although rooting was observed in full strength and half strength MS media, longer and profuse rooting system was observed on half strength MS media containing 2 mg/l IBA. Sherkar and Chavan (2014) also reported maximum number of roots (17) from half strength MS medium containing 2 mg/l IBA.

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.

The maximum root length, 5.92 ±0.22 cm for Valouro, 5.5 ± 0.2 cm for Uwezo and 6.55± 0.01 cm for Shelter variety, was recorded from half strength MS medium supplemented with 0.5 mg/l IBA. The lowest root length was found in the highest level of IBA (1.5mg/l) which was 1.82 ± 0.01 cm for Valouro, 1.5±0.08 cm for Uwezo and 2.98 ±0.02 cm for Shelter variety, respectively. The result of the present study is consistent with that reported by Papry *et al*. (2016) who found the longest root (8.785 cm) from leaf explants at 0.5 mg/l on half strength MS medium. The shortest length of root (5.106 cm) was observed on half strength MS medium supplemented with 0.1 mg/l IAA. Similarly, El-Shafey *et al*. (2017) observed the highest root length at lower dose of IBA. The authors found maximum root length (9.1 cm) at 0.25 mg/l IBA.

Contrary to this study, Pampanna (2009) reported the highest root length (6.8 cm) was obtained on MS medium supplemented with IBA at 1.0 mg/l, followed by MS + IBA at 1.5 mg/l (6.12 cm). The lowest root length (2.06 cm) was observed with control treatment. This author found that the concentration of IBA had a marked influence on the mean length of roots.

The highest rooting percentage (100.00±0.00) was recorded from an IBA levels ranging from 0.0-0.5 mg/l IBA in all the three varieties (Table 2). Therefore using IBA free MS medium could be better for reducing the cost of PGRs. whereas, the lowest percentage of rooting (56.67±3.51, 46.67±2.08 and 60.00±2) was obtained from Valouro, Uwezo and Shelter varieties, respectively grown on half strength MS medium supplied with 1.5 mg/l IBA. The present result indicated that each genotype responded differently, which may be due to differences in their endogenous auxin concentration. Similar result was reported by Ishag *et al*. (2009) who found regenerated shoots were excised and rooted on half-strength MS medium supplemented with 0.5 mg/l IBA. These authors showed that 100% rooting was obtained in both full and half-strength MS medium supplemented with IBA ranging from 0.0-0.5mg/l. On the other study, Otroshy *et al.* (2013) obtained lower concentration of auxin to be the best for root induction. These authors showed that 100% rooting was observed on MS medium supplemented with IAA (0.1 and 0.2 mg/l). Devi *et al*. (2008) found the best rooting percentage in half strength MS medium supplemented with lower concentration of IBA. On the other study, Ouyang *et al.* (2003) observed the highest rooting percentage in media supplied with 0.5 mg/l NAA. The authors suggested that tomato possesses high levels of endogenous auxins.

This result contrasts with that report by Jamous (2013) who found that rooting percentage was higher when the dose of auxin was increased. In another experiment, El-Shafey *et al.* (2017) showed that transferring the regenerated shoots to MS media supplemented with auxins (IAA or IBA) resulted in higher percentage of rooting. These authors obtained the highest rooting percentage (72.2%) from MS medium supplemented with 0.25 mg/l IBA. The two hormone free media (½ MS or full MS) showed less effectiveness (55.5 and 57.1%) in percentage of rooting. On the other hand, Sherkar and Chavan (2014) reported that regenerated shoots were transferred for rooting to full strength and half strength MS media containing different

concentrations of (0.0, 0.5, 1,2 and 3 mg/l) IBA. There was 100% rooting response in all the media combinations as well as medium without IBA. However, half strength MS media containing 2 mg/l IBA resulted in the highest number of roots. 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 response are dependent on the amount of their endogenous auxin concentration.

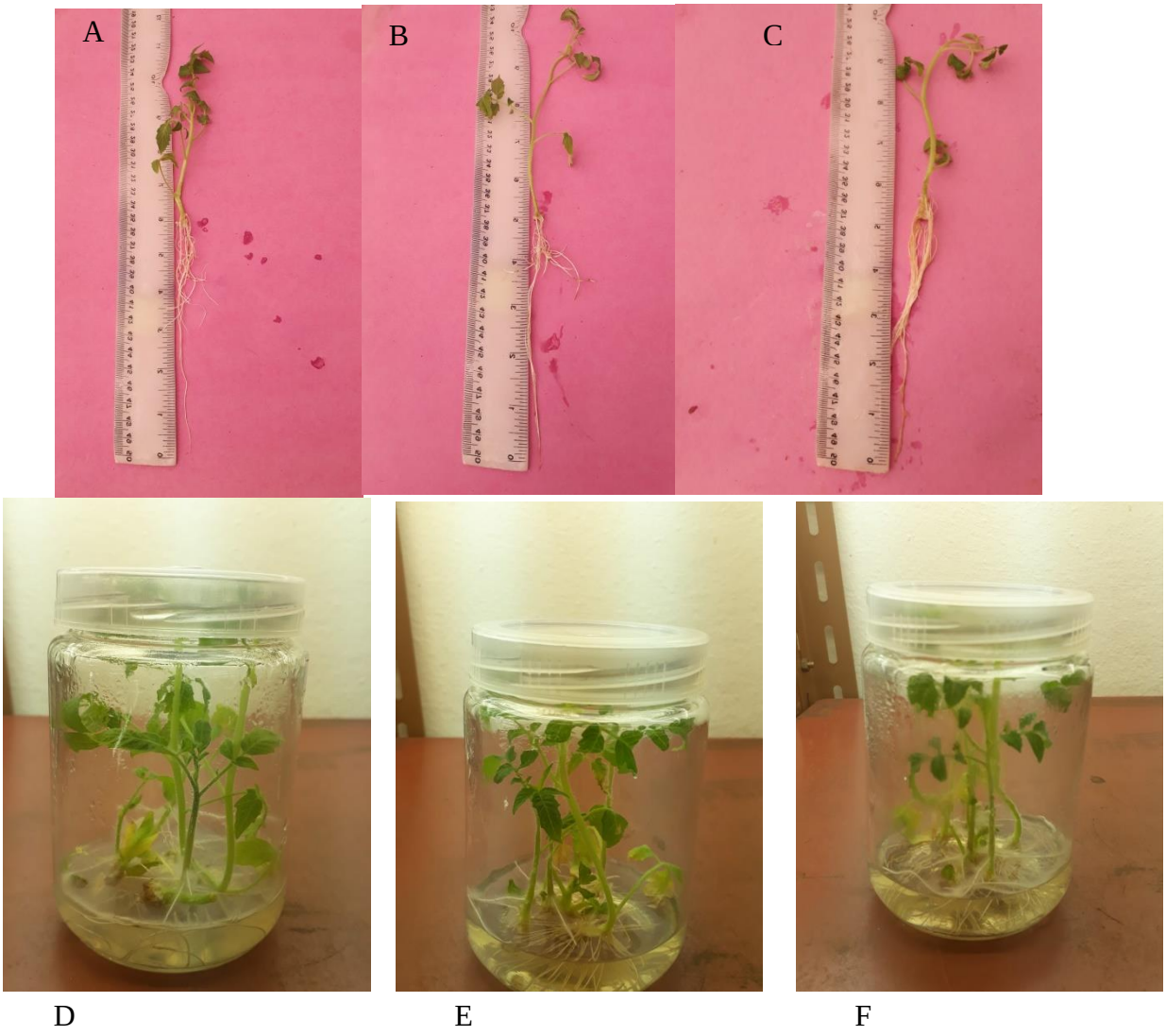


Figure 4 Invitro rooting of the three Tomato varieties; A) root morphology of Valouro, B) Root morphology of Uwezo and C) root morphology of shelter tomato variety, *D) In vitro* rooting of Valouro variety at 0.5 mg/l IBA; *E) In vitro* rooting of Uwezo variety at 0.5 mg/l IBA; F) *In vitro* rooting of Shelter variety at 0.5 mg/l IBA**.**

4.4. Acclimatization

Out of fifty three plantlets acclimatized, the survival percentages were 81.13%, 73.58% and 69.8% for Valouro, Uwezo and Shelter varieties from 1:2:1 ratio respectively (Figure 5). The survival percentage obtained from 2:1:1 ratio were 67.92%, 58.49% and 62.26 for Valouro Uwezo and Shelter varieties, respectively while the lowest survival percentage (54.71%, 49.05% and 56.6%) were achieved from different mixture of soil at the ratio of 2:1:0 for valouro, Uwezo and Shelter varieties, respectively. From this finding, different composition of soil containing an oven sterilized soil, sand, and compost at the ratio of 1:2:1 was optimum for highest survival percentage for all the three varieties (figure 7). Some plantlets failed to survive after being transferred to the lath house. This may be due to the change in the environmental condition which might have affected the growth performance of the plants. Similarly, Namitha and Negi (2013) reported that the highest survival percentage of *in vitro* grown tomato varieties was 70%-80%. In another finding, Das (2011) also reported 80%-90% survival for five tomato varieties. On the other hand, Banu *et al.* (2017) reported that regenerated plantlets of tomato were transferred in plastic pots containing garden soil and compost-sand mixed soil after hardening and were subsequently acclimatized to *in vivo* condition. These authors observed that 90% plantlets survived in external environment.

Table 4 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%

Valouro **Uwezo** **Shelter**

Figure 5 Acclimatized plantlets of three tomato varieties

5. SUMMARY AND CONCLUSION

Tomato (*Solanum lycopersicum L.*) is one of the most important vegetables. It is a perennial plant, but due to its economic and commercial importance it is cultivated as an annual crop and grows almost in every country of the world.

The addition of BAP wasn't essential for days to shoot initiation and percentage of initiation for all the three varieties. Early shoot initiation took 5 days for Valouro in MS medium without BAP (control). The highest percentage of initiation (92%) was obtained from MS medium without growth regulators. For shoot initiation, hormone free MS medium was optimum for Valouro variety. In multiplication stage, the highest number of shoot (5) was recorded from MS medium augmented with 2 mg/l BAP. The highest shoot length (5.8) was recorded at lower dose of (0.5 mg/l) BAP. The maximum numbers of leaves/shoot (4) was obtained at 1.5 mg/l BAP. For Valouro multiplication, 2 mg/l BAP was optimum for shoot multiplication. The minimum time required for root induction was recorded on hormone free media, which were 5 days for Valouro variety. Concentration of IBA ranging from 0.0-0.5 mg/l resulted in 100% rooting. The maximum numbers of roots (15.36) with an average root length (5.92 cm) was achieved at 0.5 mg/l IBA. Half strength MS medium supplemented with 0.5 mg/l IBA was optimum for in vitro rooting for valouro varieties. During acclimatization the highest survival percentage (81.13%) was obtained from 1:2:1 ratio for Valouro variety.

For Uwezo variety, the earliest shoot initiation (6.4 days) was recorded from hormone free MS medium. The highest percentage (92%) of initiation was also achieved from hormone free MS medium. For Uwezo variety hormone free MS medium was optimum for shoot initiation. The addition of BAP was essential for shoot multiplication. Increasing the concentration of BAP (up to 2 mg/l) significantly increased the multiplication efficiency of Uwezo variety. The highest number of shoots (4.3) was recorded from MS medium supplemented with 2 mg/l BAP. The highest shoot length (5.3 cm) was recorded at lower dose of BAP (0.5 mg/l). The maximum number of leaves/shoot (3.6) was found at 1.5 mg/l BAP. For Uwezo variety, MS medium supplemented with 2 mg/l BAP was optimum for shoot multiplication. The multiplied shoots of Uwezo variety was transferred to half strength MS medium supplemented with different concentrations of IBA. The minimum time required for root

induction was recorded on hormone free media, which was 7.67days for Uwezo variety. The highest numbers of roots (13.06) with an average root length of 5.5 cm was recorded on 0.5 mg/l IBA. Concentration of IBA ranging from 0.0-0.5 mg/l resulted in 100% rooting. For Uwezo variety 0.5 mg/l was optimum for *in vitro* rooting. During acclimatization the highest survival percentage was 73.58% obtained from 1:2:1 ratio for Uwezo variety.

For shoot initiation of Shelter variety the earliest shoot initiation (5.2 days) was obtained from hormone free MS medium. Additionally the maximum percentage of shoot (92%) was obtained from hormone free MS medium. For shelter variety hormone free MS medium without growth regulator was optimum for shoot initiation. The maximum number of shoots/explant (4) was recorded from MS medium supplemented with 1.5 mg/l BAP. The highest shoot length (5 cm) was recorded at lower dose of BAP (0.5 mg/l). The maximum numbers of leaves/shoot (3.4) was found at 1.5 mg/l BAP. For Shelter variety MS medium supplemented with 1.5 mg/l BAP was optimum for shoot multiplication. The minimum time required for root induction (5 days) was recorded on hormone free media for Shelter variety. The maximum numbers of roots (18.26) with an average root length of 6.55 cm was recorded on 0.5 mg/l IBA. Concentration of IBA ranging from 0.0-0.5 mg/l resulted in 100% rooting. For Shelter variety 0.5 mg/l IBA was optimum for root induction. During acclimatization the highest survival percentage was 69.8% obtained from 1:2:1 ratio for Shelter variety.

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

Future line of work

This study focused only on the effect of BAP hormones alone for shoot multiplication, further research is suggested to be done on multiplication performance by using other combinations of hormones and by taking other explant sources.

6. REFERENCES

Abdellatef, E. and Khalafallah, M.M., 2007. Adventitious shoot formation and plant regeneration in medium staple cotton (Gossypium hirsitum L.) cultivar (Barac B-67), *International journal of biology. J. Agri. Biol*, **9(6):** 913-916.

Abdu, J., 2016. *A Comparative Study on Selected Tomato Varieties for Manufacturing Paste* (Doctoral dissertation, Addis Ababa University).

Abu-El-Heba, G.A., Hussein, G.M. and Abdalla, N.A., 2008. A rapid and efficient Tomato regeneration and transformation system. *Landbauforschung Volkenrode*, **58(1-2):** 103.

Alatar, A.A., Faisal, M., Abdel-Salam, E.M., Canto, T., Saquib, Q., Javed, S.B., El-Sheikh, M.A. and Al-Khedhairy, A.A., 2017. Efficient and reproducible in vitro regeneration of Solanum lycopersicum and assessment genetic uniformity using flow cytometry and SPAR methods. *Saudi journal of biological sciences*, **24(6):** 1430-1436.

Ashakiran, K., Sivankalyani, V., Jayanthi, M., Govindasamy, V. and Girija, S., 2011. Genotype specific shoots regeneration from different explants of Tomato (Solanum lycopersicum L.) using TDZ. *Asian Journal of Plant Science and Research*, **1(2):** 107-113.

Atherton, J.G. and Rudich J., 1988. In Tomato crop; Scientific Basis for Improvement. Chapman and Hall, London, New York. 661p.

Bahurupe, J.V., Patil, S.C., Pawar, B.D., Chimote, V.P. and Kale, A.A., 2013. Callus Induction and Plantlet Regeneration in Tomato (*Solanum Lycopersicum* L.). *Journal of Cell & Tissue Research*, **13(2)**.

Bai, Y. and Lindhout, P., 2007. Domestication and breeding of Tomatoes: what have we gained and what can we gain in the future. *Annals of botany*, **100(5):** 1085-1094.

Banks, P., 1984. A new diploid chromosome number for tomato (Lycopersicon esculentum)?. *Canadian journal of genetics and cytology*, ***26*(5)**: 636-639.

Banu, N. A. Islam, S., Islam, M.A. and Alam, M.K., 2017. *In vitro* propagation from shoot tip and nodal segment in summer Tomato (*L. esculentum* Mill.). *Plant Environment Development.* **6(1):** 31-38.

Bhatia, P., 2003. Regeneration, micropropagation, and somatic embryogenesis in Tomato *(Lycopersicon esculentum* Mill*.)*. A thesis submitted for the degree of Doctor of Philosophy, School of Biological and Environmental Sciences, Central Queensland University, Queensland, Australia.305p.

Bhatia, P., Ashwath, N. and Senaranta, T., 2004. Effect of cytokinins on organogenesis and callus induction in cotyledonary explants of Tomato (*L. esculentum* Mill). *Enfield,* 17-24p.

Bhushan, A. and Gupta, R.K., 2010. Adventitious shoot regeneration in different explants of six genotypes of Tomato. *Indian Journal of Horticulture*, **67*(1)*:** 224-227.

Bhushan, A., 2001. Micropropagation Studies in Selected Genotypes of Tomato *(Lycopersicon Esculentum Mill.)* (Doctoral dissertation, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu).

Brandt, S., Pék, Z., Barna, É. Lugasi, A. and Helyes, L., 2006. Lycopene content and color of ripening Tomatoes as affected by environmental conditions. *Journal of the Science of Food and Agriculture*, **86(4):** 568-572.

Carimia F, Terzi M, De Michele R, Zottini M & Lo Schiavo F., 2004. High levels of the cytokinin BAP induced PCD by accelerating senescence. *Plant Science* **166(4):** 963–969.

Chaudhry, Z., Abbas, S., Yasmin, A., Rashid, H., Ahmed, H. and Anjum, M.A., 2010. Tissue culture studies in Tomato (Lycopersicon esculentum) var. moneymaker. *Pakistan journal of botany*, **42(1):** 155-163.

Chaudhry, Z., Habib, D., Rashid, H. and Qureshi, A.S., 2004. Regeneration from various explants of in vitro seedling of Tomato (Lycopersicon esculentum L., cv. Roma). *Pakistan journal of biology. Sci*, **7(2):** 269-272.

Cheema, D.S. and Dhaliwal, M.S., 2005. Hybrid Tomato breeding. *Journal of New Seeds*, **6(2-3):** 1-14.

Chen, H., Zhang, J., Zhuang, T. and Zhou, G., 1999. Studies on optimum hormone levels for Tomato plant regeneration from hypocotyl explants cultured in vitro. *Shanghai Nongye Xuebao*, **15(2):** 26-29.

Clinton, S.K., 1998. Lycopene: chemistry, biology, and implications for human health and disease. *Nutrition reviews*, **56(2):** 35-51.

Costa, G.M., Nogueira, F.T.S., Otoni, W.C. and Brommonschenkel, S.H., 2000a, In vitro regeneration of processing Tomato (Lycopersicon esculentum Mill.)'IPA-5'and'IPA-6'. *Ciencia e Agrotecnologia*, **24(3):** 671-678.

Costa, M.G.C., Nogueira, F.T.S., Figueira, M.L., Otoni, W.C., Brommonschenkel, S.H. and Cecon, P.R., 2000b. Influence of the antibiotic timentin on plant regeneration of tomato (Lycopersicon esculentum Mill.) cultivars. *Plant Cell Reports*, **19(3):** 327-332.

CSA, 2011. Report on area and production of crops. Central Statistical Agency, Agricultural Sample Survey, Addis Ababa, Ethiopia, Statistical Bulletin 12-18p.

CSA, 2018. Agricultural sample survey. Volume I. Report on area and production of major crops (private peasant holdings, meher season) Addis Ababa, Ethiopia. Statistical bulletin 586p

Das, A., 2011. *Effect of growth regulators on shoot induction and plant regeneration in Tomato (Lycopersicon esculentum Mill.)* (Doctoral dissertation, BRAC University).

Debela, K.B., Belew, D. and Nego, J., 2016. Evaluation of Tomato (Lycopersicon Esculentum Mill.) Varieties for Growth and Seed Quality under Jimma Condition, South Western Ethiopia. *International Journal of Crop Science and Technology*, **2(2)**.

Devi, R., Dhaliwal, M.S., Kaur, A. and Gosal, S.S., 2008. Effect of growth regulators on in vitro morphogenic response of Tomato.

Dorais, M., A. P. Papadopoulos, and A. Gosselin. 2001. Greenhouse Tomato fruit quality. *Horticultural Review* **26:** 239-27.

Durrani, N.U.S., Ahmad, D., Jalal, A., Rajab, H. and Khan, M.S., 2017. The effect of explant sources and growth regulators on callus induction and regeneration in different Tomato cultivars. *JAPS: Journal of Animal & Plant Sciences*, **27(2)**.

Dwivedi, K., Srivastava, P., Verma, H.N. and Chaturvedi, H.C., 1990. Direct regeneration of shoots from leaf segments of Tomato (Lycopersicon esculentum) cultured in vitro and production of plants. *Indian Journal of Experimental Biology*, **28(1):** 32-35.

El-Bakry, A.A., 2002. Effect of genotype, growth regulators, carbon source, and pH on shoot induction and plant regeneration in Tomato. *In Vitro Cellular & Developmental Biology-Plant*, **38(5):** 501-507.

Ellul, P.B., Sogo, G., Pineda, B., Rios, G., Roig, L.A. And Moreno, V., 2003,The Ploidy Level Of Transgenic Plants In *Agrobacterium*-Mediated Transformation Of Tomato Cotyledons (*Lycopersicon Esculentum* Mill.) is Genotype and Procedure Dependent. *Theoretical and Applied Genetics*, **106:** 231-238.

El-Shafey, N.M., Hassan, N., Khodary, S.E.D.A. and Badr, A., 2017. Differential In vitro Direct Regeneration of Tomato Genotypes on Various Combinations of Growth Regulators. *Biotechnology*, **16(4-6):** 155-164.

FAO STAT, 2012. Statistical database of the food and agriculture organization of the United Nations. Food and Agriculture Organization (FAO),Rome, Italy. *http://faostat.fao.org/.*

FAO STAT, 2016. Food and Agriculture Organization of the United Nations data.(http://www.fao.org/faostat/en/#data/QC/visualize (19/12/2017).

Fentik, D.A., 2017. Review on genetics and breeding of Tomato (Lycopersicon esculentum Mill.). *Advances in Crop Sciences and Technology*, **5(5):** 306.

Foolad, M.R., 2007. Genome mapping and molecular breeding of Tomato. *International journal of plant genomics, 2007*.

Fowler, M.R., 2000. Plant cell culture, laboratory techniques. In: Spier, RE. Encyclopedia of Cell Technology. New York: John Wiley and Sons, 994-1002.

Frankenberger, E. A., Hasegawa, P. M., and Tigchelaar, E. C. 1981. Influence of environment and developmental state on the shoot-forming capacity of Tomato genotypes. *Z. Pflanzenphysiol,* **102:**221–232.

Gemechis, A.O., Struik, P.C. and Emana, B., 2012. Tomato production in Ethiopia: constraints and opportunities. *Tropentag 2012, International Research on Food Security, Natural Resource Management and Rural Development. Resilience of Agricultural Systems against Crises: Book of Abstracts*, 373.

George, E.F. and P.D. Sherrington. 1984. Plant propagation by tissue culture. Exegetic Ltd., Basingtoke. U.K. 709p.

George, E.F., Hall, M.A. and De Klerk, G.J., 2008. The components of plant tissue culture media I: macro-and micro-nutrients. In *Plant propagation by tissue culture Springer, Dordrecht.* 65-113.

George, R. A.T., 2009. Vegetable seed production (3rd ed). CABI International, Nosworthy Way, Oxfordshire, USA. 203-214.

Georgiev, H., 1991. Heterosis in Tomato breeding. In *Genetic improvement of Tomato.* Springer, Berlin, Heidelberg. 83-98.

Gerster, H., 1997. The potential role of lycopene for human health. *Journal of the American College of Nutrition*, **16(2):**109-126.

Gerszberg, A., Hnatuszko-Konka, K., Kowalczyk, T. and Kononowicz, A.K., 2015. Tomato (Solanum lycopersicum *120*(3), L.) in the service of Biotechnology. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 881-902.

Giovannucci, E., 1999. Tomatoes, Tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *Journal of the national cancer institute*, **91(4):** 317-331.

Goode, D. A., 1989. Quality of life, quality of work life. In W. E. Kiernan and R. L. Schaloch (Ed), Economics, industry and disability: *A look abead Baltimore: Brookes*. 337-349.

Gresshoff, P.M. and Doy, C.H., 1972. Development and differentiation of haploid Lycopersicon esculentum (Tomato). *Planta*, **107(2):**161-170.

Gubis, J., Lajchová, Z., Faragó, J. and Jureková, Z., 2004. Effect of growth regulators on shoot induction and plant regeneration in Tomato (Lycopersicon esculentum Mill.). *Biologia-Bratislava-*, **59(3):**405-408.

Gubiš, J., Lajchova, Z., Klčová, L. and Jureková, Z., 2005. Influence of growth regulators on plant regeneration in Tomato. *Journal of Horticultural Science (PRAGUE)*, **32(2005):**3.

Gunay, A.L. and Rao, P.S., 1980. In vitro propagation of hybrid Tomato plants (Lycopersicon esculentum L.) using hypocotyl and cotyledon explants. *Annals of Botany*, **45(2):**205-207.

Hamza, S. and Chupeau, Y., 1993. Re-evaluation of conditions for plant regeneration and Agrobacterium-mediated transformation from Tomato (Lycopersicon esculentum). *Journal of Experimental Botany*, **44(12):** 1837-1845.

Hanus Fajerska, E., 2005, September. Variation in Tomato Plants Regenerated from Cucumber Mosaic Virus Infected Tissue. In *XV Meeting of the EUCARPIA Tomato Working Group* **789:** 299-302.

Harish, M.C., Rajeevkumar, S. and Sathishkumar, R., 2010. Efficient in vitro callus induction and regeneration of different Tomato cultivars of India. *Asian journal of Biotechnology*, **2(3):** 178-184.

Hattab, Z.N. Al., Qaudhy, W. K. Al., Razaq, A. A., Kaaby, E. A. Al. and Ani, J.A. A.l., 2015. *Invitro* regeneration of Tomato (*Lycopersicon esculentum* Mill.) Plants under drought Stress. *International Journal Multidisciplinary and Current Research.* **3:** 1194-1198.

Hu, W. and Phillips, G.C., 2001. A combination of overgrowth-control antibiotics improves Agrobacterium tumefaciens-mediated transformation efficiency for cultivated Tomato (L. esculentum). *In Vitro Cellular & Developmental Biology-Plant*, **37(1):** 12-18.

Hussey, G., 1971. In vitro growth of vegetative Tomato shoots apices. *Journal of Experimental Botany*, **22(3):** 688-701.

Indrani, C., Priyanka, S., Arijit, B., Priya, S., Sana, J. and Autashi, S., 2013. In vitro callus induction, regeneration and micropropagation of Solanum lycopersicum. *International Journal of Current Microbiology and Applied Sciences*, **2(12):**192-197.

Ishag, S., Osman, M.G. and Khalafalla, M.M., 2009. Effects of growth regulators, explant and genotype on shoot regeneration in Tomato (Lycopersicon esculentum cv Omdurman). *International Journal of Sustain Crop Prod*, ***4***, 7-13.

Izadpanah, M. and Khoshkhoy, M., 1989. Comparisons of in vitro propagation of Tomato cultivars. *Iran Agricultural Research*, **8(1):** 37-47.

Jamous, F.H., 2013. *In Vitro* Regeneration of selected local Tomato Cultivars in Palestine (Doctoral dissertation).

Janani, C., Girija, S. and Kumari, B.R., 2013. In vitro culture and Agrobacterium mediated transformation in high altitude Tomato (Lycopersicon esculentum Mill.) cultivar Shalimar. *International Journal*, *4*, 483-488.

Jatoi, S.A., Sajid, G.M., Sappal, H.U., Baloch, M.S., Quraishi, A. and Anwar, R., 2001. Differential in vitro response of Tomato hybrids against a multitude of hormonal regimes. *OnLine Journal of Biological Sciences (Pakistan).*

Jawahar, M., Mohamed, S.V. and Jayabalan, N., 1997. In vitro callus culture and plant regeneration from different explants of Lycopersicon esculentum Mill. *Journal of Phytol logical Research*, **10(1-2):** 75-78.

Jehan, S. and Hassanein, A.M., 2013. Hormonal requirements trigger different organogenic pathways on Tomato nodal explants. *American Journal of Plant Sciences*, **4(11):** 2118.

Jenkins, J.A., 1948. The origin of the cultivated Tomato. *Economic Botany*, **2(4):** 379-392.

Kahsay, Y., 2016. Open access Scope and Status of Vegetable Seed Production in Ethiopia. pp. 1-15.

Kalimuthu, K., Saravanakumar, M. and Senthilkumar, R., 2007. In vitro micropropagation of Musa sapientum L.(Cavendish Dwarf). *African Journal of Biotechnology*, **6(9)**.

Kalloo G 1991. Genetic Improvement of Tomato. Kalloo G (Eds) monographs of Theoretical and Applied Genetics Springer-verlag, Berlin, Heidelberg, New York, 1-9

Kalloo, G., 2012. Genetic improvement of Tomato. Vol. 14. *Springer Science and Business Media*.

Kelley, W. T. and Boyhan, G., 2014. Commercial Tomato production handbook. *UGA Extension* . 1-48.

Kerstetter, R. A. and Hake, S., 1997. Shoot meristem formation in vegetative development. *The Plant Cell*. **9**:1001-1010.

Khan, M.S., Usman, M. and Lilla, M.I., 2006. Facile plant regeneration from Tomato leaves induced with spectinomycin. *Pakistan Journal of Botany*, **38(4):** 947.

Khuong, T.T.H., Crété, P., Robaglia, C. and Caffarri, S., 2013. Optimisation of Tomato Micro-tom regeneration and selection on glufosinate/Basta and dependency of gene silencing on transgene copy number. *Plant cell reports*, **32(9):** 1441-1454.

Kimura, S. and Sinha, N., 2008. Tomato (Solanum lycopersicum): a model fruit-bearing crop. *Cold Spring Harbor Protocols*, **2008(11):** 105.

Kiran, B.U., 2007. Studies on the development of transgenic Tomato (*L.esculentum* Mill. var Pusa Ruby) Via Agrobacterium and biolistic methods, 163

Kubota, C., Kakizaki, N., Kozai, T., Kasahara, K. and Nemoto, J., 2001. Growth and net photosynthetic rate of Tomato plantlets during photoautotrophic and photo mixotrophic micropropagation. *Horticultural Science*. **36(1):** 49-52.

Kumar, N. and Reddy, M.P., 2011. In vitro plant propagation: a review. *Journal of forest and environmental science*, **27(2):** 61-72.

Kurtz, S.M. and Lineberger, R.D., 1983. Genotypic differences in morphogenic capacity of cultured leaf explants of Tomato [Lycopersicon esculentum]. *Journal-American Society for Horticultural Science (USA).*

Lee JM, Oda M., 2003. Grafting of Herbaceous vegetables and Ornamental Crops. Hort. Revi. In: Janick J. Horticultural reviews. *John Wiley & Sons. New York, NY*. **28:** 61-124.

Li, T., Sun, J.K., Lu, Z.H. and Liu, Q., 2011. Transformation of HBsAg (hepatitis B surface antigen) gene into Tomato mediated by Agrobacterium tumefaciens. *Czech Journal of Genetics and Plant Breeding*, **47(2):** 69-77.

Ling, H.Q., Kriseleit, D. and Ganal, M.W., 1998. Effect of ticarcillin/potassium clavulanate on callus growth and shoot regeneration in Agrobacterium-mediated transformation of Tomato (Lycopersicon esculentum Mill.). *Plant cell reports*, **17(11):** 843-847.

Liza, L.N., Nasar, A.N.M., Zinnah, K.M.A., Chowdhury, M.A.N. and Ashrafuzzaman, M., 2013. In vitro growth media effect for regeneration of Tomato (Lycopersicon esculentum) and evaluation of the salt tolerance activity of callus. *Journal of Agriculture and Sustainability*, **3(2)**.

Mamidala, P. and Nanna, R.S., 2011. Effect of genotype, explant source and medium on in vitro regeneration of Tomato. *International Journal of Genetics and Molecular Biology*, **3(3):** 45-50.

Mandal, A.B. and T. E. Sheeja. 2003. *In vitro* flowering and fruiting in Tomato (*Lycopersicon esculentum* Mill.) Asia Pac.J.Mol.Biol.Biotechnology, 37-42.

MARDPR, 2016. Plant and Animal Quarantine Regulatory, Ethiopian Ministry of Agriculture. Variety Release Booklet. Addis Ababa, Ethiopia

Mensuali-Sodi, A., Panizza, M. and Tognoni, F., 1995. Endogenous ethylene requirement for adventitious root induction and growth in Tomato cotyledons and lavandin microcuttings in vitro. *Plant Growth Regulation*, **17(3):** 205-212.

MoANR (Minister of Agriculture and Natural Resource). 2016. Plant variety release, protection and seed quality control directorate. Addis Ababa, Ethiopia. 188-189**.**

Moghaieb, R.E., Saneoka, H. and Fujita, K., 1999. Plant regeneration from hypocotyl and cotyledon explant of Tomato (*L.* esculentum Mill.). *Soil science and plant nutrition*. **45(3):** 639-64638.

Moghaieb, R.E., Saneoka, H. and Fujita, K., 2004. Shoot regeneration from GUS-transformed Tomato (Lycopersicon esculentum) hairy root. *Cellular and Molecular Biology Letters*, ***9*(3):** 439-450.

Mohamed, A.A.N., Ismail, M.R. and Rahman, M.H., 2010. In vitro response from cotyledon and hypocotyls explants in Tomato by inducing 6-benzylaminopurine. *African Journal of Biotechnology*, **9(30):** 4802-4807.

Mohamed, A.N., Ismail, M.R., Kadir, M.A. and Saud, H.M., 2011. In vitro performances of hypocotyl and cotyledon explants of Tomato cultivars under sodium chloride stress. *African Journal of Biotechnology*, **10(44):** 8757-8764.

Mukta, F.A., 2014. *Study of in vitro regeneration and transformation parameters for the development of transgenic Tomato (Solanum Lycopersicum L.)* (Doctoral dissertation, BRAC University).

Murashige, T. And Skoog, I., 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. Plant pathology **15:** 473-479

Namitha, K.K. and Negi, P.S., 2013. Morphogenetic Potential of Tomato (Lycopersicon esculentum) cv. 'Arka Ahuti'to Plant Growth Regulators. *Notulae Scientia Biologicae*, **5(2):** 220-225.

Norton, J.P. and Boll, W.G., 1954. Callus and shoot formation from Tomato roots in vitro. *Science*, **119(3085):** 220-221.

Onduso, J.N., 2014. *Management of bacterial wilt of Tomato by use of resistant rootstock* (Doctoral dissertation, University of Nairobi).

Osman, M.G., Elhadi, E.A. and Khalafalla, M.M., 2010. Callus formation and organogenesis of Tomato (Lycopersicon esculentum Mill, CV Omdurman) induced by thidiazuron. *African Journal of Biotechnology*, **9(28):** 4407-4413.

Otroshy, M., Khalili, Z., Ebrahimi, M.A., Nekoui, M.K. and Moradi, K., 2013. Effect of growth regulators and explant on plant regeneration of Solanum lycopersicum L. var. cerasiforme. *Russian agricultural sciences*, **39(3):** 226-235.

Ouyang, B., Li, H. and Ye, Z., 2003. Effects of Zeatin and IAA on plant regeneration of Tomato cotyledon explants. *Plant Physiology Communications*, **39(3):** 217-218.

Palana, D., Marta, A., Regla, M.L., Marilyn, F., Alvarez, F., and Moya, C. 2005. A new *in vitro* regeneration protocol in Tomato (*Lycopersicon esculentum*). *Cultivos Tropicales,* **26(2)** 17-20.

Pampanna, Y., 2009. *Studies on In Vitro Regeneration And Transformation of Tomato (Lycopersicon Esculentum L.) CV. VYBHAV with Chitinase Gene* (Doctoral Dissertation, University of Agricultural Sciences Gkvk, Bengaluru).

Papry, M., Ahsan, S.M., Shahriyar, S., Sathi, M.A., Howlader, P., Robbani, M., Akram, S. and Biswas, M.J.H., 2016. In vitro regeneration protocol development via callus formation from leaf explants of Tomato (Solanum lycopersicon Mill.). *Tropical Plant Research*, **3(1):** 162-171.

Peralta, I.E., Spooner, D.M., Razdan, M.K. and Mattoo, A.K., 2006. History, origin and early cultivation of Tomato (*Solanaceae*). *Genetic improvement of solanaceous crops*, ***2*:** 1-27.

Rahman, M.M. and Kaul, K., 1989. Differentiation of sodium chloride tolerant cell lines of Tomato (Lycopersicon esculentum Mill.) cv. Jet Star. *Journal of plant physiology*, **133(6):** 710-712.

Rahman, M.Z, Rahman, M.H, Mullah, M.U, Nahar, N., Sultana, R.S, Bari, M.A and Hossain, M., 2005.*In vitro* multiplication and rooting of a dessert banana (*musa spp, cv.* Anupom).*Pakistan journal of biological science***, 8 (9):** 1298-1302.

Rashid, R. and Bal, S.S., 2010. Effect of hormones on direct shoot regeneration in hypocotyl explants of Tomato. *Notulae Scientia Biologicae*, **2(1):** 70-73.

Reddy, M.P., 2011. *In vitro* Plant Propagation: A Review. *Journal Forest Science*. **27(2):** 61-72.

Ribeiro, T.O. and Carneiro, M.F., 1989. Micropropagation by nodal culture of cultivars Caturra, Geisha and Catimor regenerated in vitro. In *13 Colloque Association Scientifique Internationale du café*. Paris: asic.

Rick, C. M., 1980. Hybridization of Crop Plants. Am. Soc. Agron./Crop Sci. Soc. Am. Madison, WI, USA, 669-680.

Ružić, D.V. and Vujović, T.I., 2008. The effects of cytokinin types and their concentration on in vitro multiplication of sweet cherry cv. Lapins (Prunus avium L.). *Horticultural Science*, **35(1):** 12-21.

Ruzick, k.,Ljung, K.,Vanneste,S., Podhorska, R., Beeckman,T., Frim, J.,2007 ethylene regulates root growth through effect on auxin biosynthesis and transport dependent auxin distribution. Plant cell 19, 2197- 2212. *doi:101105/tpc.107.052126*

Sarker, I., Islam, J., Shaekh, M. P. E., Rahman, M. M., Khan, H., Chowdhury, K. and Haque, R., 2015. Establishment of a Standard Protocol for *In Vitro* Meristem Culture and Plant Regeneration of Citrus. *Aurantifollia*. **10(2):** 61-69.

Sedaghat, S. and Rahemi, M., 2012. Root Regeneration in Pistachio Rootstock is affected by Auxin and Polyamines. *Journal of Nuts.* **3(3):** 55-61.

Shah, S.H., Ali, S., Jan, S.A. and Ali, G.M., 2014. Assessment of carbon sources on in vitro shoot regeneration in Tomato. *Pakistan Journal of Agricultural Sciences*, **51(1):** 197-207.

Sherkar, H.D. and Chavan, A.M., 2014. Studies on callus induction and shoot regeneration in Tomato. *Science Research Reporter*, **4(1):** 89-93.

Shibli, R.A., 1995. Jordan's Tissue Culture Industry Makes Great Strides. *Diversity Arlington then washington-*, **11:** 77-77.

Shtereva, L.A., Zagorska, N.A., Dimitrov, B.D., Kruleva, M.M. and Oanh, H.K., 1998. Induced androgenesis in Tomato (Lycopersicon esculentum Mill). II. Factors affecting induction of androgenesis. *Plant Cell Reports,* **18(3-4):** 312-317.

Sink, K. C. and Reynolds, J.F., 1986.Tomato (*Lycopersicon esculentum* Mill.). Biotechnology in agriculture and forestry. *Springer-Verlag Berlin Heidelberg.* **2**:319-344.

Soressi, G.P., Cammareri, G. and Picarella, M.E., 2007, September. Improvement of in vitro

vegetative propagation technique in Tomato (Solanum lycopersicum). In *III International Symposium on Acclimatization and Establishment of Micropropagated Plants,* **812*:*** 283-288.

Takashina, T., Suzuki, T., Egashira, H. and Imanishi, S., 1998. New molecular markers linked with the high shoot regeneration capacity of the wild Tomato species Lycopersicon chilense. *Japanese Journal of Breeding*, **48(2):** 109-113.

Thiem, B., 2003. In vitro propagation of is flavone producing Pueraria lobata (Willd.) Ohwi. *Plant science*, **165(5):** 1123-1128.

Tilye, F., Welander, M. and Negash, L., 2005. Micropropagation of Hagenia abyssinica: a multipurpose tree. *Plant cell, tissue and organ culture*, **80(2):** 119-127.

Tindall, H.D., 1983. *Vegetables in the Tropics*. Macmillan International Higher Education. *London*, 354-359

Tindall, H.D., 1986. Vegetables in the Tropics: Mac-Millan Education Ltd. Hound mills. *Hampshire*, 533.

Tindall, H.D., 1993. Vegetables in the tropics The Macmillan press Ltd. *Hound mills, Basingstoke, Hampshire*, 553.

Ulrich, J.M. and Mackinney, G., 1969. Callus Cultures of Tomato Mutants: I. Nutritional Requirements. *Physiologia Plantarum*, **22(6):** 1282-1287.

Venkatachalam, P., Geetha, N., Priya, P., Rajaseger, G. and Jayabalan, N., 2000. High frequency plantlet regeneration from hypocotyl explants of Tomato (Lycopersicon esculentum Mill.) via organogenesis. *Plant Cell Biotechnology and Molecular Biology*, **1(3/4):** 95-100.

Vikram, G., Srikanth, K. and Swamy, N.R., 2011. Effect of plant growth regulators on in vitro organogenesis in cultivated Tomato (Solanum lycopersicum L.). *Journal of Biological research*, **1(4):** 263-268.

Wayase, U.R. and Shitole, M.G., 2014. Effect of plant growth regulators on organogenesis in Tomato (Lycopersicon esculentum Mill.) cv. Dhanashri. *International Journal of Pure and Applied Sciences and Technology*, **20(2):** 65.

Wickson, M. and Thimann, K.V., 1958. The antagonism of auxin and Kinetin in apical dominance. *Physiologia Plantarum.* **11**:62-74.

Wijbrandi, J., Vos, J.G.M. and Koornneef, M., 1988. Transfer of regeneration capacity from Lycopersicon peruvianum to L. esculentum by protoplast fusion. In *Progress in Plant Protoplast Research*. Springer, Dordrecht. 227-230.

Wing, R.A., Zhang, H.B. and Tanksley, S.D., 1994. Map-based cloning in crop plants. Tomato as a model system: I. Genetic and physical mapping of jointless. *Molecular and General Genetics MGG*, **242(6):** 681-688.

Worku, M. and Sahe, S., 2018. Review on Disease Management Practice of Tomato Wilt Caused Fusarium oxysporum in Case of Ethiopia. *Journal of Plant Pathol Microbiol*, **9(460):** 2.

Yarra, R., He, S.J., Abbagani, S., Ma, B., Bulle, M. and Zhang, W.K., 2012. Overexpression of a wheat Na+/H+ antiporter gene (TaNHX2) enhances tolerance to salt stress in transgenic Tomato plants (Solanum lycopersicum L.). *Plant Cell, Tissue and Organ Culture (PCTOC)*, **111(1):** 49-57.

Yasmeen, A., 2009. An improved protocol for the regeneration and transformation of Tomato (cv Rio Grande). *Acta physiologiae plantarum*, **31(6):** 1271.

Zhang, W., Hou, L., Zhao, H. and Li, M., 2012. Factors affecting regeneration of Tomato cotyledons. *Bioscience Methods*, *3*.

7. APPENDICES

Appendix Table 1. Components of Murashige and Skoog (1962) stock solutions with their concentrations

Stock type	Components	concentration Mg/l	Stock solution	Amount required in liter
Stock I /Macro Stock			**x50/1000ml**	**20ml/l**
	$KN0_3$	1900	95.g	
	NH_4N0_3	1650	82.5g	
	KH_2P0_4	170	8.5g	
	$MgS0_4 \cdot 7H_2O$	370	18.5g	
Stock II /Micro stock			**x100/1000ml**	**5ml/l**
	$MnS0_4 \cdot 4H_20$	22.3	2.23g	
	KI	0.83	83mg	
	H_3B0_3	6.2	0.62g	
	$ZnS0_4 \cdot 7H_20$	10.6	1.06g	
	$Na_2Mo0_4 \cdot 2H_20$	0.25	25mg	
	$CuS0_4 \cdot 5H_20$	0.025	2.ml	
	$CoCl_2 \cdot 6H_2O$	0.025	2ml	
Stock III /Iron stock			**x50/500ml**	**10ml/l**
	$FeS0_4 \cdot 7H_2O$	27.8	1.39g	
	Na_2EDTA	37.3	1.865g	
Stock IV / Vitamins Stock			**X100/500ml**	**5ml/l**
	Nicotinic acid	0.5	50mg	
	Pyridoxine-HCI	0.5	50mg	
	Thiamine-HCI	0.1	10mg	
	Myo-inositol*	100	10g	
	Glycine	2	200mg	
			X50/500ml	**10ml/l**
V	$CaCl_2 \cdot 2H_20$	440	22g	

Appendix Table 2. 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 3. 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 4. 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.