IN VITRO PROPAGATION OF TWO ANCHOTE *[Coccinia abyssinica* (Lam.) Cogn] GENOTYPES USING SHOOT-TIP CULTURE

BY:

MIGNOTE HIRKO

JUNE 2020

JIMMA, ETHIOPIA

IN VITRO PROPAGATION OF TWO ANCHOTE *[Coccinia abyssinica* (Lam.) Cogn*]* GENOTYPES USING SHOOT-TIP CULTURE

BY:

Mignote Hirko

Submitted to Jimma University College of Agriculture and Veterinary Medicine in Partial Fulfilments of the Requirements for Degree of Master of Science in Plant Biotechnology

Advisor: Temesgen Matiwos (Asst. Prof.)

Co-advisor: Techale Birhan (Asst. Prof.)

June 2020

Jimma, **Ethiopia**

APPROVAL SHEET

Jimma University College of Agricultural and Veterinary Medicine

Thesis Submission Request Form Request form (F-08)

Name of student: **Mignote Hirko** ID RM **1344/09**

Program of Study: - **Plant Biotechnology**

Title: *in vitro* propagation of two anchote *[Coccinia abyssinica(lam.) Cogn]* genotypes using apical shoot-tip culture

I have completed my thesis research work as per the approved proposal and it has been evaluated and accepted by my advisors. Hence, I hereby kindly request the Department to allow me to present the findings of my work and submit the thesis.

Mignote Hirko _____
Name Student Signature

We, the thesis advisers, have evaluated the content of the thesis and found to be satisfactory executed according to the approved proposal, written according to the standard and format of the University and is ready to be submitted. Hence, we recommend the thesis to be submitted.

Advisor: Temesgen Matiwos (Asst. Prof.) _____ 10/06/2020
Name Signature Date

Co- advisor: Techale Birhan (Asst. Prof.) _____ 10/06/2020
Name Signature Date

Decision / suggestion of Department Graduate Council/
DGC/_____

_____

Chairperson, DGC: _____ Signature: _____ Date: _____

Chairperson, CGS: _____ Signature: _____ Date: _____

DEDICATION

This thesis is dedicated to my beloved father P.G. Biswas for his encouragement, and unforgettable support in the success of my academic career

STATEMENT OF THE AUTHOR

I declare and confirm that this thesis is my original work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation. This Thesis is submitted for partial fulfillment of the requirements of Master of Science degree in Plant Biotechnology at Jimma University. The Thesis will be deposited in the Jimma University Library and will be made available to borrowers under the rules of the library. I solemnly declare that this Thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

A brief quotation from this Thesis is done without special permission provided that accurate and complete acknowledgement of the source is made. Requests for Permission for extended quotations from or reproduction of this Thesis in whole or in part granted was by the Head of the School or Department when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission from the author of the Thesis must be obtained.

Name: Mignote Hirko

Date: June, 2020

Signature: 

ACKNOWLEDGEMENTS

All praises and thanks for Almighty GOD who is the entire source of all knowledge and wisdom endowed to mankind.

I would like to express my deepest gratitude to my advisor Mr. Temesgen Matiwos and co-advisor Mr. Techale Birhan for their support, help and excellent guidance starting from the proposal development to the end of this thesis write up.

It gives me great pleasure to express my profound indebtedness and heartfelt thanks to Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) for offering me the financial support to pursue my thesis. I am also thankful to all the staff members of Horticulture and plant sciences department for their positive responses as they were always there when I want them for help.

My heartfelt and deepest thanks are also extended to all of my family members, especially to my husband Amanuel Kebede, my brother; Mesele Hirko, Wakjira Tesfahun and to my sister Hawi Deressa for their inspiration, encouragement and support all the time. I love you all and I am so honored to have you beside me.

I am very thankful to Jimma Agricultural Research Center (JARC) for allowing me to use their tissue culture laboratory facility for my research work. My gratitude extends to Mr. Berihun Menges who technically supported me during laboratory session. I also thank Mr. Desta Fekadu for providing me the seeds of the anchote genotypes used in the study.

Finally, I would like to thank all the individuals who were beside me during both the course and research work.

BIOGRAPHICAL SKETCH

Mignote Hirko was born on 28 June, 1992 in Nekemte, Wolega Zone of Oromia Region from her father Hirko Muleta and her mother Wosene Hunde. She attended her elementary and junior secondary education at Model School in Shambu. She attended her high school and preparatory education at Lalo kile and compressive school in Nekemte, respectively. She then joined Jimma University College of Agriculture and Veterinary Medicine in 2014 and graduated with a BSc degree in Plant Sciences in 2016. After graduation she joined Jimma University, College of Agriculture and Veterinary Medicine in September 2016 to pursue her study leading to the degree of Master of Science in Plant Biotechnology.

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BAP	6-Benzyle Amino Purine
CRD	Completely Randomized Design
EDTA	Ethylene Diamine Tetracetic Acid
EIAR	Ethiopian Institute of Agricultural Research
IBC	Institute of Biodiversity Conservation
IAA	Indole Acetic Acid
IAR	Institute of Agricultural Research
Kin	Kinetin
LSD	Least Significant Difference
MS	Murashige and Skoog
NAA	α-Naphthalene acetic acid
NaOCL	Sodium Hypochlorite
NaOH	Sodium Hydroxide
PGRs	Plant Growth Regulators
SAS	Statistical Analysis System
TDZ	Thidiazuron

TABLE OF CONTENTS

Contents **Pages**

LIST OF TABLES

Tables **Pages**

LIST OF FIGURES

Figures **Pages**

LIST OF TABLES IN THE APPENDIX

Appendix **Page**

ABSTRACT

*Anchote [*Coccinia abyssinica *(Lam.) Cogn.] is an endemic plant with high calcium content grown for its edible tuberous roots in Ethiopia. It is difficult to produce true-to-type anchote plants to sustain tuber quality through propagation by seeds as the plant pollinates by both self and cross. The study was carried out at Jimma agricultural research center at tissue culture laboratory with the objectives of in vitro propagation of two anchote [*Coccinia abyssinica *(Lam.) Cogn.] genotypes using shoot-tip culture. A completely randomized design (CRD) in factorial arrangement with five replications was used. The treatments were different concentrations of BAP 0.0,0.5,1.0,1.5,2.0,2.5 and 3.0 mg/l BAP for shoot initiation and 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 mg/l BAP for shoot multiplication were used. While different concentrations of IBA 0.0, 0.5, 1.0, 1.5, 2.0, 2.5mg/l IBA were used for root induction. The result revealed that different concentrations of BAP and IBA significantly influenced (P<0.01) shoot initiation and multiplication as well as root induction traits in both genotypes. The early initiation of shoot was 6.3 days for Desta variety and Acc.24/99 (9.3 days) in MS medium supplemented with 1.0 mg/l BAP and 1.5 mg/l BAP respectively. Besides, the highest percentage of shoot induction 98% of cultured explants was obtained at 1 mg/l BAP for Desta variety and at 1.5 mg/l BAP for Acc.24/99 (90%).The highest number of shoot 10.8 was recorded on MS medium supplemented with 1 mg/l BAP for Desta variety and 9.00 at 1.5 mg/l BAP for Acc.24.The lowest number of shoot was obtained from control (hormone free medium) for both genotypes. The maximum shoot length 7.3 and 5.48 was obtained from medium containing 0.5 mg/l BAP for both genotypes. The maximum number of leaves/shoot 13.0 leaves was recorded in MS medium supplemented with 1.0 mg/l BAP for Desta and 9.8 leaves at 1.5 mg/l for Acc.24/99. Early date of root induction was 6.5 days for Desta and 7.3 days for Acc.24/99 recorded on 0.5 mg/l IBA. Maximum numbers of root 13.20 was recorded on MS medium supplemented with 0.5 mg/l IBA for Desta and 12.1 at 0.5 mg/l IBA for Acc.24/99 and 12.1 and highest root length 7.1 cm and 6.4 cm) were observed on medium containing 1.5 mg/l for both genotypes respectively. Well rooted shoots were transferred to greenhouse and 82.14 % and 73.3 % Desta and Acc.24/99 survived, respectively. Generally, MS medium supplemented with 1.0 mg/l BAP is found to be optimum for shoot initiation and shoot multiplication for Desta variety in rooting experiment, half strength MS medium augmented with 0.5 mg/l IBA was optimum for both genotypes. Moreover, 1.0 mg/l BAP and 1.5 mg/l was found to be the best for shoot initiation and multiplication; whereas, in vitro rooting 0.5mg/l IBA was found to be the best. 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: Accession.24/99, BAP, Desta variety, IBA, Micro-propagation.

1. INTRODUCTION

Anchote [*Coccinea abyssinica* (Lam.) Cogn.] is one of the endemic root and tuber crops of Ethiopia cultivated for human consumption (Abera, 1995; Beruk and Fikre, 2015; Habtamu, 2014; Tilahun *et al.*, 2014). It is grown widely in the Western and South-Western parts of the country. It belongs to Cucurbitaceae family, one of the major families from the plant kingdom encompassing about 115 genera and 960 species (Schaefer, *et al*., 2009). Anchote is a vine like cucurbit with a high yield and short crop cycle used as an important dietary and medicinal plant (Girma and Hailu 2007; Yassin *et al*. 2013). The crop is known for its tuberous root and tender leaves which are used for food (Abera, 1995).

Anchote has a significant contribution for the cultural and social values of the Oromo's (the largest tribe in Ethiopia) since long ago (Abera 1995; Desta, 2011; Daba *et al*., 2012). The plant has been grown over a wide range of environments for a long time, and its cultivation and utilization have been passed from generation to generation through oral tradition, with very little recorded information (Abera, 1995; Girma and Hailu, 2007). The unique characteristic of the plant is the edibility of its different parts such as its tuber, leaf, and fruit which makes the plant ideal as potential food security crop (Amare 1973; Endashaw 2007; Desta, 2011).

According to Daba *et al*. (2012), the root yield of different anchote accessions ranges from 42-76 t ha^{-1}. Abera (1995) reported that a farmer in Western parts of Wollega usually allocate 400 to 600 square meters of land for anchote production mainly for home consumption. Its productivity show a discrepancy based on genotypes, soil fertility level, location and cultural practices used. Under farmers condition it can yield 20 - 30 tha^{-1} (Abera, 1995; BARC, 2004). However, under research condition it has a potential to yield up-to 73 tha^{-1} (Desta, 2011) and 76.45 tha^{-1} (Daba *et al.*, 2012). The total yield of anchote is 15-18 /ton, which is in the range of total yield of sweet potato and potato (IAR, 1986)

According to Fekadu (2011) the raw anchote tuber contains organic (carbohydrate, crude protein, crude fiber) and inorganic substances (calcium, magnesium, iron) as well as low levels of anti-nutrients (Oxalate, tannin, and cyanide) except phytate, when compared to other tuberous crop

plants. The root juice of anchote contains saponin; an active ingredient used to treat gonorrhea, tuberculosis and tumor cancer. A good content of calcium in anchote also helps in fast mending of broken bones (Dawit and Estifanos, 1988; Abera, 1995). Among the major tuberous vegetables such as sweet potato, Oromo potato, and others cultivated in the area, anchote is widely recognized as cultural, social and economic crop for the local and farming communities in southwest Oromia, Ethiopia with its annual yield of 25,000 tones (Anonymous, 2011).

The crop seems to have a wider ecological adaptation as it grows well in lowland, midland and highland, the traditional agro-ecological zones of Ethiopia based on relative altitudinal ranges; 500-1500 m, 1500-2300 m and 2300-3200 m above sea level, respectively (Zemede Asfaw, 1997). In the growing regions, very fertile soils in homestead areas are used for anchote cultivation. The information on other soil types is lacking. According to Abera (1995), southwestern parts of Ethiopia with oxisol, ultisol and vertisol soil types, altitudinal range of 1500-2400 m.a.s.l, receiving an annual rain fall of about 1500 mm to over 2000 mm might be suitable for anchote. According to Holstein and Norbert (2011), *Coccinia* species can grow on lateritic soils (i.e. red tropical soils), and are distributed in semi-arid, wood-lands and forest habitats of Sub-Saharan Africa.

Anchote can be propagated both vegetative and from seeds. Vegetative propagation is carried out either by planting the whole tuber or by slicing it into two or more pieces, each piece having rootlets and an external covering (Abera, 1995). The pollination and fertilization of anchote is limited by reducing its natural regeneration and population (Daba *et al*., 2012). Micropropagation is used as a means to improve crop varieties and it has been getting attention and being applied in agricultural research centers. One of the most important application of micropropagation as a tool of biotechnology is its application in the production of disease free plant materials. Moreover, it enables production of large number of plantlets in short period of time as well as maintenance of germplasm under controlled conditions in small spaces and with reduced labor requirement (Hartmann *et al.*, 2002). There has been progress in tissue culture studies in many Cucurbitaceae members. Studies to promote adventitious shoot from callus of some Cucumis have been unsuccessful or has occurred sporadically and at low frequency (Anugulati, 1988; Rakhi *et al*., 2010). The regeneration of cucurbits is largely dependent on the

nature of explants, the cultivar, the genotype, growth regulator concentrations and physical conditions of culture (Rakhi e*t al.,* 2010). However, stimulation of several axillary meristems were able to produce highly increased number of shoots and rooted plantlets within a short period of time (Lee *et al.,* 2003; Tarek *et al*., 2008). According to Fekadu (2011), micropropagation is important for the production of large number of plants of the same clone within relatively short period of time. *In vitro* regeneration and genetic transformation as well as disease-free plant production also require micropropagation. The high level of some anti-nutritional factors in anchote may be removed through genetic transformation.

Despite the high importance of anchote, the accessibility of its seed for cultivation is limited. Because it takes long time to obtain the seed. The seeds are also eaten by birds and the roots are eaten by wild animals. Furthermore, little attention has been given to research and development in anchote and there have been no breeding programs to develop new varieties (Daba *et al*., 2012). Anchote is conventionally propagated by both vegetative and seeds.via seeds and as both cross and self - pollination may occur in anchote, it is difficult to obtain true- to- type plants (Jeffrey, 1995). The other constraint of anchote production both pollination and fertilization is limited, thereby, reducing its natural regeneration and population (Daba *et al*., 2012). These problems present crucial challenges for the farmers to cultivate anchote as much as they would want to scale-up its production. The second way of anchote propagation is vegetative propagation, in the vegetative propagation; tubers are planted and used as seed sources during the next growing season. Some tubers may also be left in soil for regrowth (as *'guboo'*) for the coming season. When this method is used from generation to generation, the plants are attacked by diseases due to accumulation of fungi, bacteria, viruses and nematodes (Yosef and Tileye 2013). Therefore, there is a need to discover alternative propagation methods. To overcome these problems, the recent advancement in tissue culture especially *in vitro* propagation allows an alternative method of propagation. Micropropagation is advantageous over traditional propagation, as it can be used to provide a rapid, reliable system for the production of large numbers of genetically uniform plantlets. It offers a method to increase valuable accessions rapidly and expedite the release of improved varieties. In addition, micro-propagation ensures healthy seedlings with desirable characters that substantially improve production. There has been progress in micro-propagation studies in many plant species members of the Cucurbitaceae

family such as *Momordica dioica* (Shiragave and Chavan, 2001), *Coccinia indica* (Venkateshwaralu, 2001), *Citrullus vulgaris* (Dong and Jia, 1991), *Cucumis melo* (Mackay et al., 1989) and *Coccinia grandis* (Gulati, 1988). Moreover, some tissue culture techniques to micropropagation *Coccinia* species have been reported using different explants including hypocotyl of *Coccinia grandis* (Gulati, 1998), shoot tip and nodal segments of *Coccinia grandis* (Sarker *et al*., 2009), and nodal and leaf explants of *Coccinia indica* (Josckutty et al., 1993) and nodal explant of *Coccinia grandis* (Thiripurasundary and Rao, 2012). Folla *et al.* (2013), and Yosef and Tileye (2013) have reported a limited research has been done on anchote *in-vitro* propagation and using *in vitro* germinated seedlings through using 6-Benzyle Amino Purine (BAP) and kinetin (Kin) plant growth regulator in MS (Murashige and Skoog, 1962) (MS) medium. However, there is no research has been made to exploit tissue culture technique for Desta variety and accession 24/99. Therefore, this research work was initiated with the following objectives.

General objective

- To develop *in vitro* propagation protocol of two anchote [*Coccinia abyssinica* (Lam.) Cogn.] genotypes using apical shoot-tip culture.

Specific objectives

- To determine effect of different BAP concentrations for shoot initiation in two anchote genotypes.
- To determine effect of different BAP concentrations of BAP for shoot multiplication in two anchote genotypes.
- To determine effect of IBA concentrations for root induction in two anchote genotypes.

2. LITERATURE REVIEW

2.1. Description and Taxonomy of Anchote [*Coccinea abyssinica* (Lam.) Cogn.]

Cucurbitaceous (commonly called cucurbits) is one of the most diversified plant families consisting of 120–130 genera (Edwards *et al*., 1995) and 940 to 980 species (Schaefer and Renner, 2011). *Cucurbitaceae* is probably named after the chemical *cucurbitacin,* which was first identified from this group of plants. *Cucurbitacin* is oxygenated tetracyclic triterpenoids known with bitter taste and toxic to most organisms but, at the same time, can attract some specialized herbivorous insects (Da Costa and Jones, 1971; Gibbs, 1974; Balkerna-Boomstra *et al*., 2003).

The leaves are alternate and usually palmately five lobed or divided; and stipules are absent (Phan, 1999). Members of the *Cucurbitaceae* are including some important crop species such as cucumber, squash, pumpkin, luffa and melons (Andres, 2004). Winter squash populations show great diversity in morphological characteristics, particularly fruit length, fruit diameter, fruit shape, fruit brightness, skin thickness, flesh thickness and color (Balkaya *et al.,* 2009). In cucurbits normally the seed setting and germination is low, probably due to the presence of a thin nuclear membrane lending impermeability to water and gas and make them dormant for many days (Thripurasundari and Rao, 2012). Globally, the family of *Cucurbitaceae* has two large subfamilies: Zanonioideae and Cucurbitoideae, which consists of more than 20 and 100 genera (or 75 spp. and 750 spp.), respectively.

Ethiopia and Eritrea, as one flora region, are represented by more than 24 genera of *Cucurbitaceae* (2 from *Zanonioideae* and 22 from *Cucurbitoideae*) and 71 species (2 from Zanonioideae and 69 from Cucurbitoideae) (Edwards *et al*., 1995, Serbessa, 2017). The genus *Coccinia* belongs to subfamily *Cucurbitoideae* and possesses about 30 extant species in the palaeotropics, all restricted, in their distribution, to Africa except *Coccinia grandis* [(L.) Voigt.], which is disseminated to other continents such as America, Asia, and Australia (Berndt, 2007; Schaefer and Renner, 2011).

A total of 10 *Coccinia* species have been recorded in Ethiopia and Eritrea: eight in the Flora of Ethiopia and Eritrea (Edwards *et al*., 1995), and two more species, *C. ogadensis* Thulin described by Thulin, (2009), and *C. microphylla* Gilg by Holstein and Renner, (2011). Among the eight species found in the Flora, only five were fully named according to the rules for giving scientific names to plants in the Flora of Ethiopia and Eritrea. Those correctly named include *C. schliebenii* Harms (1932), *C. adoensis* (Hochst. Ex. A. Rich.) Cogn., *C. abyssinica* (Lam.) Cogn., *C. megarrhiza C. Jeffrey*, and *C. grandis* (L) Voigt (syn. *C. indica* Wight and Arn.); and those not fully named are: C. sp= Bally 12989, C. sp. = Burger 2947A, and C. sp. = Gilbert and Jones 129 (Edwards *et al*., 1995).

2.2. Origin, Domestication and Distribution

Understanding a crop plant's geographic distribution and/or center of origin is very important for breeding, genetic improvement, and conservation managements activities of the crop (Villa *et al.*, 2005). This is because of the nearby availability of the wild type and related species, which can provide adaptive value as well as broaden the genetic base of a crop species via outcrossing. In fact, the center of origin is, usually considered as center of diversity (Acquaah, 2007; Sebastian, 2011). Therefore, determination of center of origin of a crop plant is important to conserve its genetic diversity, especially for those species, which are vulnerable to ecosystem fragmentation (degradation) and other anthropogenic pressures. Cucurbitaceous species are distributed in the tropics and subtropics of both the Old and New Worlds, with hotspots of diversity in Southeast Asia, West Africa, Madagascar and Mexico (Schaefer and Renner, 2011).

However, Schaefer *et al*. (2009) observed the great disjunction between related genera in their geographical distribution. They reasoned out that there have been many successful long distance dispersals (by different mechanisms, usually by birds) between Asia and Africa, back to Asia, between Africa and South America, and from Asia to Australia (Schaefer *et al*., 2009; Schaefer and Renner, 2011).

Holstein and Renner (2011) used molecular technology to estimate the center of origin and diversification dates of wild *Coccinia* species to be, in eastern and southern Africa, around 6–7 million years ago. The genus has a broad range of agro–ecology from semi–arid habitat to moist forest (Holstein and Renner, 2011). Since Ethiopia is endowed with such highly diversified agro ecologies, the genus *Coccinia* is suitably occurring in wide range of areas including the western, central, south eastern and northern parts of the country (Edwards *et al*., 1995).

However, the well-known species for its food, medicine, and other socio-cultural values, in western and southwest Ethiopia, is only anchote [*Coccinia abyssinica* (Lam.) Cogn. (Amare Getahun, 1973). In different parts of Ethiopia, the plant is known by its vernacular names such as anchote (Afaan Oromo) (Amare Getahun, 1973), ushushe (Welayita), wushish (Tigrinya), shushe/ushushe (Dawuro), ajjo (Kefinya) (Wolde Michel, 1987). This indicates that anchote was originated and endemic to Ethiopia, where it is found as cultivated and in wild form (Amare Getahun, 1973; Edwards *et al*., 1995). The crop is cultivated in backyard, for its rootstock, particularly in southwestern part of Ethiopia, namely Wollega, Illu Aba Bora, and Jimma Zones. Other parts, such as Shoa and Harerghe Zones of the Oromia Regional State had also started cultivation of anchote recently (Tesfaye and Abebe, 1988; Karin, 2002). Recent work by Bula Sirika, (2016) shows that anchote crop is also being cultivated in other countries by Oromo Diasporas, including in various states of Canada and United States of America.

However, the time of domestication of *C. abyssinica*, more probably by Oromo (Cushitic) people (Bula Sirika, 2016), was not well known. The diploid chromosome number reported, so far, for six *Coccinia* species revealed that it varies between 2n = 20 to 2n = 24, but exceptionally *C. grandis* contained heteromorphic sex chromosomes (2n = 22+XX/XY). However, the chromosome number and the existence of sex chromosomes for other species including *C. abyssinica* have not been reported. For rehmannii clade, a reduced chromosome number (2n = 20) was implied from phylogeny not from practical observation (since only two species, *C. trilobata* and *C. rehmannii*, were examined to have 2n =20) and the mechanisms of reduction was not explained (Holstein, 2012).

2.3. Ecology and agriculture of anchote

The crop seems to have a wider ecological adaptation as it grows well in 'Qolla', 'Weinadega' and 'Dega' (Amh.), the traditional agro-ecological zones of Ethiopia based on relative altitudinal ranges; 500-1500 m, 1500-2300 m and 2300-3200 m above sea level, respectively (Zemede Asfaw, 1997). But, there is almost no research information on the ecological adaptation of the crop. In the growing regions, very fertile soils in homestead areas are used for anchote cultivation. The information on other soil types is lacking. According to Abera (1995), southwestern parts of Ethiopia with oxisol, ultisol and vertisol soil types, altitudinal range of 1500-2400 m.a.s.l, receiving an annual rain fall of about 1500 mm to over 2000 mm might be suitable for anchote. But, on the contrary, he also found anchote growing in areas outside the indicated ecological limits. According to Holstein and Norbert (2011), Coccinia species can grow on lateritic soils (i.e. red tropical soils), and are distributed in semi-arid, wood-lands and forest habitats of Sub-Saharan Africa.

Tubers vary in shape depending on environmental conditions, but generally spherical or elongated at maturity. Almost all activities associated to anchote culture are done by women (Abera, 1995; Endashaw Bekele, 2007). Land preparation, sowing, weeding, staking, harvesting, processing, storing and marketing are mainly accomplished by women, and usually, home gardens are used for anchote growing. Pests such as porcupines, wild pigs, and wart hogs hunt anchote tubers. The vicinity of anchote plots to home area helps family members and guarding dogs in protection. Parasites of fungal, bacterial, viral, and/or nematode origin and insect pests may also attack the aerial and/or underground parts of the plant

2.4 Traditional processing of anchote

Aerial parts and fibrous roots are removed from the tubers. After thorough washing, they are boiled in a clay pot called 'xuwwee' (Oro.). To achieve rapid cooking, the pot is covered by leaves of enset, maize, sorghum, or pumpkin. Final sealing may be by a lid or cow dung. This arrangement minimizes heat loss. The well-boiled tubers are then, cooled, peeled and processed in different ways. Anchote tubers are, most of the time, consumed boiled (Abera, 1995).

Habtamu (2011) mentioned the consumption of raw anchote, but this is uncommon. Boiling decreases both nutritional and anti-nutritional (substances that interfere with food utilization and affect health in animals including humans) contents of foods by leaching and/or decomposition (FAO, 1990; Makkar, 1993). But, boiling makes most foods palatable, increases digestibility and bioavailability of some nutrients, inactivates some anti-nutritionals and enzyme inhibitors, and increases consumer preferences (FAO, 1990; Habtamu, 2011). Except for moisture, crude fiber andiron, all contents were seen reduced after boiling. But, boiling after peeling caused more reduction in both nutritional and anti-nutritional contents. Reduction in the anti-nutritionals such as phytate, oxalate, tannin and cyanide is desirable. Loss of the nutrition is disadvantageous. So, boiling before peeling is recommended for anchote as this minimizes the nutritional losses and unnecessary moisture gain. Dish preference may also depend on processing. According to Habtamu (2011), 66% of anchote consumers given, at a time, both 'boiled-after-peeling' and 'boiled-before-peeling' anchote dishes, have preferred dishes from 'boiled-before-peeling' anchote tubers.

2.5. Importance of Anchote

Most of tuber and root crops are drought tolerant and can play a key role in supporting rural livelihoods and in addressing food shortage in many parts of the country. Anchote can be safely stored under the ground, which thus gives added food security to the population at times of main crop failures and also farmers in Ethiopia gain a substantial income from the sale of anchote seedlings (Asfaw *et al,* 1992). Anchote is a unique root crop in its uses and the parts consumed. All parts of anchote such as root, leaves and the immature fruit are used even though the root is the most economic concern in most growing areas of Ethiopia. In addition to its nutritional importance, anchote is a cultural and medicinal crop widely used in growing areas (Amare, 1973).

Anchote tuber contains appreciable quantity of carbohydrate, crude protein, crude fiber, calcium, magnesium, iron and low levels of antinutrients (Oxalate, tannin, and cyanide) except phytate. The traditional processing methods of anchote were very important because that increased in crude fiber content and improved the bioavailability of zinc contained in the anchote tubers

(Habtamu, 2011). Anchote contained good nutrient composition with good supplements of vitamins and minerals. Its anti-nutritional contents are probably of little nutritional significance and they may be still minimized or destroyed during cooking processes (Habtamu and Kelbessa, 1997). Dawit and Estifanos (1991) reported that the juice prepared from tuber of anchote has saponin as an active substance and is used to treat gonorrhea, tuberculosis and tumor cancer. Anchote when sliced, dried in the sun and ground, its flour remains in good conditions for a long time. The flour is used to prepare a soup when boiled with bone-marrow from animals. Such soup is particularly served to patients with broken or fractured bones or sick people.

2.6. *In vitro* propagation of Cucurbitaceous family and Anchote

Tissue culture contributed as an important tool that offers many approaches for propagation conservation and genetic improvement of any plant species. Among these, micropropagation is a powerful tool for fast multiplication of selected genotypes at faster rates; therefore, it has been adopted in agriculture sector (Kumar and Kumar, 1996). Tissue culture techniques are used extensively to grow many different plants for commercial and research purposes (Hussain *et al*, 2012). New plants are grown from small pieces of plant tissue in a nutrient medium under sterile conditions. When conditions are suitable, plants can be induced to rapidly produce new shoots, which can be subdivided to produce more plants. The addition of suitable hormones can then induce root growth, and the plants can then be placed in soil and grown in the normal manner (Rand, 2001).

Micropropagation is used as a means to improve crop varieties and it has been getting attention and being applied in agricultural research centers. One of the most important application of micropropagation as a tool of biotechnology is its application in the production of disease free plant materials. Moreover, it enables production of large number of plantlets in short period of time as well as maintenance of germplasm under controlled conditions in small spaces and with reduced labor requirement (Hartmann *et al*., 2002).

2.6.1. Type of explant in anchote micropropagation

Type of explant is also one of the important factors in optimizing the tissue culture protocol. Type of explants like leaf, petiole, cotyledonary leaf, hypocotyle, epicotyle, embryo, internode and root explant significantly affect tissue culture process of plants (Ali and Mirza, 2006; Kumar *et al*., 2011b). This may be due to the different level of endogenous plant hormones present in the plant parts. Leaf is the most commonly used explant for regeneration due to more surface area available (Tyagi *et al*., 2001). According to Tyagi *et al*. (2001) root, shoot, and leaf explant and maximum regeneration efficiency was observed from leaf explants in Cajanuscanian. Similarly, Alagumanian *et al*. (2004) also used leaf and stem for *in vitro* propagation. Source of explant is an important for regeneration (Reddy *et al*., 2008; Kumar *et al*., 2010a). In vitro explant is considered to be the most suitable for organogenesis (Reddy *et al*., 2008). The fact that source of explant has different capacity of regeneration are well documented (Feyissa *et al*., 2005). The difference may be due to the level of endogenous hormones present in the plant explant. Seedling explant is more responsive or meristematic than mature plants (Feyissa *et al*., 2005) due to different level of plant hormones present in the plants.

2.6.2. Effects of plant growth regulators on anchote regeneration

Growth regulators are organic compounds naturally synthesized in higher plants, which influence growth and development. Apart from the natural compounds, synthetic chemicals with similar physiological activities have been developed which correspond to the natural ones (Pierik, 1997). There are several classes of plant growth regulators, as e.g. cytokinins, auxins, gibberellins, ethylene and abscisic acid. Growth and morphogenesis in vitro are regulated by the interaction and balance between the growth regulators supplied in the medium, and the growth substances produced endogenously (George, 1993). A balance between auxin and cytokinin is most often required for the formation of adventitious shoots and roots.

Auxin is a plant growth regulator which is known to be distributed universally in higher plants. This compound is secreted by the apical meristems of both shoots and roots and controls the expansion of the tissue cells. Auxin was also proved to be responsible for apical dominance (the

inhibition of lateral bud and lateral root development by the active apical meristem), in the retention or falling of leaves and flower buds, in flower development and in the initiation and continuance of fruit development (Davies, 1995). At the cellular level, auxins control basic processes such as cell of initiating cell division and they are involved in the formation of meristems giving rise to either unorganized tissue, or defined organs. In organized tissues, auxins are involved in the establishment and maintenance of polarity and in whole plants their most marked effect is the maintenance of apical dominance and mediation of tropisms. Auxins are required for induction of cell division, cell expansion and frequently for rooting (Gamborg and Phillips, 1995).

Among plant growth regulators, cytokinins have proven to be the most important factor affecting shoot regeneration, and their significant effects may be related to the histological changes in induced tissues (Magyar-Tabori *et al*., 2010). Cytokinins stimulate plant cells to divide, and they were shown to affect many other physiological and developmental processes (Howell *et al*., 2003). These effects include the delay of senescence in detached organs, the mobilization of nutrients, chloroplast maturation, and the control of morphogenesis (Taiz and Zeiger, 1991). The success of a culture is affected by the type and concentration of applied cytokinins, because their uptake, transport, and metabolism differ between varieties and they can interact with endogenous cytokinins of an explant (van Staden *et al*., 2008.)

2.6.2.1 Shoot induction

The purpose of shoot initiation is to establish axenic culture. It involves the selection and cultivation of explant under aseptic condition (Kumar and Reddy, 2001). Shoot induction and multiplication attained through supplementation of different cytokinins concentration into the culture medium (Abdulla *et al.*,2003). According to reports of Danci and Danci (2007) regeneration response of *in vitro* is generally species and often accession specific. Following the attainment of multiple shoot formation in the cultured shoot tips, the newly formed shoots are induced to root in an auxin containing medium (Varshney *et al.*, 2013). However, the number of shoots and roots produced at the multiplication and rooting stages of plant propagation through tissue culture dependent on type and concentration of plant growth regulators. Also the chemical

formulation and physical status of nutrient medium and the incubation conditions are of major significance.

In terms of multiple shoot proliferation nodal explants responded better than other explants *viz.,* Internodes and leaves (Biswas *et al.,* 2009). Similar findings of axillary bud proliferation have also been reported in medicinal plants (Chandramu *et al.,* 2003; Sultana and Handique 2004). Exogenous application of different cytokinins, *viz*., BAP, Kinetin, etc. has become obligatory for induction of multiple shoot in many plants (Khadiga *et al*. 2009). When used singly BAP showed stronger effect than Kin in terms of shoot induction. Of the different treatments 3.0 mg/l BAP was most effective (Biswas *et al*., 2009). BAP is considered to be one of the most useful Cytokines for achieving the micropropagation and showed highest effect in respect of multiplication of axillary buds (Joshi and Dhar, 2003).

Synergistic effect of BAP in combination with an auxin has been reported and most of the cases BAP and NAA were used for the induction of multiple shoots of various medicinal plants (Chen *et al*., 2001). The number of shoots per explant increased when the media were replaced after every 14 days of inoculation. A rapid rate of propagation depends on the sub culturing of proliferating shoot culture (George *et al*., 2007). In the control no plants produced any shoots. However, Roy (2008) reported that 90% shoot induction at 1.5 mg/l BAP and 0.5 mg/l NAA and in this combination the highest number, 12 shoots regenerated from nodal explants.

Regeneration through organogenesis *via* shoot formation in *Cucumis sativum* was reported on the best shoot growth obtained through a balance of both auxins and cytokinins. Cytokinin (0.5 – 6 mg/l) produced shoots after 20 days in culture and best response was observed on media containing 0.5 mg/l cytokinin and 2 mg/l BAP (Agarwal and Kamal, 2004). Multiple shoot buds originated from nodal explants when MS was supplemented with different concentrations of BAP along with IAA. The nodal explants showed slight swelling prior to the emergence of shoot buds developing from the pre-existing material 15 days after inoculation. Initially two to four shoot buds per explant emerged 25 days after inoculation at 5.0 mg/l BAP along with combination of 0.5 mg/l IAA (Anand and Jeyachandran, 2004).

According to Fazlima (2011) distinct variations were found in respect of number of leaves per plantlet due to the interaction effect of variety and cytokinins (BAP and Kin) concentrations. The maximum number of leaves per shoot (7.75) was observed in the potato variety with 1.0 mg/l BAP. When kinetin was used, the highest number of leaves per plantlet (10.25) was recorded with 0.5 mg/l kinetin. Highest percentage (71.3 ± 0.69) of shoot lets formation was achieved in *Coccinia indica* on MS medium supplemented with 1.5 mg/l of BAP in combination with 0.5 mg/l Kin (Ghanthikumar *et al.,* 2013). Significantly higher number of shoots per explant (5.78), number of nodes/shoot (6.11) and longest shoot (3.57 cm) were observed on the medium containing 8.88 µM BA (Sanjeev *et al*, 2003).

2.6.2.2 Root induction

Shoots produced from the previous stage are separated and individually rooted in a relatively high Auxin -containing media. In this stage, a good root system is initiated and complete plants are achieved. Rooting of micro shoots is critical in plant production systems *in vitro*. Induction of rooting for a long time has been considered as a single-phase process but successively there were several reports where the adventitious rooting depended on a series of interdependent phases (induction, initiation and expression) (Heloir *et al*., 1996). Various studies on adventitious root formation in micro shoots have shown the fundamental role played by peroxidases in controlling rooting *in vitro (*Rival *et al.,* 1980). The role of auxin in relation to peroxidase activity in rooting of various plant species was also reported by Kevers *et al.* (1997).

Rooting of shoots was usually attained at low concentration of auxins in many *in vitro* cultures (Nitzche and Wenzel, 1984) and also the beneficial effect of using IBA for *in vitro* rooting has already been reported for potato by Khatun *et al.* (2003). In order to induce roots the individual *in vitro* grown shoot buds were cultured on half-strength MS fortified with different concentrations of IBA. Without growth regulators no induction of roots was achieved. 100% micro cuttings rooted in the medium supplemented with 1.0 mg/l IBA. Maximum number (10.48) and the longest (5.33 cm) roots were obtained in this concentration within three weeks of inoculation. It indicates that stress condition favors the root induction in *B. diffusa*. Induction of roots in IBA supplemented medium has also been reported in some other medicinal plants (Lee

1994, Rout et al. 1999, Liu and Li 2001). Roy (2008) observed maximum of 90 % micro shoots rooted in MS fortified with 1.0 mg/l each of IBA and IAA.

2.6.3. Acclimatization

The ultimate success of in vitro propagation depends on a reliable acclimatization protocol, ensuring low cost and high survival rates. In vitro protocols provide minimal stress and optimum conditions for shoot/plant multiplication (Hazarika 2006). As a consequence of these special conditions (e.g., high air humidity, low irradiance, low CO_2 during photoperiod, high levels of sugars as carbon source and growth regulators), in vitro-grown plantlets usually exhibit abnormal morphology, anatomy, and/or physiology (Hazarika, 2006). Under these conditions, in vitro plantlets can develop specific features (e.g., nonfunctional roots and/or stomata) that are inconsistent with the development under field conditions. Also, the heterotrophic mode of nutrition and poor mechanism to control water loss render micro propagated plants vulnerable to the transplantation shocks when directly placed in a field (Omar and Aouine, 2007).

In vitro plantlets grow generally under low level of light, with plenty of sugar and nutrients to favor heterotrophic growth (Hazarika, 2003). Due to these factors, in vitro plants have low rates of photosynthesis and an incipient photosynthetic apparatus. Thus, they need hardening and acclimatization, where they receive a special treatment before they can be transferred to the soil in order to stimulate photosynthesis, cuticle development and their stomata's starts functioning. Probably this is the most important stage in the whole process. Thus, it should be conducted under proper conditions regarding soil, light, temperature and irrigation. About 85% of the transplanted plants of *Coccinia cordifolia* (Linn.) Cogn. survived if the plants in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and reared for three weeks (Roy et al., 2012).

3. MATERIALS AND METHODS

3.1. Plant materials

Seeds of two anchote genotypes named as Desta and acc.24/99 were kindly obtained from Debere Zeit Agricultural Research Center. The genotypes were selected based on their best performances. Desta is newly released and being widely distributed variety to farmers. The next one is known by its early maturity. They are having soft texture and easy of cooking. Desta has larger tuber, higher nutrient content, its maturity time is 120 days and known to give high yield (325 q/ha) on the research field. Whereas Acc.24/99 is also has larger tuber, leaves used as vegetables, its maturity time is 90 days and gives yield 292 q/ha on the research field. Seeds were planted at greenhouse in pots containing mixture of soil, coffee husk and sand in the ratio of 1:2:1 respectively. Plants were watered as needed with tap water and all the necessary agronomic managements and practices were taken for better establishment of plants to be used as explant source. Four week old seedlings were used as a source of explants and cut from the mother plant and taken to the laboratory for sterilization and culture initiation.

3.2. Media preparation

MS medium supplemented with PGRs were used. Stock solution of macro salt, micro salt, vitamins, iron source and PGRs (1mg: 1ml) were prepared and stored in refrigerator at 4^{o}C. PGRs, auxin (IBA) were dissolved using a drop of ethanol and cytokinin (BAP) by 1N NaOH before making up the final volume with distilled water. Iron EDTA (ethylene Di-amine Tetra Acetic Acid) stock solution was covered with Aluminum foil.

Culture medium was prepared from all solution. The medium was solidified with 0.7% (w/v) agar, 3% sucrose was added as energy supply. The pH was adjusted to 5.8 1N NaOH or 1N HCI prior to the addition of agar. PGRs were added according to the concentration required. Then 50 ml media were dispensed in to washed and sterilized cultured 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. Finally, the autoclaved media were taken out of the autoclaving chamber and put on the shelf for four days until used.

3.3 Plant growth regulators preparation

Plant growth regulators, 6-benzyl amino-purine (BAP) for shoot induction and indole-3-butryic acid (IBA) for rooting were used in this study. Both plant growth regulator powder of BAP and IBA were weighed and dissolved in 1N NaOH and1N HCl at 1mg/ml concentration using double distilled water. Then, the stock solution volume was adjusted in 100 ml by adding double distilled water. Finally, growth regulator stock solutions were stored in a refrigerator at 4^{o}C.

3.4. Sterilization and preparation of explants

Healthy and young explants, shoot tip of about 3 cm were selected for sterilization. The excised explants were thoroughly washed in running tap water for about 15-20 minutes and in 5% (w/v) detergent solution of Tween 20 for 10 minutes. After thorough rinsing in sterile distilled water, the explants were surface sterilized with 70% ethanol for 30 seconds. The explant was disinfected with 20% commercial sofi bleach (1% active chlorine) for 10 minutes. Then the Sterilized explants were transferred to the inoculation chamber, where they were washed with double distilled sterile water 5 times to remove any trace of surface sterilizing chemicals present on the surface of the explants. Finally, the size of the explants was reduced to a size of 2 cm and was aseptically transferred to the initiation experiment.

3.5. Treatments and experimental design

Effects of BAP on shoot initiation

In this experiment, shoot tip of 2 cm length, which were prepared from greenhouse grown seedlings, were cultured on MS medium supplemented with BAP at various concentration (0.0,0.5,1,1.5,2,2.5,3 mg/l) for shoot initiation. The experiment was laid out using completely randomized design (CRD) in factorial combination with five replications. Four explant/jar were used. From this experiment date and percentage of initiation were recorded. Days of initiation was the average number of days counting from culturing to the date that the plants/jar took to initiate or showing new growth while the number of initiated explants was recorded after three

weeks. The cultures were grown at 25 ± 2 ºC with a photoperiod of 16 h at 2000- 3000 lux of cool white fluorescent light for 4 weeks in a growth chamber.

Effects of BAP on shoot multiplication

Prior to shoot multiplication experiment, the initiated shoot was taken out from the culture medium and cultured on hormone free MS medium for two weeks to avoid carry over effects of growth hormones. Then shoots of 3 cm length were cultured on fresh MS medium plus BAP at 0.0, 0.5, 1,1.5, 2 and 2.5 mg/l level for the purpose of shoot multiplication. The experiment was laid out using CRD in factorial combination with five replications. Four shoots were used per jar. After 30 days, the number of shoot, number of leaves and shoot length were recorded. Shoots were counted from each explant which was obtained from four explants per jar and shoot per explants determined in average of four explants. The shoot length was measured from base to the tip of the plantlet at the time of transferring to rooting media and the average length was determined in centimeters. Number of leaves were counted from four explants per jar and determined in average as leaves per explant.

Effects of IBA root formation

After multiplication, shoots that have ~ 4 cm length were excised and cultured on growth regulators free MS medium for two weeks to avoid carry over effect. Then, the shoots were transferred to MS medium supplemented with IBA (0.0, 0.5, 1, 1.5, 2, and 2.5 mg/l) concentrations. The experiment was laid out using CRD in factorial combinations with five replications. Four shoot per jar were used. After the plantlets were kept in the rooting media for three weeks, number of shoots that produced root, number of roots and root length in cm were recorded. The number of days taken for induction of roots was recorded from transplanted shoots, then the average number of days was calculated on later. Percent of root induction was calculated. For root length determination, for each shoot the length of longest roots was measured from the collar region to the highest root tip as a root length and expressed in average. The numbers of roots formed per shoot were recorded from four explants per jar and average numbers of root was taken for numbers of root determination.

3.6. Acclimatization

For acclimatization, rooted plantlets were taken out of the rooting media, washed thoroughly under running tap water to remove the adhering agar. Then transplanted on plastic pots containing soil, coffee husk and sand in the ratio of 1: 2:1. Each pot with plantlet was covered with plastic bags and maintained in greenhouse. The plastic bag was gradually removed after a week and the number of surviving plants in the glasshouse was recorded after three weeks.

3.7. Data Analysis

For the collection data analysis of variance (ANOVA) was performed using SAS software packages (version 9.3). Least significant different (LSD) was used for the comparison of significant differences between means at $p<0.01$. The model for this experimental study was;

$$Y_{ij} = \mu + T_i + Y_j + (TY)_{ij} + \varepsilon_{ijkr}$$

Where Y_{ij}= All recorded data in tissue culture

μ= overall mean effect

T_i= treatment effect at i^{th} level

Y_j= Variety effect at j^{th} level

$(TY)_{ij}$= the combination effect of both treatment and variety at ij^{th} level.

ε_{ijkr}= a random error competed for all factors.

4. RESULTS AND DISCUSSION

4.1. Effect of different concentrations of BAP on shoot initiation

Days to shoot initiation were highly significantly (P≤0.01) at different concentrations of BAP in both genotypes. In most concentrations, shoot was initiated within two weeks. The early initiation was recorded 6.0 and 9.0 days in Desta variety and Acc.24/99, respectively in medium supplemented with 1.0 mg/l and 1.5 mg/l BAP, respectively (Table 1). The late initiation of shoot was 14.3 and 16 days for Desta variety and Acc.24/99, respectively in growth regulators fee MS medium. The result showed that when concentration level was increased from 0.5 mg/l to 1.5 mg/l, shoot initiation was increased. Further, increase in concentration to 2.0-3.0 mg/l reduced the proliferation of shoots and at the highest level (3.0 mg/l) the cultured explants failed to proliferate any shoot. This might be due to cytokinins most effective for bud proliferation at low level and prevent the apical dominance of shoot at high level. In line with current result Sanjeevakumar *et al*. (2003) reported that BAP was the most effective cytokinins for bud proliferation and shoot induction in *Trichosanthes dioica* plants. Moreover, Islam *et al*. (1994 pronounced that BAP was the most effective cytokinins for bud proliferation and multiple shoot induction in *Momordica charactia* plants. In agreement the current finding Firoz *et al*. (2015) found that the regeneration of shoot from nodal explant of *Cucim sativum* was generated visible shoot structure within 6-10 days of culturing at the same concentration of BAP level.

The percentage of initiation showed a highly significant (P ≤ 0.01) difference in different concentrations of BAP (Appendix 2). The highest percentage of shoot induction (98%) of cultured explants was obtained for Desta variety at 1 mg/l BAP and for Acc.24/99 (90%) at 1.5 mg/l BAP. A jar treated with level of 1 mg/l for Desta and 1.5 mg/l for Acc.24/99 showed a 47.58 % and 44.44 % more percent of shoot initiation over control treatment. There was no significant difference between 0.5 mg/ml and 1.5 mg/l of BAP for Desta as well as 0.0 mg/l and 3.0 mg/l. on percentage of initiation likewise, there were not statistical difference between 0.0 mg/l and 2.5 mg/l of percent of shoot initiation for Acc.24/99. The current finding showed that 1 mg/l BAP could be optimum percent of shoot induction for Desta variety. This might be due to the result of BAP at high concentration level reduced apical dominance and increased lateral

growth of shoot. Sarker *et al*. (2008) who reported that highest percent of shoot induction (81.25%) was achieved on MS medium supplemented with 1.5 mg/l BAP in *in vitro* regeneration of *Coccinia grandis* this report partially agreed with current study.

Table 1. Effect of different concentrations of BAP on shoot initiation in two anchote genotypes.

Genotype	BAP (mg/l)	Days to initiation ±SD	% Initiation ±SD
Desta variety	0.0	15.00 ± 0.51^{b}	50.00 ± 0.60^{g}
	0.5	7.00 ± 0.64^{i}	79.98 ± 10.89^{cd}
	1.0	6.00 ± 0.64^{j}	95.38 ± 5.54^{a}
	1.5	8.00 ± 0.51^{h}	83.02 ± 6.99^{c}
	2.0	9.40 ± 0.12^{fg}	72.02 ± 4.77^{e}
	2.5	10.80 ± 0.51^{e}	62.04 ± 4.76^{f}
	3.0	12.98 ± 0.63^{d}	44.98 ± 0.62^{g}
Accession 24/99	0.0	16.00 ± 0.64^{a}	50.00 ± 0.51^{g}
	0.5	15.00 ± 0.51^{b}	65.00 ± 0.56^{f}
	1.0	10.00 ± 0.46^{f}	80.00 ± 0.60^{cd}
	1.5	9.00 ± 0.60^{g}	90.00 ± 0.46^{b}
	2.0	10.98 ± 0.63^{e}	75.00 ± 0.46^{de}
	2.5	13.00 ± 0.46^{d}	50.04 ± 0.59^{g}
	3.0	14.00 ± 0.51^{c}	30.00 ± 0.60^{h}
	LSD ($P\leq0.01$)	0.24	2.08
	CV (%)	4.56	6.56

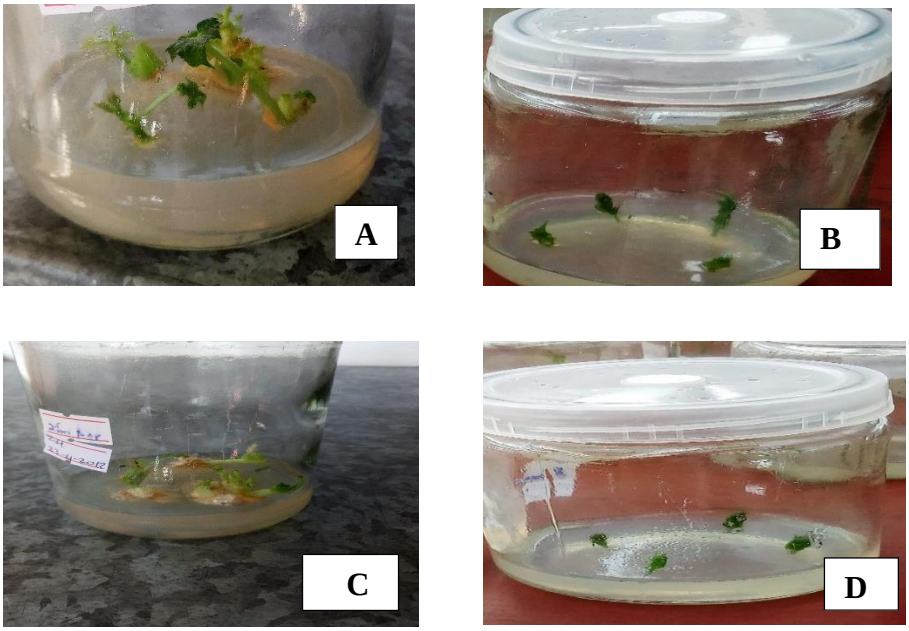

Figure 1: Shoot initiation in two anchote genotypes, A) Desta variety showing initiation B) Desta variety at 1.0 mg/L BAP, C) Acc.24/99 showing initiation and D) Acc.24/99 1.5 mg/l BAP.

4.2. Effect of different concentrations of BAP on shoot multiplication of anchote [*Coccinea abyssinica (*Lam.) Cogn.]

Number of shoot

Number of shoot for Desta variety and Acc.24/99 significantly ($P<0.01$) influenced by supplementation of different concentrations of BAP (Table 2 and Figure 2). A medium supplemented with 1.00 mg/l and 1.5 mg/l BAP showed maximum number of shoot for Desta 10.8 and 9.00 for Acc.24/99. The lowest number of shoot was recorded from a medium without growth regulator. A medium supplemented by 1 mg/l and 1.5 mg/l BAP showed 87.04% and 68.89% more numbers of shoot, respectively over control treatment. A medium supplemented with 1mg/l and 1.5 BAP was significantly different among all treatment for Desta variety and Acc.24/99 respectively. There was insignificant difference of shoot numbers between 0.5 mg/l and 2.5 mg/l concentrations of BAP for Desta variety. Shoot number of Desta variety and Acc.24/99 increases with increasing concentration of BAP at 1mg/l and 1.5mg/l respectively. However, when concentration of BAP further increased, the shoot numbers decreased. This might be due to the result of BAP at high concentration reduced apical dominance and increased

lateral growth of shoot. Keresten and Hake, 1997 reported that the number of shoots per shoot increased as the level of BAP increased that could be due to the fact that cytokinin at elevated level reduced apical and ectopic shoot formation and released lateral buds. Sundari *et al*. (2011) reported that the maximum number of shoots was found from MS medium containing 1mg/l BAP in shoot multiplication of *Coccinia grandis*.

The current finding disagree with that of Bekele *et al.* (2013) who investigated best multiplication performance from a medium supplemented with 3.0 µM BAP at *in vitro* regeneration of *Coccinia abyssinica* and observed that the highest number of shoot was 10.38. Venkateshwarlu *et al*. (2001) also reported that number of shoots per explant ranged from 2-5 to 4-5 by the addition of 2 mg/l and 3.0 mg/l BAP, respectively in *Coccinia grandis* using stem explant. Disagreement with both authors could be due to the difference in response to hormones combination that factors plant genotype and physiological states of the explants that influence shoot regeneration of a plant (Gaba, 2005).

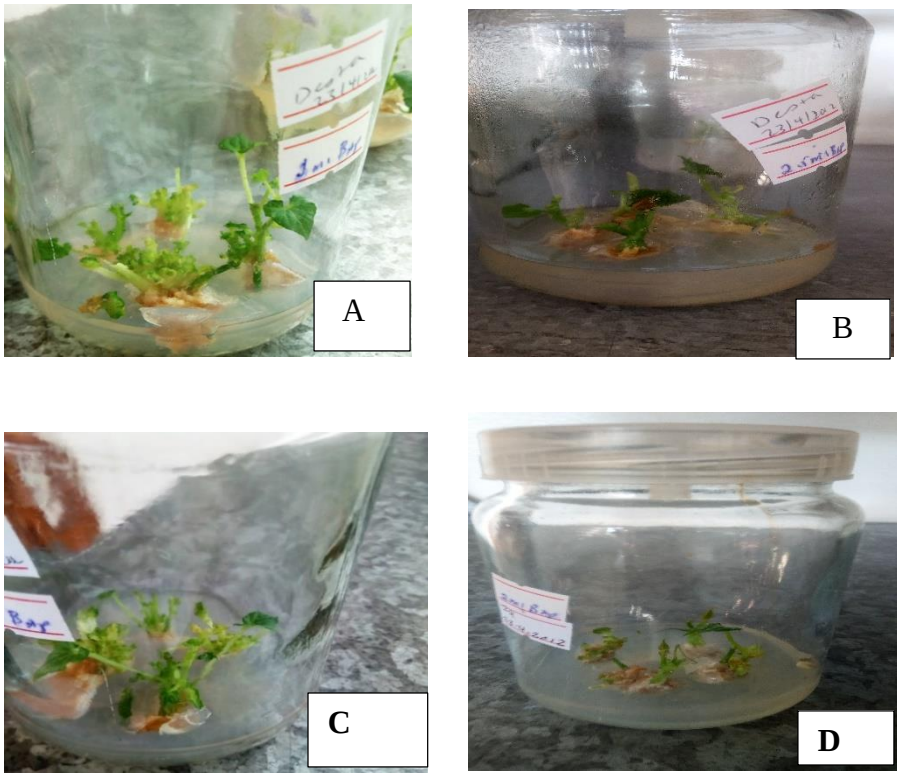

Figure 2: Effect of BAP on shoot multiplication of anchote genotypes, (A, C) high shoot multiplication for Desta variety and Acc.24/99 and (B, D) low shoot multiplication Desta variety and Acc.24/99

Shoot length

Shoot length showed highly significant difference ($P<0.01$) among BAP concentrations in both Desta variety and Acc.24/99 (Table 2). The maximum shoot length per plantlet7.3 and 5.48 cm was recorded from a medium supplemented with 0.5 mg/l BAP in both Desta variety and Acc.24, whereas the minimum shoot length 4.18 and 3.30 cm, was recorded from a medium supplemented with 2.5 mg/l and 2 mg/l of BAP respectively. A medium supplement with 0.5 mg/l of BAP was significantly different from other treatments. There was insignificant difference between a medium supplemented with 1.5 mg/l and 2.5 mg/l. The current finding showed that medium with high multiplication rate of shoots per shoot gave smaller average length of shoot per explant when compared to a medium giving more number of shoot per explant. This might be due to the cytokinin known to reduce the apical meristem dominance as well as induce both axillary and adventitious shoot formation from shoot tip of explant of *Coccinia abyssinica*. In

line with current result, Folla *et al*. (2013) reported that cytokinin such as benzyl amino purine (BAP) and kinetin are known to reduce the apical meristem dominance as well as induce both axillary and adventitious shoot formation from shoot tip explant of a *Coccinia abyssinica*. Likewise, the current finding, Patel and Ishnava (2015) found that the highest shoot length which was (7.7 cm) from a medium supplemented with 0.5 mg/l of BAP for *Coccinia grandis*. In contrary, Folla *et al.* (2013) reported that the longest average shoot length was obtained from 5.0 µM BAP that observed from shoot tip explants of *Coccinia abyssinica*.

Table 2: Effects of different concentrations of BAP on shoot multiplication of two anchote genotypes

Genotype	BAP (mg/l)	Shoot number±SD	Leaves number±SD	Shoot length (cm) ±SD
Desta variety	0.0	1.40 ± 0.10^{i}	2.20 ± 0.10^{i}	6.12 ± 0.83^{b}
	0.5	2.82 ± 0.62^{h}	4.00 ± 0.51^{g}	7.30 ± 0.07^{a}
	1.0	10.80 ± 0.46^{a}	13.00 ± 0.60^{a}	5.16 ± 0.08^{d}
	1.5	8.00 ± 0.60^{c}	9.00 ± 0.60^{c}	4.28 ± 0.08^{g}
	2.0	5.00 ± 0.46^{f}	5.00 ± 0.46^{f}	5.56 ± 0.08^{c}
	2.5	2.36 ± 0.16^{h}	3.80 ± 0.60^{g}	4.18 ± 0.08^{hg}
Accession24/99	0.0	2.80 ± 0.07^{h}	3.00 ± 0.60^{h}	5.04 ± 0.05^{e}
	0.5	4.04 ± 0.63^{g}	6.20 ± 0.10^{e}	5.48 ± 0.08^{c}
	1.0	7.02 ± 0.62^{d}	7.00 ± 0.60^{d}	4.62 ± 0.13^{f}
	1.5	9.00 ± 0.60^{b}	9.80 ± 0.60^{b}	4.08 ± 0.08^{h}
	2.0	5.80 ± 0.60^{e}	2.20 ± 0.10^{i}	3.14 ± 0.05^{j}
	2.5	5.00 ± 0.51^{f}	1.40 ± 0.07^{j}	3.30 ± 0.07^{i}
	LSD (P≤0.01)	0.63	0.60	0.10
	CV (%)	8.55	7.41	1.65

Note: %R =rooting percentage, NR =average number of roots, DR=days to rooting and RL =average root length in cm.

Number of leaves

Number of leaves showed highly significant difference ($P<0.01$) among BAP concentrations in both Desta variety and Acc.24/99 (Table 2, Appendix 3). Desta variety gave the maximum mean number of leaves (13.0) on a medium supplemented with1 mg/l BAP as well as Acc.24/99 (9.8) at 1.5 mg/l BAP. The second highest number of leaves was recorded from a medium supplemented with 1.5 mg/l and 1 mg/l BAP for Desta variety and Acc.24/99, respectively. The lowest number of leaves was obtained from a medium supplemented with 0.0 mg/l and 2.5 mg/l of BAP for Desta and Acc.24, respectively. Number of leaves for Desta variety and Acc.24/99 increased with increasing concentration of BAP at 1 mg/l and 1.5 mg/l respectively. However, when concentration of BAP further increased, the numbers of leaves decreased. In line with the current result, Abdulla *et al*. (2003) indicated that shoot multiplication has been achieved through supplementation of culture medium with various concentrations of different cytokinins, BAP and Kin were found to be effective among other cytokinins for inducing shoots. Moreover, Karrupusamy and Senthilkumar *et al.* (2007) suggested that BAP is most active for number of leaves at concentration of 1.0 mg/l to 2.0 mg/l plant species of c*ucurbit*. The current result showed that Desta variety was found to be the best variety over Acc.24/99 regarding micropropagation capacity for shoot tip explants under *in vitro* regeneration experiment. This might be due to regeneration response in *in vitro* is species and accession specific which are influenced by endogenous and exogenous factors. In line with the current finding, Danci and Danci (2007) reported that regeneration response in vitro is species and accession specific.

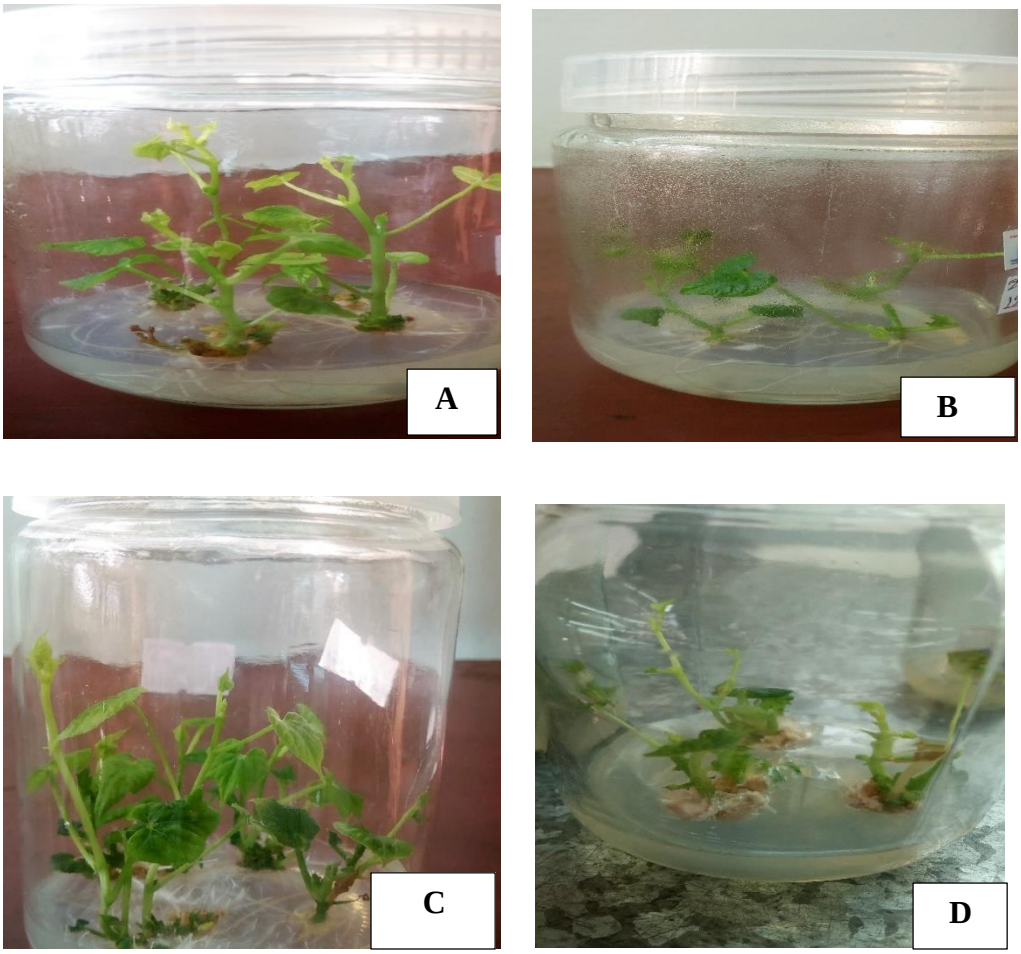

Figure 3 Effects of different concentrations of BAP on shoot multiplication of anchote genotypes, (A, C) the highest number of leaves for Desta variety and Acc.24/99 and (B,D) lowest number of leaves for Desta variety and Acc.24/99.

4.3. Effects of IBA on *in vitro* rooting of anchote genotypes

Days to rooting

Days to rooting showed highly significant difference ($P<0.01$) among IBA concentrations in both Desta variety and Acc.24/99. (Table 3). The earliest root induction was 6.6 and 7.4 days for Desta variety and Acc. 24/99, respectively that recorded from a medium supplemented with 0.5 mg/l of IBA. The late root induction was 18.4 and 19.2 days for Desta and Acc.24/99 which was recorded on the control treatment. Desta variety showed rooting in fewer days than Acc.24/99.

All concentrations of IBA were significantly different within treatment for Desta variety whereas there was insignificant difference between 1.5 mg/l and 2 mg/l of IBA for Acc.24/99. The current result showed that root induction was occurred early at lower concentration of IBA. This might be due to exogenous application of IBA that has been reported to enhance the speed of translocation and movement of sugar to the stem cuttings and promote root growth. In line with the current result Haissig (1974) reported that auxin has enhanced the speed of translocation and movement of sugar to the stem cuttings and promote root growth or root induction. Similarly, Haque *et al*. (2008) reported that IBA gave better response than NAA in root formation which was also observed in pumpkin.

Rooting percentage

Rooting percentage showed highly significant difference ($P<0.01$) among IBA concentrations in both Desta variety and Acc.24/99 (Table 3, Appendix 4). The highest rooting percentage (100.0) was obtained from 0.5 mg/l IBA in Desta variety whereas the lowest percentage of rooting (25.0) and (30.0) was obtained from both Desta variety and Acc.24/99 in IBA free MS medium. There was no statistical difference between 0.5 mg/l and 1 mg/l of IBA for Desta variety. The current result showed that the highest rooting percentage for Desta variety and Acc.24/99 was obtained at lower concentration specifically at 0.5 mg/l IBA and when the concentration of IBA further increased, rooting percentage was decreased. This might be due to the increase or decrease in concentration of these hormones led to variability of root induction percentage. Induction percentage was less at high concentration; shoots were longer at low concentration; number of roots, was high at optimum low concentration; and root length was also based on the auxins concentrations. Similar observation was made by Roy *et al*. (2012) who reported the highest rooting percentage when micro shoot cultured on root induction medium consisted of half strength MS medium containing 0.5 mg/l IBA in *Coccinia cordifolia.* Similarly, Kahia (2016) also reported that microshoots cultured on medium supplemented with 0.5 mg/l IBA produced the highest number of shoots. Moreover, Bekele *et al.* (2013) revealed that there was highly significant effect among the concentrations of the given auxins for all experimental variables; namely, rooting percentages, root number, except root length.

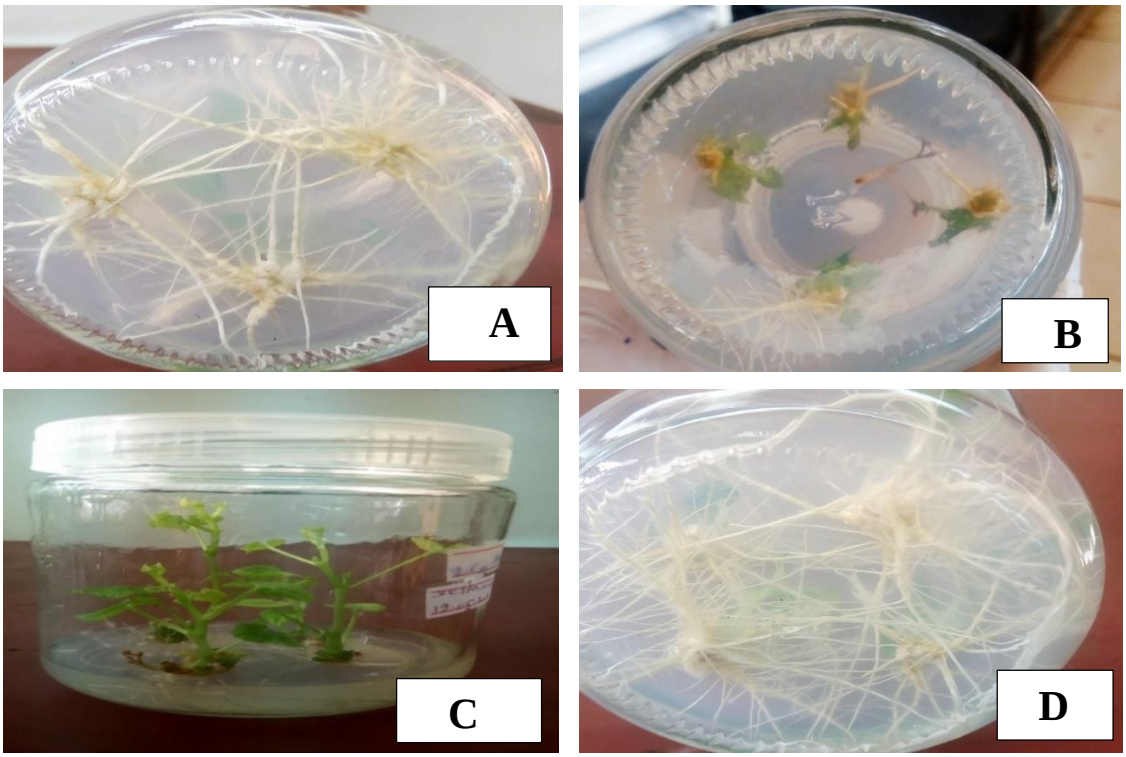

Figure 4. Effect of different concentrations of IBA on rooting of two anchote genotypes (A) Acc.24/99 at 0.5 mg/l of IBA, (B) Control, (C) 0.5 mg/l of IBA and (D) Desta at 0.5mg/l of IBA

Number of roots

Number of roots showed highly significant difference ($P<0.01$) among IBA concentrations in both Desta variety and Acc.24/99 (Table 3, Appendix 4).). The maximum number of roots (13.20 and 12.02) were recorded on medium supplemented with 0.5 mg/l IBA for Desta variety and Acc.24/99, respectively The minimum numbers of roots for Desta (4.10) and for Acc.24/99 (2.56) were found on control treatment. Number of roots increased with the application of IBA and decreased when shoots were on growth regulators free medium. The number of roots decreased when the concentration of IBA increased beyond 1 mg/l. Rooting of shoots was usually attained at low concentration of auxins in many in vitro cultures (Nitzche and Wenzel, 1977). The effectiveness of using IBA for in vitro rooting has been reported in *Momordica dioica* (Hoque *et al*., 1995; Nabi et al., 2002). This finding is in agreement with Roy *et al*. (2012). They found more number of root (8.8) on half strength MS + 0.5 mg/l IBA in *Coccinia cordifolia*. Similarly, Sarder (2009) in his study of regeneration of *Solanum tuberosum L*.

indicated that the highest number of roots was found at 0.5 mg/l IBA and the root induction gradually decreased with increasing concentration of IBA. Likewise, Nabi *et al*. (2002) observed that 1/2-MS with 0.5 µM IBA showed a better response for rooting on *Momordica dioica* Roxb.

Root length

Root length showed highly significant difference ($P<0.01$) among IBA concentrations in both Desta variety and Acc.24/99 (Table 3). The maximum root length per plantlet 6.12 and 5.82 cm was recorded from a medium supplemented with 1.5 mg/l IBA in Desta variety and Acc.24/99 respectively, whereas the minimum root length was found in the control treatment which was 3.10 and 2.70 cm for Desta variety and Acc.24/99, respectively. A medium supplemented with 1.5 mg/l IBA was significantly different from other treatments for both genotypes. The current finding showed that medium with high number of roots per shoot gave smaller average length of root per explant. This might be due to auxins promote adventitious root with low level of an auxins in most of *cuccurbits*. Abhayankar and Reddy 2007 reported that Root development was slower at higher concentration of IBA (0.5-2.0 mg/l). Grossmann (2000) also reported that an endogenous or exogenous auxins over dose is phytotoxic and typically causes plant deformation such as epinasty and growth inhibition. In line with current result, Sirous *et al*. (2012) obtained the highest root length from medium supplemented with 1.5 mg/l IBA.

Table 3 Effect of different concentrations of IBA on rooting of two anchote genotypes

Genotypes	IBA in mg/l	DRMean±SD	%R Mean±SD	NR Mean±SD	RL Mean±SD
Desta variety	0.0	18.40±0.10^{a}	25.00±0.50^{e}	4.20±0.07^{e}	3.10±0.10^{i}
	0.5	6.60±0.07^{g}	100.00±0.00^{a}	13.20±0.07^{a}	4.08±0.04^{h}
	1.0	11.20±0.01^{f}	98.00±2.46^{a}	11.20±0.04^{b}	4.44±0.08^{g}
	1.5	12.40±0.10^{d}	74.00±14.75^{b}	10.20±0.10^{b}	6.12±0.13^{a}
	2.0	14.40±0.15^{c}	63.00±2.78^{c}	7.40±0.07^{c}	5.50±0.07^{c}
	2.5	16.20±0.10^{b}	35.00±0.50^{d}	6.20±0.10^{d}	5.06±0.05^{e}
Accession24/99	0.0	19.20±0.10^{a}	30.00±0.50^{f}	2.56±0.46^{e}	2.70±0.14^{j}
	0.5	7.40±0.07^{e}	93.90±0.58^{a}	12.02±0.10^{a}	4.38±0.08^{g}
	1.0	10.20±0.07^{d}	84.20±0.54^{b}	10.20±0.10^{b}	4.66±0.11^{f}
	1.5	11.40±0.07^{c}	75.00±0.50^{c}	8.20±0.10^{b}	5.82±0.08^{b}
	2.0	13.20±0.10^{c}	55.00±0.50^{d}	6.20±0.10^{c}	5.20±0.10^{d}
	2.5	15.40±0.07^{b}	44.80±0.44^{e}	4.20±0.10^{d}	5.08±0.04^{e}
	LSD(P≤0.01)	0.12	6.30	0.20	0.11
	CV (%)	0.73	7.64	1.89	1.98

Note: %R =rooting percentage, NR =average number of roots, DR=days to rooting and RL =average root length in cm.

4.4. Acclimatization of plantlets

As indicated in table 4, the plantlet transferred for acclimatization, the survival rate of 83.92 % and 73.07% were recorded for Desta variety and Acc.24/99, respectively. Plantlets taken from the rooting medium containing 0.5 mg/l of IBA showed better survival rate and growth than the others during acclimatization. This may be due to the better anchoring and absorptive capacities of the vigorous roots produced in that medium. Yosef and Tileye (2013) reported that well developed root systems are important for the successful development of acclimatized plantlets.

The current result also indicated that Desta variety had best performance on rapid survival rate as compared to Acc.24/99

Table 4. The survival rate of two anchote genotypes plantlets during acclimatization in greenhouse

Genotypes	Number of transferred plantlet	Number of survived plantlet	Survival rate (%)
Desta variety	56	47	83.92
Accession 24/99	52	38	73.07

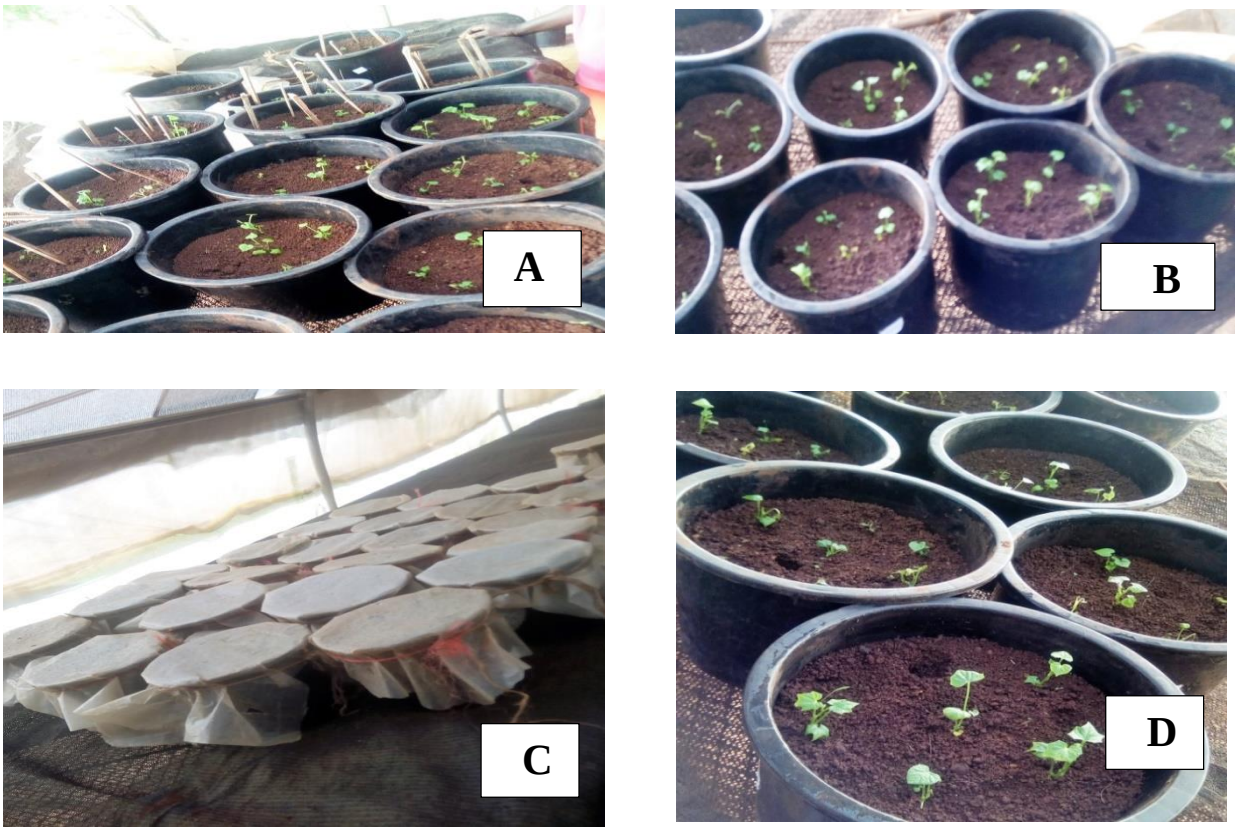

Figure 5. Acclimatized plantlet of the two anchote genotypes: A) Acc.24/99 after one week; B) Desta variety after one week, C) anchote genotypes covered by polyethylene, D) Desta after five days.

5. SUMMARY AND CONCULUSION

Micropropagation is an alternative means of propagation that can be employed in mass multiplication of plants in relatively shorter time. Recently, modern techniques of propagation have been developed which could facilitate large scale production of true –to-type plants and for the improvement of the species using genetic engineering techniques. The present study was conducted on the micropropagation of two Anchote genotypes (*Coccinia Abyssinica*). The addition of BAP was found to be essential for shoot initiation in both anchote genotypes. 1.0 mg/l BAP was found to be the best for shoot initiation and multiplication. For in vitro rooting of anchote 0.5mg/l IBA was found to be the optimum for the two anchote genotypes.

The current finding pointed out that different concentrations of BAP influenced days of shoot induction, percentage of shoot initiation, shoot number, leaf number and shoot length for Desta Variety and Acc.24/99. The highest shoot initiation percentage, shoot number, and leaf number were recorded at lower BAP concentration for Desta variety and Acc.24/99 specifically at 1 mg/l and 1.5 mg/l respectively except shoot length. However, at further increase of BAP concentration, shoot induction, percentage of shoot initiation, shoot number, leaf number and shoot length was decreased.

The current result also indicated that early days of rooting and highest rooting percentage, numbers of rooting was obtained from 0.5 mg/l of IBA concentration for both Desta and Acc.24/99, but the highest root length was recorded from 1 mg/l and 1.5 for Desta and Acc.24/99, respectively. In conclusion, 1.0 mg/l BAP and 1.5 mg/l was found to be the best for shoot initiation and multiplication for Desta variety and Acc.24/99, respectively. For in vitro rooting of Desta variety and Acc.24/99, 0.5mg/l IBA was found to be the best.

6. FUTURE LINE OF WORK

The effect of sub-culturing on the multiplication of shoots should be further studied, to determine whether the sub-culturing increases or decreases shoot multiplication to a significant extent.

Explants other than shoot tips such as leaf, stem and bud culture for in vitro regeneration of anchote should be considered to see if they can offer better and efficient responses.

The effect of different accessions on regeneration of shoots and roots should be further studied.

7. REFERENCES

Abdullah, G.R., Al-Khateeb, A.A. and Serage, M., 2003. Effect of different concentrations of growth regulators on Gardenia jasminoides cv. Veitchii micropropagation by tissue culture technique. *Journal of Agricultural and Marine Sciences*, *8*(1): 35-40.

Abera, H., 1995. Anchote-An Endemic Tuber Crop. (S. Edwards, G. Mirutse, and T. Yilma, Eds.). Jimma College of Agriculture, Jimma, Ethiopia.

Aga, H.F. and Bada, K.U., 1997. Nutritional and antinutritional characteristics of anchote (Coccinia abyssinica). *The Ethiopian Journal of Health Development*, *11*(2).

Agarwal, M. and Kamal, R. 2004. In vitro clonal propagation of Momordica charandia L. *Indian Journal of biotech*, 3(4): 26 – 430.

Alagumanian, S., Perumal, V.S., Balachandar, R., Rameshkannan, K. and Rao, M.V., 2004. Plant regeneration from leaf and stem explants of Solanum trilobatum L. *Current Science*, *86*(11): 1478-1480.

Acquaah, M., 2007. Managerial social capital, strategic orientation, and organizational performance in an emerging economy. *Strategic management journal*, *28*(12): 1235-1255.

Ali, S. and Mirza, B., 2006. Micropropagation of rough lemon (Citrus jambhiri Lush.): Effect of explant type and hormone concentration. *Acta Botanica Croatica*, *65*(2): 137-146.

Amare, G., 1973. Developmental Anatomy of Tubers of Anchote: A potential dry land crop. Acta Horticulture, Technical Communications of (ISHS)

Anand, S.P. and Jeyachandran, R., 2004. In vitro multiple shoot regeneration from nodal explants of Zehneria scabra (Lf) Sonder-An important medicinal climber. *Plant tissue cult*, *14*(2): 101-106.

Andres, T.C., 2004. Web site for the plant family Cucurbitaceae and home of The Cucurbit Network.

Arulanandam, L., Peter, J. and Ghanthikumar, S., 2011. Indirect organogenesis of Vitex trifolia Linn. -An important medicinal plant. *Indian J. Biotechnology.*, 10: 238-241

Asfaw, B., Beyene, Y., Suwa, G., Walter, R.C., White, T.D., WoldeGabriel, G. and Yemane, T., 1992. The earliest acheulean from konso-gardula. *Nature*, *360*(6406): 732-735.

Asfaw, Z. and Woldu, Z., 1997. Crop associations of home gardens in Welayita and Gurage in Southern Ethiopia. *SINET: Ethiopian Journal of Science*, *20*(1): 73-90.

Asfaw, Z., 1997. Conservation and use of traditional vegetables in Ethiopia. In *Traditional African vegetables: Proceedings of the IPGRI International Workshop on genetic resources of traditional vegetables in Africa Conservation and use. ICRAF-HQ, Nairobi, Kenya* (57-65).

Ayalew, Y., 2016. *Nutritional and Phytochemical Evaluation of Anchote (Coccinia abyssinica) (Lam.) (Cogn.) Accessions to Promote its Contribution for Food Security and Medicinal Use* (Doctoral dissertation, Addis Ababa University).

Balkaya, A., M. Ozbakir and E.S. Kurtar, 2009. The phenotypic diversity and fruit characterization of winter squash (Cucurbita maxima) populations from the Black Sea Region of Turkey. African J. of Biotech. 9(2):152-162.

Balkema-Boomstra, A.G., Zijlstra, S., Verstappen, F.W.A., Inggamer, H., Mercke, P.E., Jongsma, M.A. and Bouwmeester, H.J., 2003. Role of cucurbitacin C in resistance to spider mite (Tetranychus urticae) in cucumber (Cucumis sativum L.). *Journal of chemical ecology*, *29*(1): 225-235.

Bekele, A., Feyissa, T. and Tesfaye, K. 2014. Genetic diversity of anchote (Coccinia abyssinica (Lam.) Cogn.) from Ethiopia as revealed by ISSR markers. *Genetic resources and crop evolution*, 61(3): 707-719.

Bekele, E., 2007. Study on actual situation of medicinal plants in Ethiopia. *Japan Association for International Collaboration of Agriculture and Forestry*: 54-60.

Bekele, F., B. Abera, and M. Getahun. 2013. In vitro propagation of Anchote (Coccinia abyssinica) (Lam.) Cogn. *Afr. J. Plant Sci,* 7(6): 253–264.

Berndt, R. 2007. A global survey of Puccinia-rust on Cucurbitaceae. Mycol. *Progress*, 6: 151-178.

Beruk, B. D., and Fikre, T. T., 2015. Physical and Proximate Characterization of Anchote (Coccinia abyssinica) Accessions Grown under Hawassa and Wondo Genet Conditions, Southern Ethiopia. Food Science and Quality Management, 42: 62–75.

Biswas, A., Bari, M.A., Roy, M. and Bhadra, S.K., 2009. Clonal propagation through nodal explant culture of Boerhaavia diffusa L.-a rare medicinal plant. *Plant Tissue Culture and Biotechnology*, *19*(1): 53-59.

Borah, A.R., Anbumalarmathi, J. and Sharmili, S.A., 2019. In vitro propagation of Coccinia indica (L.) Voigt. from internodal segments. *Indian Journal of Agricultural Research*, *53*(2): 202-207.

Chandramu, C., D.M. Rao and V.D. Reddy, 2003. High frequency induction of multiple shoots from nodal explants of Vitexnegundu L., using sodium sulphate. *J. Plant Biotech,* 5(2): 107-113.

Chandramu, C., Rao, D.M. and Reddy, V.D., 2003. High frequency induction of multiple shoots from nodal explants of Vitex negundo L. using sodium sulphate. *JOURNAL OF PLANT BIOTECHNOLOGY-DAEJEON-*, *5*(2): 107-114.

Chen, C.C., S.J. Chen., A.P. Sagare and H.S. Tsay, 2001. Adventitious shoot regeneration from stem internode explants of *Adenophoratriphylla* (Thumb) A. DC. (Campanulaceae) –An important medicinal herb. *Bot. Bull. Acad. Sin*. 42: 1-7.

Czynczyk, A. and T. Takubowski, 2007. Value of standard and new selected rooted root stocks for apples in Poland. *Acta Hort.*, 732-751.

Da Costa, C. P. and Jones, C.M. 1971. Cucumber Beetle Resistance and Mite Susceptibility.

Daba, M., Derebew, B., Wosene, G., And Waktole, S., 2012. Growth and Yield Performance of Anchote in Response to Contrasting Environment.pdf. Asian Journal of Plant Sciences.

Danci, M. and O. Danci., 2007. Ghidpractic de culturi*in vitro*, Ed. Eurobit, Timisoara. DeRopp R. 1956 Kinetin and auxin activity. *Plant Phy*. 31: 253-254.

Davies, P.J, 2004. Regulatory factors in hormone action: Level, location and signal transduction. In: Davies PJ(ed) plant Hormones, Kluwer Academic Publishers, Dordrecht: 16–35

Dawit, A., and Estifanos, H., 1991. Plants as a primary source of drugs in the traditional health practices of Ethiopia. In J. M. M. Engels, J. G. Hawkes, and W. Melaku (Eds.), Plant Genetic Resources of Ethiopia. Cambridge University Press

Derso, C. and Feyissa, T., 2015. Micropropagation of Yeheb from Shoot Tip: An Endangered Multipurpose Shrub. Plant Science International Journal, 2(1): 01-12.

Desta, F., 2011. Phenotypic and Nutritional Characterization of Anchote [Coccinia abyssinica (Lam.) Cogn] Accessions of Ethiopia. M.Sc. Thesis for the Degree of Master of Science in Horticulture (Vegetable Science). Jimma University, Ethiopia

Devendra, N.K., Rajanna, L., Sheetal, C. and Seetharam, Y.N., 2008. In vitro Clonal Propagation of Trichosanthes cucumerina L. var. cucumerina. *Plant Tissue Culture and Biotechnology*, *18*(2): 103-111.

DeVore, R.A. and Lorentz, G.G., 1993. *Constructive approximation* (Vol. 303). Springer Science and Business Media.

Edwards, R.A. and Bryan, J., 1995. Fascins, a family of actin bundling proteins. *Cell motility and the cytoskeleton*, *32*(1): 1-9.

Edwards, S., Tadesse, M. and Hedberg, I., 1995. *Flora of Ethiopia and Eritrea vol. 2, Part 2: canellaceae to euphorbiaceae*. AAU.

Emmanuel, S., S. Ignacimuthu and Kathiravan K., 2000. Micropropagation of Wedelia calendulacea Less., a medicinal plant. Phytomorphology, 50: 195-200

Endashaw, B., 2007. Study on Actual Situation of Medicinal Plants in Ethiopia. A report Prepared for JAICAF.

Fazlima, P., 2011. *In vitro* regeneration of three local potatoes *(Solanum tuberosum* L.) varieties of Bangladesh. M.Sc. Thesis, BRAC University, BRAC.

Fekadu, H., 2011. Nutritional and Anti-nutrition's of Anchote (Coccinia abyssinica) tubers: lap lambert Academic Publishing, Addis Ababa Ethiopia: 124.

Fekadu, H., 2011. *Nutritional and Anti-nutritions of Anchote (Coccinia Abyssinica) Tubers*. Lap Lambert Academic Publ.

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

Firoz Alam M., Ruhul Amin, Ekhlas Uddin M., Sudhangshu kumar Biswas and Monirul Islam M.,2015. Regeneration of Shoot from Nodal explants of Cucumis sativum considering different Hormonal concentration 4: 48-52.

Folla, B., Abera, B., and Getahun, M., 2013. In vitro propagation of Anchote (Coccinia abyssinica) (Lam.) Cogn.], 7(6), 253–264

Gamborg, O. L. and G. C. Phillips., 1995. Plant cell, tissue and organ culture. Spring-Verlag Berlin Heidelberg: 21- 22.

Ganasan, K. and F. Huyop, 2010. *In vitro regeneration* of *Citrulluslanatus*cv. Round Dragon. *J. Biol. Sci.* 10:131–137.

Ganasan, K. and Huyop, F., 2010. The Sensitivity of Plant Tissue Culture and Plant cell of Citrullus lanatus cv. Round Dragon Against BASTA®. *International Journal of Agricultural Research*, *5*(1): 11-18.

Gaspar, T., C. Kevers, J. F. Hausman, J. Y. Berthon and V. Ripetti., 1994. Peroxidase activity and endogenous free auxin during adventitious root formation. In: P. J. Lumsden,

Gemede, H.F., 2014. Nutritional composition, antinutritional factors and effect of boiling on nutritional composition of Anchote (Coccinia Abyssinica) tubers. Journal of Scientific and Innovative Research, 3(2): 177-188.

George, E.F., Hall, M.A. and De Klerk, G.J. eds., 2007. *Plant propagation by tissue culture: volume 1. the background*. Springer Science and Business Media,1: 1–5

Getahun, A. 1969. Developmental Anatomy of Seedlings and Tuber of Anchote, Coccinia abyssinica (Cucurbitaceae). Univ. of Florida, Gainesville, FL, PhD Diss.

Ghanthikumar, S., L.J.P. Arulanandam and L.H. Joseph, 2013. Micropropagation of Coccinia indica wight and Arn: A medicinal plant. Int. J. Applied Bioresearch, 18: 1-4.

Gibbs, R.D., 1974. Chemo taxonomy of flowering plants. McGill-Queens University Press.Montreal and London 2: 829–843

Girma, A., and Hailu, G., 2007. Response of Anchote (Coccinia Abyssinica) to Organic and Inorganic Fertilizers Rates and Plant Population Density in Western Oromia, Ethiopia. East African Journal of Sciences, 1(2), 120–126.

Guma, T. B., Jane, K., Justus, O., Kariuki, P. N., 2015. Standardization of in vitro sterilization and callus induction protocol for leaf explants of anchote: Coccinia abyssinica. International Journal of Research and Development in Pharmacy and Life Sciences, 4(2), 1427-1433

Habtamu, F. G. 2014. Effects of Boiling Methods on Anti-Nutritional Factors of Anchote (Coccinia Abyssinica (lam.) Cogn) tubers Grown in Western. Food Science and Quality Management, 27: 39–45.

Hazarika, B.N, 2006. Morpho-physiological disorders *in vitro* culture of plants. SciHortic 108:105–120

Hazarika, B.N., 2003. Acclimatization of tissue-cultured plants. *Current science*, 1704-1712.

Helmke, B.P., Rosen, A.B. and Davies, P.F., 2003. Mapping mechanical strain of an endogenous cytoskeletal network in living endothelial cells. *Biophysical journal*, *84*(4): 2691-2699.

Heloir, M.C., Kevers, C., Hausman, J.F. and Gaspar, T., 1996. Changes in the concentrations of auxins and polyamines during rooting of in-vitro-propagated walnut shoots. *Tree Physiology*, *16*(5): 515-519

Holstein, N. and Renner, S.S., 2011. Coccinia intermedia–a new Cucurbitaceae species from West Africa. *PhytoKeys*, (7): 27.

Howell, S.H., Lall, S. and Che, P., 2003. Cytokinins and shoot development. *Trends in Plant Science*, *8*(9): 453-459.

Huq, A., Akter, S., Islam, S. and Khan, S., 2012. In vitro micropropagation of pointed gourd (Trichosanthes dioica Roxb.) from shoot tip and nodal segment. *Bangladesh Journal of Scientific and Industrial Research*, *47*(2): 217-222.

Hussain, A., Qarshi, I. A., Nazir, H., Ullah, I., 2012. Recent Advances in Plant in vitro Culture. In A. Leva and L. M. R. Rinaldi (Eds.), Plant Tissue Culture: Current Status and Opportunities (1-28). Intech open access publisher: Croatia.

Hussain, A., Qarshi, I.A., Nazir, H. and Ullah, I., 2012. Plant tissue culture: current status and opportunities. *Recent advances in plant in vitro culture*, 1-28.

IBC (Institute of Biodiversity Conservation), 2007. Second Country Report on the State of PGRFA to FAO. Addis Ababa, Ethiopia.

Islam AS, Haque MM, Sarker RH and Hoque MI (Eds). Proc. Sixth Intl. Plant Tissue Cult. and Biotech. Conf., December 3-5, 2010, BangladeshAssoc. Plant Tiss. Cult. Biotech. 27- 40.

Jafari, N., Othman, R.Y. and Khalid, N., 2011. Effect of benzylaminopurine (BAP) pulsing on in vitro shoot multiplication of Musa acuminata (banana) cv. Berangan. African Journal of Biotechnology, 10(13): 2446-2450.

Jane Kahia, 2016. Improved Micropropagation of Plantlets from Nodal Explants of Anchote (Coccinia abyssinica .51(7): 905–909

Jeffrey, C. (1995). Cucurbitaceae. In S. Edwards, M. Tadesse, and I. Hedberg (Eds.), Flora of Ethiopia and Eritrea. National Herbarium, Addis Ababa University, and Uppsala University, Sweden, 2: 52-55.

Joshi, M. and U. Dhar, 2003. *In vitro* propagation of *Saussureaobvallata*(DC.) Edgew. an endangered ethno-religious medicinal herb of Himalaya. Plant Cell Rep. 21: 933-939.

Kahia, J., Njenga, P. and Kirika, M., 2016. Improved Micropropagation of Plantlets from Nodal Explants of Anchote (Coccinia abyssinica)—A Calcium- and Protein-rich Tuber, HortScience, 51: 905-909.

Karin, Z. 2002. Enset (Ensetventricosum (Welw.) Cheesm.) in subsistence farming systems in

Kelbessa, E. and Demissew, S., 2014. Diversity of vascular plant taxa of the flora of Ethiopia and Eritrea. *Ethiopian Journal of Biological Sciences*, *13*: 37-45.

Kevers, C., J. F. Hausman, O. Faivre-Rampant, D. Evers, and T. Gaspar, 1997. Hormonal control of adventitious rooting: Progress and Questions. *Angew. Bot*. 71: 71–79.

Khadiga, G.A.E., Rasheid, S.M. and Mutasim, M.K., 2009. Effect of cultivar and growth regulator on in vitro micropropagation of Potato (Solanum tuberosum L). *Am. Eurasian J Sustain Agric*, *3*: 487-492.

Khatun, N., M.A. Bari, R. Islam, S. Huda, N.A. Siddque, M.A. Rahman and M.U. Mullah, 2003. Callus induction and regeneration from nodal segment of potato cultivar Diamant. *J. Biol. Sci.,* 3: 1101-1106.

Kishor, R.H. and S. Devir, 2009. Induction of multiple shoots in a monopodial orchid hybrid (*AeridesvandarumReichb*.f X *Vanda stangeanaReichb*.f) using Thidiazuron and analysis of their genetic stability. *Plant Cell Tiss Organ Cult* 97: 121-129.

Kubitzki, K. ed., 2010. *Flowering Plants. Eudicots: Sapindales, Cucurbitales, Myrtaceae* (Vol. 10). Springer Science and Business Media.

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

Kumar, N., Vijayanand, K.G., Reddy, M.P. 2010a. Shoot regeneration from cotyledonary leaf explants of *Jatropha curcas*: a biodeselplant. *ActaPhysiol Plant* 32: 917-924.

Kumar, N., Vijayanand, K.G., Reddy, M.P. 2011b. Plant regeneration in non-toxic *Jatropha curcas*-impacts of plant growth regulators, source and type of explants. *J Plant BiochemBiotechnol*. 20: 125-133.

Lee Wai- Leng, Chan Lai-Keng, 2004. Plant regeneration from stem nodal segments of Orthosiphon stamineus benth, a medicinal plant with diuretic activity. Plant cell Tissue and Organ Culture; 78(2):101-106

Lui, Z. and Le Z., 2001. Micropropagation of *Camptothecaacuminata*Decaisne from axillary buds, shoot tips and seed embryos in tissue culture system. *In Vitro Cell Dev. Biol.Plant*37: 84-88.

Magyar-Tabori K, Dobranszki J, Teixeira da Silva JA, Bulley SM, Hudak I, 2010. The role of cytokinins in shoot organogenesis in apple. Plant Cell Tiss Org Cult101:251–267

Makkar, H.P.S., 1993. Antinutritional factors in foods for livestock. *BSAP Occasional Publication, 16*: 69-85.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiologia Plantarum.15: 473–497

Nabi, S.A., M. Rashid Al-Amin and M.G. Rasul, 2002. Organogenesis in teasle gourd (*MomordicadioicaRoxb*.). *Plant Tissue and organ Cult.,*12: 173-180.

Nitzche, M.W. and G. Wenzel, 1977. Haploid in Plant Breeding. Berlin, Hamburg, Parey: 90.

Omar, M.S. and Aouine, M., 2007. Commercial in vitro mass propagation of plants: current status and future investment prospects. *Kuwait J Sci*, *34*: 94-99.

Padmapriya, H., Karthikeyan, A.V.P., Jahir Hussain, G., Karthi, C. and Velayutham, P., 2011. An efficient protocol for in vitro propagation of Solanum nigrum L. from nodal explants, Journal of Agricultural Technology, 7:1063-1073.

Pareek, A.; A. Kantia and S. L. Kothari, 2004. In vitro cloning of ornamental species of Dianthus. *Indian J. Biotech.* 3: 363-266

Parveen, F., 2011. *In Vitro r*egeneration of three local potatoes *(Solanum tuberosum L.) varieties of Bangladesh* (Doctoral dissertation, BRAC University).

Pierik, R.L.M. 1997., In vitro culture of higher plants. Martinus Nijhoff Publ, Dordrecht, Boston, Lancaster, 364-370.

Purseglove, J.W., 1966. Tropical crops, Dicotylendons I. Longmans Green and Co. Ltd., Lond and Harlow, 100–138

Rakhi C, Rekha HR, Kumar, M.V., 2010. Assessment of regenerative potentiality of cotyledon explants of some indigenous varieties of Cucurbits using varied concentrations of Cytokinins. In: Role of Biotechnology in Food Security and Climate Change.

Reddy, M.P., Kumar, N., Vijayanand, G., Singh, A.H. Singh, S. 2008. Method for micropropagation of *Jatropha curcas*plantsfrom leaf explants (Patent filed US and PCT, Application No.2537.

Regassa, T., 2016. Vascular Plant Diversity and Ethnobotanical Study of Medicinal and Wild Edible Plants in Jibat, Gedo and Chilimo Forests, West Shewa Zone of Oromia Region, Ethiopia (Doctoral dissertation, Addis Ababa University).

Rival, A., F. Bernard and Y. Mathieu, 1980. Changes in peroxides' activity during *in vitro* rooting of oil palm (*Elaeisguineensis*JACQ.). ScientiaHortic Roca. WM Annual Report. Genetic Resources unit, Tissue Culture section. CIAT, Cali. Columbia.

Rout, GR., S. Samantaray and P. Das, 2000. *In vitro* manipulipulation and propagation of medicinal plants. *Biotech. Advances* 18: 91-120.

Roy, P.K., 2008. Rapid Multiplication of *Boerhaaviadiffusa*. Through *in vitro* Culture of Shoot tip and Nodal Explants. *Plant Tiss. Cult. and Biotech.*18(1): 49-56.

Sanjeevkumar, Majorsingh, Singh AK, Srivastava K and Banerjee MK, 2003. In vitro propagation of pointed gourd (Trichosanthes dioica Roxb.) Cucurbit Genetics Cooperative Rep. 26: 74-75.

Sarker, P.F.M, S.R. Jahan, and M. Rahmatullah, 2008. In vitro regeneration of Coccinia grandis (L.) Voigt, An Indigenous Medicinal Plant of Bangladesh. Afr. J trad comp Alt med, World Congers On Medicinal and Aromatic Plants, Held at Cape Town South Africa.

Saxena, C., Samantaray, S., Rout, G.R. and Das, P., 2000. Effect of auxins on in vitro rooting of Plumbago zeylanica: Peroxidase activity as a marker for root induction. *Biologia Plantarum*, *43*(1) 121-124.

Schaefer, H. and Renner, S.S., 2011. Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxon*, *60*(1) 122-138.

Schaefer, H., Heibl, C., Renner, S.S., 2009. Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events.Proc.R.Soc. B276: 843–851.

Sebastian, P.M. 2011. Phylogenetics and biogeography of two clades of Cucurbitaceae: one New World, one Old World, and both with economically important species. Ph.D. Dissertation, Munich University, Munich

Serbessa, B., 2017. Morphological and Molecular Genetic Diversity and Cytogenetics of Cultivated Anchote (Coccinia abyssinica (Lam.) Cogn) from Ethiopia (Doctoral dissertation, Addis Ababa University).

Shebabaw, H., 2013. Effect of Processing on Physicochemical and Antinutritional Factors of "Anchote" (Coccinia Abyssinica) and Development of Value Added Biscuit (Doctoral dissertation, Addis Ababa University).

Shekhawat, K., Gupta, P.C., Kumar, R., Kumar, A., Kumari, R. and Kumawat, S., Role of tissue culture techniques in social forestry. *Recent Advances in Chemical Sciences and Biotechnology*,113.

Sultana, S. and PJ. Handique, 2004. Micropropagation of *Wedeliachinensis*through high frequency shoots multiplication using nodal explants. J. *Curr. Sci*. 5: 447-452.

Taiz, L. and Zeiger, E., 1991. Plant physiology. Redwood City. *Plant Physiology: Redwood City*.

Tarek AS, Soliman AO, Yousry, A.B., 2008. *In vitro* propagation of two triploid hybrids of watermelon through adventitious shoot organogenesis and shoot tip culture Acta Biologica Szegediensis. 5(1):27-31.

Tesfaye Mesele, Abebe Demissie, 1988. Root and tuber crops collecting expedition in southwestern Ethiopia.PGRC/E ILCA Germplasm Newsletter18:17-23

Thiruvengadam, M., Rekha, K.T., Yang, C.H., Jayabalan, N. and Chung, I.M., 2010. High-frequency shoot regeneration from leaf explants through organogenesis in bitter melon (Momordica charantia L.). *Plant Biotechnology Reports*, *4*(4): 321-328.

Tilahun, W., Sentayehu, A., Amsalu, A., and Weyessa, G., 2014. Genetic Diversity Analysis among Anchote (Coccinia abyssinica) Accessions in Western Ethiopia. International Journal of Agricultural Research, 9(3): 149–157.

Tyagi, A.P., Comai, L., Byers, B. 2001. Comparison of plant regeneration from root, shoot and leaf explants in pigeon pea(Cajanuscajan) cultivars. SABRAO J. Breed. Gen. 33: 59-71.

Uddin, S.N., 2006. In vitro propagation of Elite Indigenous Potato (Solanum tubeosum L Var. Indurkani) of Bangladesh. Jornal of plant Sciences 1 (3) 212-216. Academic Jornal Inc., USA.

Ugandhar, T., M. Venkateshwarrlu, G. Begum, T. Srilatha and K. Jaganmohanreddy, 2011. In vitro plant regeneration of Cucumber (Cucumis sativum (L.) from cotyledon and hypocotyl explants. Sci. Res. Rep., 1: 164-169

V Sheena, Gabriel Jeya Jothi ,2015. In vitro propagation of Orthosiphon stamineus Benth using nodal and leaf explants 4: 06-10

Van Staden J, Zazimalova E, George EF, 2008. Plantgrowth regulators II: Cytokinins, their analoguesand antagonists. In: George EF, Hall MA, De KlerkGJ (eds) Plant propagation by tissue culture, 3rdedn.Springer, Dordrecht, 205–226

Van Staden, J., Zazimalova, E. and George, E.F., 2008. Plant growth regulators II: cytokinins, their analogues and antagonists. *Plant propagation by tissue culture*, *1*: 205-226.

Varshney, A., Anis, M. and Aref, I.M., 2013. Potential role of cytokinin–auxin synergism, antioxidant enzymes activities and appraisal of genetic stability in Dianthus caryophyllus L.—an important cut flower crop. *In Vitro Cellular and Developmental Biology-Plant*, *49*(2): 166-174.

Veatch-Blohm, M.E., 2007. Principles of plant genetics and breeding. *Crop Science*, *47*(4): 1763.

Villa, T.C.C., Maxted, N., Scholten, M. and Ford-Lloyd, B., 2005. Defining and identifying crop landraces. *Plant Genetic Resources*, *3*(3): 373-384.

Wayessa, B.S., 2016. Toward a history of the Oromo of Wollega in southwestern Ethiopia: an ethno archaeological study of ceramic technological style and tuber crop domestication.

Woldegiorgis, G., Gebre, E. and Lemega, B., 2008. Root and tuber crops: The untapped resources. *Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia*.

Yassin, H., Mohammed, A., Fekadu, D. and Hussen, S., 2013. Effect of flower bud removal on growth and yield of anchote root (Cocciniaabyssinica (Lam.) Cogn.) accessions at bishoftu. Advanced Research Journal of Plant and Animal Sciences, 1(1): 7-13.

Yosef Yambo and Tileye Feyissa, 2013. Micropropagation of anchote[Cocciniaabyssinica(Lam.) Cogn.]: High calcium content tuber crop of Ethiopia. *Afr. J. Agr. Res*. 8(46):5915–59

Zenkteler, M. and Nitzsche, W., 1984. Wide hybridization experiments in cereals. *Theoretical and Applied Genetics*, *68*(4): 311-315.

8. APPENDIX

Appendix Table 1. MS stock solutions

Stock solution	Ingredient	Concentration of ingredient in the medium(mgl-1)	Stock (mgl-1)	Volume of stock solution taken for one line of medium(mg-l)
Inorganic macronutrient				
			X50/10000ml/l	
stock I	NH_4NO_3	1,650		
	KNO_3	1,900	33.000	
	$MgSO_47H_2O$	370	38.000	
	$KH2PO_4$	170	7,400	**20ml/l**
			3,400	
			X100/500ml	
stock II	$CaCl_22H_{2O}$	440	8,800	
Micronutrient	$MnSO_{4,}4H_2O$	22..3	1,115	
	$ZnSO_{4,}7H_2O$	8.6	430	
	H3BO3	6.0	310	**5ml/l**
	KI	0.83	41.5	
	$Na_2M_OO4,2H_2O$	0.25	12.25	
	$CuSO_45H_2O$	0.025	1.25	
	$CoCl_{2,}6H_2O$	0.025		
			X50/500ml	
Stock III	$Na_2EDTA.2H_2O$	37.3	373	**10ml/l**
	$FeSO_4,7H_2O$	27.3	278	
			X100/500ml	
Stock V	Thiamine-HCl	0.1	10	
	Pyridoxine-HCl	0.5	50	**5ml/l**
	Nicotinic acid	0.5	50	
Organics/			**X50/500ml**	
Vitamins	Myo-inositol	100	10,000	**10ml/l**
stock VI	Glycine	2	200	

Appendix Table 2. ANOVA for the effect of BAP on shoot initiation

Parameters					
		DI		**PI**	
Source	**DF**	**MS**	**Pr>f**	**MS**	**Pr>f**
Var	1	126.22	<.0001	801.73	<.0001
IBA	6	71.34	<.0001	3595.38	<.0001
V*IBA	6	16.61	<.0001	235.93	<.0001
Error	52	0.26	<.0001	18.90	<.0001
CV		4.56		6.56	

Appendix Table 3. ANOVA table for shoot multiplication

Parameters							
		NS		**NL**		**SL**	
Source	**DF**	**MS**	**Pr>f**	**MS**	**Pr>f**	**MS**	**Pr>f**
Var	1	4.48	<.0001	22.81	<.0001	2.28	<.0001
IBA	5	79.27	<.0001	112.07	<.0001	49.68	<.0001
V*IBA	5	12.27	<.0001	23.29	<.0001	12.58	<.0001
Error	44	0.20	<.0001	0.16	<.0001	0.10	<.0001
CV		8.55		7.41		1.65	

Appendix Table 4. ANOVA table for root induction

Parameters									
		DR		**NR**		**RL**		**P**	
source	**DF**	**MS**	**Pr>f**	**MS**	**Pr>f**	**MS**	**Pr>f**	**MS**	**Pr>f**
Var	1	2.40	<.0001	33.75	<.0001	0.013	0.25	63.03	0.1196
IBA	5	169.24	<.0001	121.10	<.0001	44.95	<.0001	7739.20	<.0001
V*IBA	5	2.20	<.0001	0.49	<.0001	1.08	<.0001	197.01	<.0001
Error	44	0.008	<.0001	0.025	<.0001	0.01	<.0001	25.01	<.0001
CV		0.73		1.89		1.98		7.64	