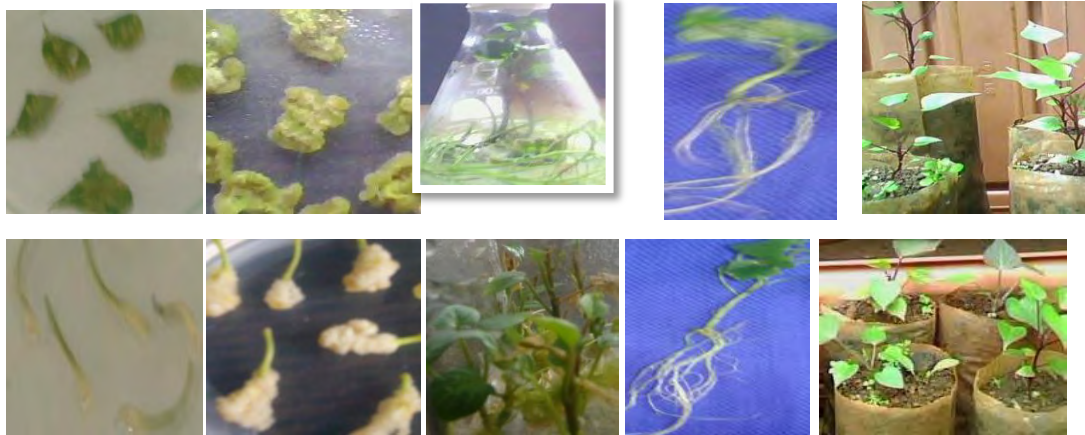


ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
FACULTY OF LIFE SCIENCES
MICROBIAL, CELLULAR AND MOLECULAR BIOLOGY
PROGRAM UNIT



***In vitro* regeneration of sweet potato (*Ipomoea batatas* (L.) Lam.)
from leaf and petiole explants**



By

Tasew Getu

**A Thesis Submitted to the School of Graduate Studies of the Addis Ababa
University in Partial Fulfillment of the Requirements for the Degree of Master of
Science in Biology (Applied Genetics)**

October 2011

Addis Ababa, Ethiopia

TABLE OF CONTENTS

CONTENTS	PAGE
Table of contents.....	i
List of tables.....	iii
List of figures.....	iv
List of appendices.....	v
List of abbreviations.....	vi
Acknowledgments.....	vii
Abstract.....	viii
1. Introduction	1
2.Objectives	3
2.1. General objective	3
2.2. Specific objectives	3
3. Literature review.....	4
3.1 Description of sweet potato.....	4
3.2 Origins of sweet potato.....	5
3.3 Significance and uses of sweet potato.....	5
3.4 Growing conditions of sweet potato.....	7
3.5 Harvesting of sweet potato.....	8
3.6 Global production of sweet potato.....	8
3.7 Major problems associated with sweet potato production.....	9
3.7.1 Viral disease.....	9
3.7.2 Damage by insects.....	10
3.7.3 Fungal and bacterial diseases.....	10
3.8 Tissue culture.....	11
3.8.1 <i>In vitro</i> regeneration of sweet potato.....	11
4. Materials and methods.....	13
4.1 Plant material.....	13

4.2 Media preparation.....	13
4.2.1 Stock solution preparation.....	13
4.2.2 Plant growth regulators preparation.....	13
4.2.3 Culture medium preparation.....	14
4.3 Callus initiation.....	14
4.4 Plant regeneration.....	15
4.5 Shoot multiplication	15
4.6 Rooting and acclimatization.....	15
4.7 Experimental design and data analysis.....	16
5. Results.....	17
5.1 Effect of 2,4-D and kinetin on callus induction.....	17
5.2 Effect of BAP on shoot regeneration.....	24
5.3 Effect of BAP on shoot multiplication.....	24
5.4 Effect of BAP and GA₃ on shoot multiplication.....	26
5.5 Rooting and acclimatization.....	29
5.5.1. Rooting.....	29
5.5.2. Acclimatization	30
6. Discussion.....	32
6.1 Effect of 2,4-D and kinetin on callus induction.....	32
6.2 Effect of BAP on shoot regeneration.....	33
6.3 Shoot multiplication.....	34
6.4 Rooting and acclimatization.....	35
7. Conclusion.....	37
8. Recommendations.....	38
9. References.....	39

LIST OF TABLES

	Page
Table 1. Percentage of calli induced from petiole and leaf explants of Beletech and Awassa-83 varieties of sweet potato	18
Table 2. Percentage of regenerated shoots from calli derived from leaf and petiole of Beletech and Awassa-83 sweet potato varieties.....	22
Table 3. Mean number of shoots per explant at different concentrations of BAP from calli derived from petiole and leaf of Beletech and Awassa-83 sweet potato varieties.....	23
Table 4. Mean height of regenerated shoots from petiole and leaf.....	23
Table 5. Mean number of shoots produced per node from plantlets regenerated from petiole and leaf calli of Beletech and Awassa-83 varieties of sweet potato at different concentrations of BAP.....	24
Table 6. Mean height of multiplied shoots	25
Table 7. Mean number of shoots per node of shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 varieties of sweet potato at different concentrations of BAP in combination with GA ₃	27
Table 8. Mean height of shoots regenerated from leaf and petiole calli of Beletech and Awassa-83 varieties of sweet potato at different concentrations of BAP in combination with GA ₃	28
Table 9. Effect of different IBA (mg/l) concentrations on number of main roots of shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 varieties of sweet potato.....	29

LIST OF FIGURES

	Page
Figure 1. The different skin color of sweet potato.....	4
Figure 2. Sweet potato flower.....	5
Figure 3. Sequential events showing shoot regeneration from calli derived from leaf of Beletech variety.....	19
Figure 4. Stages showing shoot regeneration from calli derived from petiole of Beletech variety.....	20
Figure 5. Stages showing shoot regeneration from calli derived from leaf of Awassa- 83 variety.....	20
Figure 6. Stages showing shoot regeneration from calli derived from petiole of Awassa- 83 variety.....	21
Figure 7. Multiplied shoots regenerated from petiole and leaf calli of Beletech and Awassa- 83 varieties.....	25
Figure 8. Shoot multiplication medium supplemented with 2 mg/l BAP resulted in degeneration of shoots to callus	25
Figure 9. Dwarf shoots of Awassa-83 (A) degenerated shoots of Beletech variety to callus (B).....	28
Figure 10. Rooting of plantlets regenerated from calli derived from petiole and leaf of Beletech and Awassa-83 varieties.....	30
Figure 11. Acclimatized plantlets of Beletech and Awassa-83 varieties in the glasshouse.....	31

LIST OF APPENDICES

	Page
Appendix 1. Average constituents of the sweet potato: values per 100 g.....	50
Appendix 2. Full MS basal medium stock solution.....	51

LIST OF ABBREVIATIONS

2,4-D	Dichlorophenoxy acetic acid
ABA	Absciscic acid
BAP	6-Benzyle Amino Purine
CIP	International Potato Center
CMV-	Cucumber Mosaic Virus
CRD	Completely Randomized Design
CTA	Technical Center For Agricultural And Rural Cooperation
FAO	Food and Agricultural Organization
GA ₃	Gibberellic Acid
IAA	Indol Acetic Acid
IBA	Indol-3-Butyric Acid
Kpa	kilo Pascal
MS	Murashige and Skoog
NAA	α -Naphthalene acetic acid
RDA	Recommended Daily Allowance
SPCSV	Sweet Potato Chlorotic Stunt Virus
SPFMV	Sweet Potato Feathery Mottle Virus
SPVD	Sweet Potato Virus Disease
TDZ	1-phenyl -3-(1, 2, 3-thiadiazol-5-yl) urea
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
WFP	World Food Program

ACKNOWLEDGMENTS

Above all, I would like to thank the father of our ancestors, the Almighty GOD, Alpha and Omega and his blessed mother St. Mary for allowing me to start and finish this work.

I am thankful and would like to express my deepest gratitude to my advisor, Dr. Tileye Feyissa for his positive approach, advice, valuable suggestions, and providing necessary materials during this study.

My deepest and special acknowledgement goes to Professor Legesse Negash for allowing me to use all costly resources and to do my activities in the Plant Tissue Culture Laboratory. It is a place where I grasped the knowledge of tissue culture. Once more thank you Prof.

I am grateful to acknowledge Addis Ababa University and the Microbial, Cellular and Molecular Biology Program Unit for providing me financial support during the study.

I would like to express my gratitude to my colleagues Rosa Birhanu and Edris Youna for their assistance during the activities, Fikadu Kumsa for his technical advice and for his support during statistical data analysis, Firehiwot Birhane for providing me a camera and other materials, and others that I didn't mention your name all thank you.

I greatly indebted to acknowledge my family my mother, Assegedech Bekele, my uncle Teklu Bekele, my sister and brothers Zewdinesh Getu, Shimeles Getu, Alayu Getu, Mandefroh Kefyalew and Biniam Getu for support this work by walking along with me during the ups and downs.

Abstract

Sweet potato (Ipomoea batatas (L.) Lam.) is a tuber-bearing species and it is an economically important crop in tropical, sub-tropical and warm temperate regions. It is a staple root crop serving as a source of vitamins, proteins and carbohydrate. The establishment of in vitro plant regeneration system in sweet potato is of potential importance to sweet potato quality improvement and genetic transformation. The study was conducted to develop in vitro protocol for use in plant regeneration from leaf and petiole explants of Beletech and Awassa-83 sweet potato varieties through callus. For this study, twelve different combinations of 2,4-D and kinetin were used for callus induction from petiole and leaf explants of Beletech and Awassa-83 sweet potato varieties on MS medium. Among the entire combinations, the medium supplemented with 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin resulted in maximum percentage of calli formed (90 and 83) from leaf and petiole explants of Beletech variety whereas 93% and 87% from petiole and leaf explants of Awassa-83 variety, respectively. Among different concentrations of BAP used for shoot regeneration, maximum percentage (80 and 77) of shoot regeneration were obtained from leaf calli of Beletech and Awassa-83 whereas 70% and 71% were obtained from petiole calli of Beletech and Awassa-83 varieties, respectively, on media without growth regulators. BAP in combination with GA₃ was found to be less efficient for shoot multiplication than BAP alone for shoots regenerated from leaf and petiole originated calli of the two sweet potato varieties. Among the different concentrations of BAP used for shoot multiplication, 1.0 mg/l resulted in maximum of two shoots per node from shoots regenerated from petiole and leaf calli of both varieties. Maximum shoot heights recorded were 11.10 cm, 11.40 cm for shoots regenerated from Beletech petiole and leaf calli, and 10.70 cm and 11.90 cm for shoots regenerated from petiole and leaf calli of Awassa-83 varieties, respectively. Variety difference, concentration of IBA and explant difference significantly affected the average number of main root and root length of the two varieties. Among the different concentrations of IBA used for rooting, 0.00 mg/l was the best with main roots 8.10, 6.80 per shoot and root length 10.00 cm and 10.10 cm for shoots regenerated from petiole and leaf calli of Beletech variety, respectively. However, for Awassa-83, 0.1 mg/l IBA was the most suitable concentration of IBA with mean number of roots 6.07 and 4.83 per shoot and mean length of 10.40 cm and 8.70 cm for shoots regenerated from petiole and leaf calli, respectively. Among all plantlets planted in the glasshouse, 90 and 100% for shoots regenerated from petiole and leaf calli of Beletech variety and 80 % and 90% for shoots regenerated from petiole and leaf calli of Awassa-83 variety, respectively were survived.

Key words/Phrases: Awassa-83, Beletech, Rooting, Shoot Multiplication

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a gamopetalous dicot and belongs to the order Polemoniales and the family Convolvulaceae. Sweet potato ranks as the world's seventh most important crop after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), potato (*Solanum tuberosum* L.), barley (*Hordeum vulgare* L.) and cassava (*Manihot esculenta*) (Hironori *et al.*, 2007). Sweet potato is a tuber-bearing species and represents an economically important crop in tropical, sub-tropical and warm temperate regions (Sihachakr *et al.*, 1997). Luo *et al.* (2006) stated that sweet potato is grown in more than 100 countries with the world's average productivity of 15 t/ha. Sweet potato has increased attention because it can adapt to a wide range of environmental conditions and grows even on marginal areas with poor soils of limited fertility and inadequate moisture (Bioethics Nuffield Council, 2004).

In recent years, the improvement of sweet potato is becoming vital as the productivity is adversely affected by biotic stresses like insect pests, fungal and viral infections. The greatest danger has been identified as the susceptibility of sweet potato to virus diseases, which have caused extensive yield reduction of up to 80% (Abo El-Abbas *et al.*, 1998; Karyeija *et al.*, 2000a; Odame *et al.*, 2002). Moreover, conventional breeding efforts and genetic improvement have been seriously limited by difficulties in sexual crosses mainly due to self-incompatibility and male sterility within species (Martin, 1970), hexaploid nature, dichogamy, seed dormancy, abnormal seed and seedling development (Sihachakr and Ducreux, 1993), as well as specific physiological requirements for flowering (Martin and Jones, 1971).

Tissue culture techniques have opened a new frontier in agricultural science by addressing food security and emphasis on the use of biotechnological methods for genetic improvement of exacting traits in sweet potato. Little work has been achieved, particularly the exploitation of somaclonal variation (Sihachakr *et al.*, 1997) and genetic

transformation (Otani *et al.*, 2003). The application of new techniques for improvement of sweet potato crops mostly including the exploitation of gene transfer by genetic transformation and somatic hybridization requires the control of plant regeneration from tissue cultures (Sihachakr *et al.*, 1997). Therefore, development of a reliable *in vitro* plant regeneration procedure for a cultivar is a prerequisite for its improvement by creating genetic variability via biotechnological methods involving direct gene transfer (Sihachakr *et al.*, 1997). Success of any transformation strategy depends largely upon the regeneration capability of the target explants (Chugh and Khurana, 2003). Thus, the lack of efficient system for regeneration has been a bottleneck for the application of genetic engineering in sweet potato (Liu *et al.*, 2001). One of the main difficulties is the control of plant regeneration for which sweet potato is considered as a recalcitrant species (Sihachakr and Ducreux, 1993). In most cases, evidence for plant regeneration in sweet potato were restricted to few genotypes and when attempts were made to extend this into a wide range of genotypes, the majority were found to be recalcitrant (Liu *et al.*, 2001). Additionally, sweet potato is recalcitrant to regeneration because each cultivar shows different responses *in vitro* (González *et al.*, 1999) since every cultivar varies widely in its response to plant regeneration (Gosukondan *et al.*, 1995). For these reasons, genotype is one of the most important factor affecting the evolution of the *in vitro* culture regeneration (Jarret and Gawel, 1991) and consequently transformation response.

Some plant regeneration procedures through organogenesis and somatic embryogenesis have been described for sweet potato using different explants such as meristem, leaf, stems, petioles, storage roots, anthers and ovaries (Gong *et al.*, 1998). Despite many efforts, the underlying problem in these procedures is low frequency of regeneration, long periods of culture and frequent media changes (Gosukonda *et al.* 1995). According to Lowe *et al.* (1992), the overall frequency of shoot regeneration in sweet potato was 2–20%. Hence, the main objective of this study is to regenerate shoots from leaf and petiole explants through calli *in vitro*.

2. Objectives

2.1. General objective:

The general objective of the present study was to regenerate plants from leaf and petiole calli of Beletech and Awassa-83 sweet potato varieties.

2.2. Specific objectives are to:

- optimize growth regulators for callus induction from leaf and petiole explants of the two sweet potato varieties
- optimize concentrations of growth regulators for shoot regeneration, shoot multiplication and rooting
- optimize combinations of different growth regulators for callus induction and shoot multiplication
- acclimatize rooted plantlets and evaluate their survival rate

3. Literature review

3.1. Description of sweet potato

There are over 400 varieties of sweet potato. Its thin skin, smooth or rough, can be white, orange, red or purple in color. Its flesh is white, yellowish or orange (Fig. 1). The different varieties of sweet potato are divided into two groups, those with dry flesh, and those with moist flesh (Austin, 1987). Sweet potatoes are oblong or pointed oval, tuberous roots. Long, trailing stems bear funnel-shaped flowers tinged with pink or rose violet pigments (Fig. 2).

The sweet potato is distantly related to the potato (*Solanum tuberosum* L). The sweet potato is botanically very distinct from the other vegetable called yam, which is native to Africa and Asia and belongs to the monocot family Dioscoreaceae (USDA, 2001).

The number of chromosomes in *I. batatas* is $2n=6x=90$, which is known as hexaploid (Wheatley and Song, 2000), while most of the wild species are $2x$ or $4x$, although there are some species like *I. trifida* which has $2x$, $3x$, $4x$ and possibly $6x$ cytotypes (Winter *et al.*, 1992).



Fig. 1. The different skin color of sweet potato ([www.self.com/food/blogs/nutritional diet/sweet potato.gif](http://www.self.com/food/blogs/nutritional-diet/sweet-potato.gif))



Fig. 2. Sweet potato flower (en.wikipedia.org)

3.2. Origins

Scientists debate the exact place of origin of *I. batatas*, although the evidence points toward Central America. They are no longer found growing in the wild, but it is possible that the wild Mexican sweet potato *I. trifida* is an ancestor (Hakluyt, 1851). Sweet potatoes have been cultivated for more than five thousand years, and fossilized remains found in the Andes have been dated at about 8,000 years old. Genetic studies suggest the likelihood that in early times sweet potatoes were carried by the local people from island to island, spreading gradually across the Pacific from Central and South America to eastern Indonesia, New Guinea, Polynesia and New Zealand. Christopher Columbus is credited with taking sweet potatoes from the New World back to Spain, from where they spread through the warmer regions of Europe and were transported to other parts of Asia and to Africa by Spanish and Portuguese explorers and traders (de Candolle, 1959). Sweet potatoes were grown in gardens by North American, Indians and were an important staple food during the American Revolutionary War and the American Civil War. They were also an essential part of the diet of the slave population in Southern states (Rossel *et al.*, 2001).

3.3. Significance and uses of sweet potato

The sweet potato blends with herbs, spices and flavorings producing delicious dishes of several types. From processed baby foods to the main dishes, salads, breads and desserts,

sweet potatoes add valuable, appetizing nutrients and color to any meal (Nutrition Institute, 1995).

In Africa, particularly poorly educated, resource poor farmers, especially women, grow sweet potato mostly for family consumption and for cash (Bashaasha *et al.*, 1995). The crop is an important means of poverty alleviation and invaluable in times of food insecurity, especially in areas of East Africa like Tanzania, Kenya, Uganda and Ethiopia (Byamukama *et al.*, 2004).

Sweet potato is a nutritious and economical food. This nutritious vegetable provides 42% of the Recommended Daily Allowance (RDA) for vitamin C, six percent of the RDA for calcium, 10% of the RDA for iron, and 8% of the RDA for thiamine for healthy adults (FAOSTAT, 2006). It is low in sodium and is good source of fiber and other important vitamins and minerals. It is a complex carbohydrate food source that provides β -carotene and others that may be a factor in reducing the risk of certain cancers (appendix 1) (USDA, 2001; www.nlm.nih.gov). Sweet potatoes are also used in industrial processes to make alcohol and starch, and products such as noodles, candy, desserts, and flour (FAOSTAT, 2000). Tewe *et al.* (2003) also reported that sweet potato is used to prepare agar for microbial nutrient.

Sweet potato is an important source of starch, vitamin and minerals. Orange fleshed varieties are sources of the β -carotene, the precursor of vitamin A; hence, it prevents night blindness and deaths from weakened immunity due to vitamin A deficiency (Scott *et al.*, 2000). According to Duke (1983), the root of sweet potato is used as medicinal plant in folk remedy for asthma, burns, diarrhea, fever, nausea and tumor.

The leaves of sweet potato are source of nutrients including anti-oxidants, vitamin B, β -carotene, boron, calcium, copper, cystine, fiber, folic acid, iodine, iron, magnesium,

manganese, niacin, phosphorous, protein, sulfur, tryptophan, tyrosine and Zink (Gad and Kandil, 2008). Additionally, young leaves are sources of proteins for animal feed but not commonly used as food for human (Martin, 1988; CTA, 2007).

3.4. Growing conditions

Sweet potatoes are tropical plants that can also be grown in the summer in temperate regions as long as they have at least five frost-free months combined with fairly warm days and nights. They can be grown from vine cuttings or by planting pieces of the roots. It grows from underground tuberous roots with trailing, twisting stems that can be as long six meters. To grow new plants, one or more tubers of sweet potatoes are placed in a bed of sand and covered with a couple of inches of moist, sandy soil. When the sprouts reach about 25 cm, they are detached by twisting and transplanted to the place they are to grow. Sprouts of 8 cm are pushed into the ground and watered well. A distance of about 30 cm is left between plants and 1.0 m between rows. Roots grow where stem nodes touch the ground, and most develop into the edible storage roots, usually four to ten storage roots per plant (Winter *et al.*, 1992).

Sweet potatoes do best in full sun with fertile, open sandy-loam soils with a pH range of 4.5-7.0 (Woolfe, 1992). They also like some added manure (well rotted) and compost, although they should not be given too much nitrogen as this encourages leaf growth at the expense of root growth. They benefit from regular additions of potash. Roots will be bigger and easier to harvest if sprouts are planted into raised mounds about 30 cm high. This is particularly important in heavy or wet soil. Once established, apart from occasional weed, sweet potatoes need little care.

The plant doesn't tolerate frost. It grows best at an average temperature of 24°C, abundant sunshine and warm nights (Winter *et al.*, 1992). Annual rainfall of 750-1000mm is considered as the most suitable with a minimum of 500mm in the

growing season. The crop is sensitive to drought at the tuber initiation stage 50-60 days after planting and is not tolerant to water logging, as it may cause tuber rots and reduce growth of storage roots if aeration is poor (Chen *et al.*, 2006).

3.5. Harvesting

Roots are harvested within 3 to 6 months after planting (Njeru *et al.*, 2004) as the leaves begin to yellow in the fall. They are then brushed, clean and left to cure. Traditionally curing involved stacking the potatoes in the field or garden, covering them with sand, and leaving them for several weeks. Sweet potatoes in commercial production are cured in rooms with temperatures between 27°C and 30°C. Curing heals cuts and reduces decay and shrinkage during storage, and it converts some starches to sugars, improving the flavor (Alicai *et al.*, 1999).

3.6. Global production

Sweet potato is a leading root crop grown more in developing countries. World sweet potato production is around 133 million tons in an area of about 9.2 million hectares (FAO, 1997) which was about half of that of potatoes (*Solanum tuberosum* L.). China is the world's leading sweet potato producing country with about 84% of production followed by Uganda and Rwanda (World Bank, 1995; FAO, 2007). Of the 82 developing countries where sweet potatoes are grown, 36 are in Africa, 24 in Latin America and 22 in Asia. Japan and the United States are among the few industrialized countries that grow sweet potatoes (Huaman, 1996). Africa's top producers are Uganda (1.7 million tons), Rwanda (980,000 t), Malawi (960,000 t) and Kenya (725,000 t) (Ewell, 2002).

Sweet potato is a major crop in Ethiopia for at least 20 million Ethiopians although it is grown traditionally around the densely populated areas of the South, Southwestern and Eastern parts of the country and also grown around Assosa and Jimma in the West (Van

Bruggen, 1984). The annual production of sweet potato in Ethiopia is 600,000 tons (Assefa Tofu *et al.*, 2007). However, in other countries like Uganda, Kenya, Rwanda and Tanzania it is 1.7 million tons; 1 million tons; 900,000 tons and 340,000 tons, respectively (ISAAA, 2006). According to Zuger (2003), the productivity of sweet potato in Zimbabwe was 0.5 and 1.8 tons per hectare using unimproved and tissue cultured sweet potatoes, respectively.

3.7. Major problems associated with sweet potato production

3.7.1. Viral diseases

The most harmful diseases in sweet potatoes are caused by viruses (Kozai *et al.*, 1996); the spread of which is enhanced by the cultivation practices, insects and lack of resistant cultivars (Winter *et al.*, 1992). Sweet potato chlorotic stunt crinivirus (SPCSCV) breaks down resistance to other viruses in sweet potato cultivars, which results in the severe sweet potato virus disease (SPVD) and high or total yield loss (Sheffield, 1953).

Sweet potato chlorotic stunt virus (SPCSV) (Karyeija *et al.*, 1998), also known as sweet potato sunken vein virus (Cohen *et al.*, 1992) and sweet potato virus disease associated with clostero virus (Winter *et al.*, 1992), causes severe symptoms generally referred to as SPVD in mixed infection with sweet potato feathery mottle virus (SPFMV) (Gibson *et al.*, 1998; Karyeija, 1998; Karyeija, 2000a). SPVD is the main disease of sweet potato (Geddes, 1990), characterized by small, distorted leaves which are often narrow (strap-like) and crinkled with chaotic mosaic or vein clearing and stunting (Gibson *et al.*, 1998; Karyeija *et al.*, 1998; Karyeija *et al.*, 2000a). The disease has been reported to cause up to 80% yield reduction (Haahn, 1979).

3.7.2. Damage by insects

According to Carey *et al.* (1998), at least 18 species of insects feed on sweet potato root. Weevils are the most destructive insect pests of sweet potatoes. The adult weevil feeds on all parts of the sweet potato but prefers the roots. Feeding scars on tubers consists of tiny shallow holes that usually occur in patches (Chavri *et al.*, 1997). The eggs, laid in vines and roots, hatch in about one week (Clerk, 1960). The legless white grubs with brown heads begin to tunnel through potatoes, causing the potatoes to be too bitter for human consumption (Clerk, 1960; Cohen *et al.*, 1992; Chavri *et al.*, 1997). Other insects, such as beetles and caterpillars that chew on the leaves or stem and leafhoppers that withdraw juice from plants also cause damage to sweet potatoes (Cohen *et al.*, 1992).

3.7.3. Fungal and bacterial diseases

The most common fungal diseases are scurf, black rot, wilt (stem rot), soft rot and bed blight (Geddes, 1990). Scurf produces a grayish-brown to black surface discoloration on the roots. This discolored areas can occur in all shapes and sizes with no definite outline. They can be uniform over the surface but are usually worse on the stem end (Gibson *et al.*, 1998). Black rot causes a black lesion to develop on the base of young potato stems before the plants are removed from the bed. The foliage of affected transplants appears yellow and stunted (Gibson *et al.*, 1997; Gibson *et al.*, 1998; Gichuki *et al.*, 1998). A dark to nearly black spot appears on the potato in the field or in storage. These spots begin as small, round areas, but they enlarge until they cover the entire potato (Gibson *et al.*, 1998). The surface of the diseased spots has a metallic luster, and the tissue just beneath the surface is greenish (Gichuki *et al.*, 1998).

Wilt infected vines have a slight off- color of a yellowish tinge, followed by puckering of the foliage and wilting of the plant. The older leaves drop off first, leaving near the vine tip (Hahn, 1979; Hahn *et al.*, 1981). On affected young vines, many short stems grow at

the center of the hill, giving the vine a rosette appearance (Hollings *et al.*, 1976). Bed blight is caused by a soil-borne fungus that attacks the stems of young plants at the soil line, causing a white mold to develop. Infected plants turn yellow and die, creating dead areas in the plant bed (Hahn *et al.*, 1981).

Bacterial soft rot can occur in the seed bed, field, or even after harvest (Karyeija *et al.*, 1998). Infected plants usually have long black stem lesions, although potatoes on diseased vines can show various degrees of soft rot development. Good sanitation practice is the best method of preventing bacterial soft rot (Karyeia *et al.*, 1998; Karyeija *et al.*, 2000b).

3.8. Tissue culture

Plant tissue culture is the science (or art) of growing plant cells, tissue or organs isolated from the stock plant on artificial media based on the cell doctrine that states a cell is capable of autonomy and is potentially totipotent (George, 1993). *In vitro* culture has many applications like production of virus free plants, large scale micro propagation, plant improvement, transformation of plant materials, production of metabolites, genetic improvement, preservation of germplasm, somatic hybridization, haploid and dihaploid production (Dong, 1987 and George, 1993). Plant tissue cultures are done by placing one or more explants like node, stems, roots, leaves, petioles, anthers, fruits, meristem and embryos of plants into a pre-sterilized container of sterile nutrient medium.

3.8.1. *In vitro* regeneration of sweet potato

Different researches were done on regeneration of sweet potato using different plant growth regulators from different explants of stock plants. Cavalcante *et al.* (1994) regenerated plants from lateral bud derived callus of sweet potato onto regeneration medium supplemented with 2,4-D and BAP. Newell *et al.* (1995) also obtained callus and able to regenerate shoot onto medium containing NAA and BAP. Moreover, Zheng *et al.* (1996) reported that rapid and repetitive plant regeneration in only one genotype of

sweet potato i.e. PI318846-3 onto media devoid of hormones after using firstly an initiation medium supplemented with 2,4-D. Shoot regeneration from petiole-derived calli of sweet potato cv. Genki was also done using regeneration medium containing BAP only (Wang *et al.*, 1999). Furthermore, Oggema *et al.* (2007) regenerated shoots from leaf callus initiated onto medium containing 2,4-D and BAP or BAP alone and regeneration medium containing ABA and BAP.

4. Materials and methods

4.1. Plant material

About six weeks old *in vitro* grown Beletech and Awassa-83 sweet potato varieties were used as a source of petiole and leaf explants from AAU, Faculty of Life Science, Plant Propagation Laboratory. They were originally obtained from meristem culture. Stock plants were maintained by sub culturing on MS (Murashige and Skoog, 1962) medium supplemented with 1 mg/l BAP using node and shoot culture every four weeks onto a fresh medium supplemented with 3% sucrose, 7 g/l agar at pH=5.8 and maintained at $27\pm 1^{\circ}\text{C}$ under white fluorescent light of $20\ \mu\text{molm}^{-2}\text{s}^{-1}$ intensity for 16 h photoperiod.

4.2. Media preparation

4.2.1. Stock solution preparation

In the present study, MS medium was used. The stock solution of the media was prepared separately by weighing the recommended amount of macronutrients, micronutrients and vitamins (appendix 2). The solution was poured into plastic bottles and stored at -10°C until used.

4.2.2. Plant growth regulators preparation

Plant growth regulators such as gibberellic acid (GA_3), Indole-3-butyric acid (IBA), 6-Benzyl aminopurine (BAP), kinetin and dichlorophenoxy acetic acid (2,4-D) were weighed in such a way that every ml of the solution contains 1 mg of a given growth regulators and three to four drops of 1 M NaOH was added until the crystals dissolved. The dissolved solution was poured into labeled volumetric flask to be fully dissolved. Finally stored at 4°C for later use.

4.2.3. Culture medium preparation

For callus induction, 100 ml/l macronutrient stock solution, 10 ml/l vitamin stock solution, 10 ml/l micronutrient stock solution, 30 g/l sucrose, with different concentrations of Kinetin and 2,4-D (Table 1) were mixed and allowed to dissolve by using magnetic stirrer. Before addition of 7 g agar, the pH of the solution was adjusted to 5.8 using 1 M HCl or 1 M NaOH and then the medium was autoclaved for 15min at 121°C and 0.15 kpa pressure. About 25 ml of the medium was poured into 10 cm diameter Petri dish in the laminar airflow cabinet and left for about 45 minute to solidify before the leaf and petiole explants were cultured on it. For shoot regeneration, shoot multiplication and rooting, the same condition was used like that of callus induction except that the medium was boiled until became a clear solution and then about 60 ml of the solution was poured into Magenta GA-7 before autoclaving. Different concentrations of growth regulators were also used for shoot regeneration (Table 2), shoot multiplication (Tables 5 and 7) and rooting (Table 9). All prepared media were stored at 4°C for later use.

4.3. Callus induction

The leaf explants were cultured in such a way that the abaxial surface of the explants contacted with the medium and the petiole was trimmed into about 2 cm long segments in the laminar airflow cabinet. These explants were cultured on the medium supplemented with different concentrations of 2,4-D in combination with kinetin (Table 1) and maintained at $27\pm 1^{\circ}\text{C}$ under white fluorescent light of $20\ \mu\text{molm}^{-2}\text{s}^{-1}$ intensity for 16 h photoperiod.

4.4. Plant regeneration

After three weeks culture of petiole and leaf explants, the calli were subcultured on to medium containing different concentrations of BAP for shoot regeneration (Table 2) and maintained at $27\pm 1^{\circ}\text{C}$ under white fluorescent light of $20\ \mu\text{molm}^{-2}\text{s}^{-1}$ intensity for 16 h photoperiod.

4.5. Shoot multiplication

Medium supplemented with different concentrations of BAP alone or in combination with GA_3 were used for shoot multiplication (Tables 5 and 7), respectively. After 6 weeks of culture, the number of shoots produced per node and their height from each treatment was recorded.

4.6. Rooting and acclimatization

About 10 cm long shoots multiplied from shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 sweet potato varieties were cultured on a medium supplemented with different concentrations of IBA for rooting (Table 9). After about one month, the plantlets were taken out from the Magenta GA-7, washed under running tap water, the root length and number of roots per plantlet recorded. After that, the plantlets were transferred to pots containing red soil, sand and compost in the ratio of 1:2:1, respectively. The pots were covered with transparent plastic bags with random holes for air circulation and the under side of the pots were drilled for drainage. Then they were transferred to the glasshouse and watered using sprayer every day. Plastic cover were removed partially after a week and completely removed after two weeks. Finally, after about one month, percent of plantlets successfully hardened were calculated.

4.7. Experimental design and data analysis

Completely Randomized Design (CRD) was used for all the treatments. Treatments were different combinations and concentrations of plant growth regulators. Each treatment had five replicates of culture Petri dishes and set as experimental unit. Each Petri dish contained six explants. A total of thirty leaves and thirty petioles were cultured for each explant of the two varieties per treatment for callus induction. Five explants in each Magenta GA-7, a total of fifteen shoots or three Magenta GA-7s per treatment was used for shoot multiplication and rooting.

The data was analyzed using SPSS version 16.0 software. The mean separation method (LSD) was used to compare means between treatments. Mean homogeneity analysis was carried out using Tukey's B homogeneity test. For all comparisons probability level 0.05 ($p < 0.05$) was considered for statistical significance.

5. Results

5.1. Effect of 2, 4-D and kinetin on callus induction

After three weeks of culture, different changes on leaf and petiole explants of Beletech and Awassa-83 were observed. The green leaves of both varieties were changed to yellowish calli and the green petiole explants of Awassa-83 were changed to whitish calli, whereas dark red color of petiole explants of Beletech variety was changed to yellowish calli. The size and the shape of explants were also changed to dome shape calli.

The rate of callus induction was significantly different among growth regulators, explants and varieties. As shown in Table 1, the frequencies of callus induction was typically influenced by the concentrations of 2,4-D and kinetin included in the callus induction medium. It has also been observed that the wounded areas of explants induce calli faster than unwounded areas of explants. The best calli were recorded on medium supplemented with 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin. The frequencies of induced calli on this combination were 90 % and 83% for Beletech petiole and leaf explants, respectively and 93% and 87% for Awassa-83 petiole and leaf explants, respectively. The frequency of calli induction were low at high concentration of 2,4-D and kinetin except the combination that contains equal concentration of the two growth regulators.

Table 1. Percentage of calli induced from petiole and leaf explants of Beletech and Awassa-83 sweet potato varieties, values given as mean \pm SE

T	Concentrations of PGRs (mg/l)		Percentage of induced calli			
			Bel		Aw-83	
			Pet	Leaf	Pet	Leaf
	2, 4- D	Kinetin				
1	0.00	0.00	0.00 \pm 0.00 ^l	0.00 \pm 0.00 ^l	0.00 \pm 0.00 ^m	0.00 \pm 0.00 ^l
2	0.05	0.50	90 \pm 0.00 ^a	83 \pm 0.00 ^a	93 \pm 0.00 ^a	87 \pm 0.00 ^a
3	0.05	1.00	77 \pm 0.00 ^b	67 \pm 0.00 ^c	77 \pm 0.035 ^c	63 \pm 0.00 ^c
4	0.05	1.50	63 \pm 0.00 ^c	60 \pm 0.00 ^d	73 \pm 0.00 ^d	60 \pm 0.00 ^d
5	0.05	2.00	53 \pm 0.00 ^d	50 \pm 0.00 ^e	57 \pm 0.00 ^e	53 \pm 0.00 ^e
6	0.10	0.50	47 \pm 0.00 ^e	47 \pm 0.00 ^f	50 \pm 0.00 ^f	53 \pm 0.00 ^e
7	0.10	1.00	43 \pm 0.00 ^f	40 \pm 0.00 ^g	43 \pm 0.00 ^g	47 \pm 0.00 ^f
8	0.10	1.50	40 \pm 0.00 ^g	33 \pm 0.00 ⁱ	37 \pm 0.00 ⁱ	43 \pm 0.00 ^g
9	0.10	2.00	33 \pm 0.00 ^h	30 \pm 0.00 ^j	33 \pm 0.00 ^j	40 \pm 0.00 ^h
10	0.50	0.50	77 \pm 0.00 ^b	73 \pm 0.00 ^b	83 \pm 0.00 ^b	80 \pm 0.00 ^b
11	0.50	1.00	33 \pm 0.00 ⁱ	37 \pm 0.00 ^h	40 \pm 0.00 ^h	37 \pm 0.00 ⁱ
12	0.50	1.50	30 \pm 0.00 ^j	30 \pm 0.00 ^j	27 \pm 0.00 ^k	30 \pm 0.00 ^j
13	0.50	2.00	23 \pm 0.00 ^k	20 \pm 0.00 ^k	17 \pm 0.00 ^l	17 \pm 0.00 ^k

Means within each column connected by the same superscript (a-m) are not significantly different at 5% probability level. Results were recorded after three weeks of culture. T=Treatments, PGRs = Plant growth regulators, Bel=Beletech, Aw-83= Awassa-83, Pet=Petiole

5.2. Effect of BAP on shoot regeneration

Three weeks old calli were transferred to medium supplemented with different concentrations of BAP (0.00, 0.5, 1.0 and 2.0) mg/l for shoot regeneration. After one month of transfer, shoot formation was observed from those calli induced using 0.05 mg/l 2,4-D in combination with 0.5 mg/l, 1 mg/l or 1.5 mg/l kinetin. As shown in Table 2, most induced calli didn't regenerate shoot. The frequency of shoot regeneration was influenced by 2,4-D and kinetin included in the callus induction medium. The highest frequency (80 and 77%) were regenerated on medium without growth regulator from leaf calli of Beletech and Awassa-83 from 83% and 87% of induced calli with two and one mean number of shoot per callus and mean height 8.50 cm and 8.40 cm, respectively. However, for petiole calli, 1 mg/l BAP regenerated 70 % and 71% from Beletech and Awassa-83 from 90% and 93% of induced calli with one mean number of shoot per callus and mean height of 9.00 cm and 7.50 cm, respectively. Two shoots per callus were the maximum number of shoots recorded from Beletech leaf calli at 0.00 mg/l and 0.5 mg/l BAP. However, petiole calli of Awassa-83, 1 mg/l BAP produced maximum of two shoots per callus (Tables 3 and 4). At 2 mg/l BAP, only calli formation was observed.

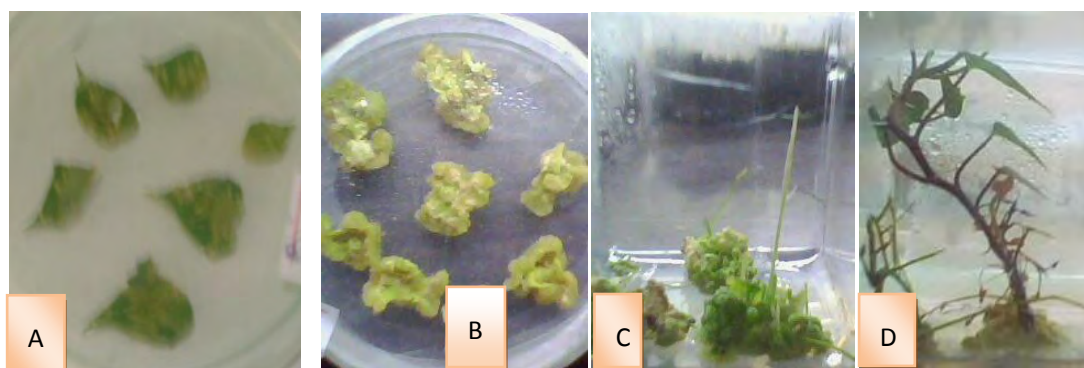


Fig. 3. Sequential events showing shoot regeneration from leaf calli of Beletech variety: leaf explants cultured on medium supplemented with 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin (A); three weeks old induced calli (B); six weeks old calli after transferred on medium without growth regulator (C); and about three- month-old regenerated shoot (D).

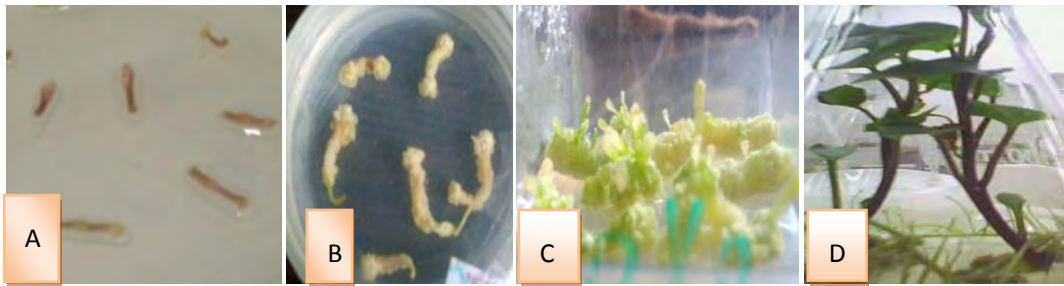


Fig. 4. Stages showing shoot regeneration from petiole calli of Beletech variety: Petiole explants cultured on medium supplemented with 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin (A); three weeks old induced calli (B); six weeks old calli after transferred to medium supplemented with 1 mg/l BAP (C); and about three- month-old regenerated shoot (D).

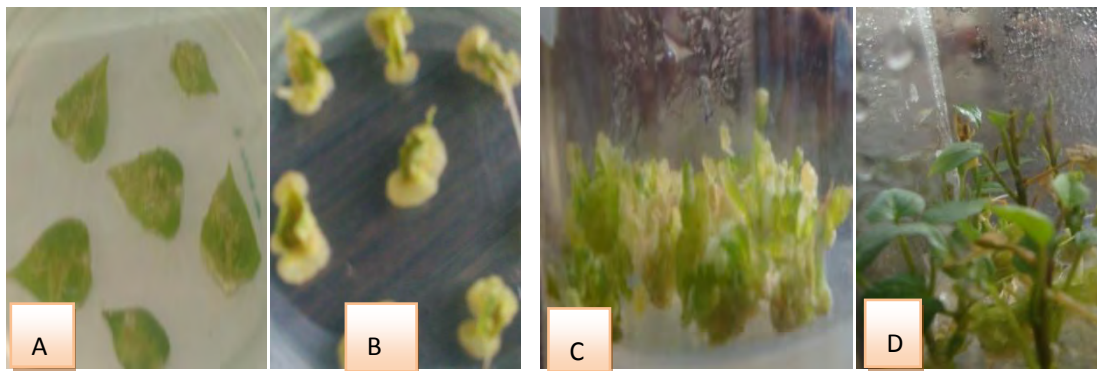


Fig. 5. Stages showing shoot regeneration from leaf calli of Awassa-83 variety: leaf explants cultured on medium supplemented with 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin (A); three weeks old induced calli (B); six weeks old calli after transferred to medium without growth regulator (C); and about three-month-old regenerated shoot (D).

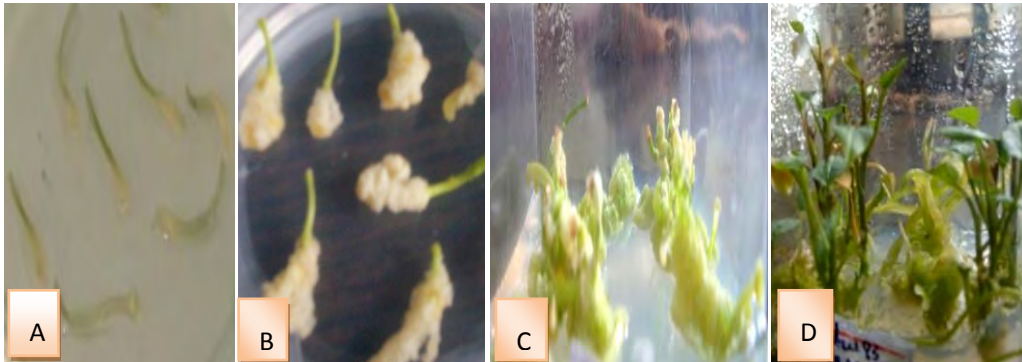


Fig. 6. Stages showing shoot regeneration from petiole calli of Awassa-83 variety: Petiole explants cultured on medium supplemented with 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin (A); three weeks old induced calli (B); six weeks old calli after transferred to medium supplemented with 1 mg/l BAP (C); and about three- month-old regenerated shoot (D).

Table 2. Percentage of regenerated shoots from leaf and petiole calli of Beletech and Awassa-83 sweet potato varieties

Variety	Explant	Callus induction PGRs (mg/l)		Concentration of BAP (mg/l)	Percentage of regenerated shoots
		2,4-D	Kinetin		
Beletech	Petiole	0.05	0.5	0.00	30 ^h
				0.50	56 ^d
				1.0	70 ^a
				2.0	0 ^j
		0.05	1.0	0.00	35 ^g
				0.5	52 ^c
	1.0			65 ^b	
	2.0			0 ^j	
	0.05	1.50	0.00	21 ⁱ	
			0.5	42 ^f	
			1.0	58 ^c	
			2.0	0 ^j	
leaf	0.05	0.5	0.00	80 ^a	
			0.5	68 ^b	
			1.0	32 ^f	
			2.0	0 ^a	
	0.05	1.0	0.00	60 ^c	
			0.5	40 ^d	
1.0			0 ^g		
2.0			0 ^g		
0.05	1.50	0.00	39 ^c		
		0.5	0 ^g		
		1.0	0 ^g		
		2.0	0 ^g		
Awassa-83	Petiole	0.05	0.5	0.00	35 ^c
				0.5	43 ^d
				1.0	71 ^a
				2.0	0 ^f
		0.05	1.0	0.00	52 ^c
				0.5	65 ^b
	1.0			0 ^f	
	2.0			0 ^f	
	0.05	1.50	0.00	0 ^f	
			0.5	0 ^f	
			1.0	0 ^f	
			2.0	0 ^f	
leaf	0.05	0.5	0.00	77 ^a	
			0.5	62 ^b	
			1.0	46 ^c	
			2.0	0 ^d	
	0.05	1.0	0.00	42 ^{cd}	
			0.5	42 ^{cd}	
1.0			0 ^d		
2.0			0 ^d		
0.05	1.50	0.00	0 ^d		
		0.5	0 ^d		
		1.0	0 ^d		
		2.0	0 ^d		

Percentage within each column connected by the same superscript (a-g) are not significantly different at 5% probability level, results were recorded after three months.

Table 3. Mean number of shoots per explant at different concentrations of BAP from petiole and leaf calli of Beletech and Awassa-83 sweet potato varieties, values given as mean \pm SE.

BAP (mg/l)	Mean number of shoots per callus			
	Beletech		Awassa-83	
	Petiole	Leaf	Petiole	Leaf
0.00	1.20 \pm 0.20 ^a	1.50 \pm 0.139 ^a	1.00 \pm 0.163 ^a	1.17 \pm 0.167 ^a
0.50	1.40 \pm 0.163 ^a	1.50 \pm 0.189 ^a	1.33 \pm 0.211 ^a	1.29 \pm 0.184 ^a
1.00	1.47 \pm 0.133 ^a	1.00 \pm 0.00 ^a	1.60 \pm 0.00 ^a	1.36 \pm 0.152 ^a
2.00	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b

Means within each column connected by the same superscript (a-b) are not significantly different at 5% probability level, results were recorded after three months

Table 4. Mean height of regenerated shoots, values given as mean \pm SE.

BAP (mg/l)	Mean shoot height (cm)			
	Beletech		Awassa-83	
	Petiole	Leaf	Petiole	Leaf
0.00	9.10 \pm 0.314 ^a	8.50 \pm 0.224 ^a	8.70 \pm 0.153 ^a	8.40 \pm 0.211 ^a
0.50	6.30 \pm 0.260 ^b	6.20 \pm 0.359 ^b	6.10 \pm 0.277 ^c	6.30 \pm 0.355 ^b
1.00	9.00 \pm 0.221 ^a	8.80 \pm 0.291 ^a	7.50 \pm 0.477 ^b	8.70 \pm 0.396 ^a

Means within each column connected by the same superscript (a-c) are not significantly different at 5% probability level, results were recorded after three months

5.3. Effect of BAP on shoot multiplication

Shoots regenerated from petiole and leaf calli with three nodes were cultured on growth regulators free medium, 0.5 mg/l, 1.0 mg/l, 1.50 mg/l and 2.0 mg/l BAP for shoot multiplication (Table 5). After two weeks of culture, each treatment has shown different responses. The difference was in terms of number of shoots per node, height of shoots and general morphologies of the shoots. Average of two shoots per node was the maximum number of shoots recorded at 1 mg/l BAP for shoots regenerated both from petiole and leaf calli of Beletech and Awassa-83 varieties with mean height of 11.10 cm, 11.40 cm, 10.70 cm and 11.90 cm, respectively (Tables 5 and 6). At 2 mg/l BAP only degenerated shoots to calli were formed (Fig 8).

Table 5. Mean number of shoots produced per node from shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 sweet potato varieties at different concentrations of BAP, values given as mean \pm SE.

BAP (mg/l)	Mean number of shoots per node			
	Beletech		Awassa-83	
	Petiole	Leaf	Petiole	Leaf
0.00	1.33 \pm 0.079 ^b	1.03 \pm 0.217 ^b	1.13 \pm 0.104 ^b	1.10 \pm 0.200 ^b
0.50	1.20 \pm 0.074 ^b	1.20 \pm 0.176 ^b	1.27 \pm 0.082 ^b	1.07 \pm 0.179 ^b
1.00	2.03 \pm 0.033 ^a	2.27 \pm 0.117 ^a	2.33 \pm 0.088 ^a	2.40 \pm 0.113 ^a
1.50	1.30 \pm 0.085 ^b	0.73 \pm 0.106 ^{ab}	1.37 \pm 0.140 ^b	0.90 \pm 0.100 ^b
2.00	0.33 \pm 0.100 ^c	0.33 \pm 0.100 ^c	0.37 \pm 0.102 ^c	0.40 \pm 0.113 ^c

Means within each column connected by the same superscript (a-c) are not significantly different at 5% probability level; results were recorded after six weeks.

Table 6. Mean height of multiplied shoots, values given as mean \pm SE

BAP (mg/l)	Mean shoot height (cm)			
	Beletech		Awassa-83	
	Petiole	Leaf	Petiole	Leaf
0.00	9.60 \pm 0.211 ^{bc}	8.50 \pm 0.200 ^c	8.40 \pm 0.267 ^c	8.50 \pm 0.224 ^c
0.5	9.50 \pm 0.224 ^b	9.80 \pm 0.133 ^b	9.30 \pm 0.153 ^b	9.50 \pm 0.167 ^b
1.00	11.10 \pm 0.379 ^a	11.40 \pm 0.306 ^a	10.70 \pm 0.396 ^a	11.90 \pm 0.314 ^a
1.50	8.00 \pm 0.471 ^c	8.70 \pm 0.153 ^c	8.30 \pm 0.300 ^c	8.30 \pm 0.153 ^c
2.00	2.50 \pm 0.500 ^d	2.70 \pm 0.213 ^d	3.30 \pm 0.260 ^d	3.10 \pm 0.233 ^d

Means within each column connected by the same superscript (a-d) are not significantly different at 5% probability level, results were recorded after six weeks

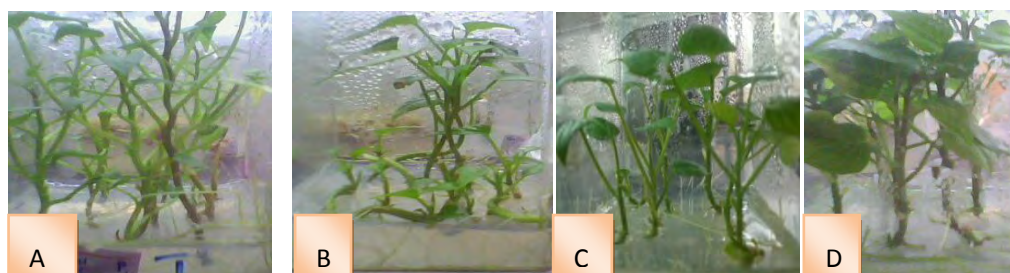


Fig. 7. Multiplied shoots regenerated from petiole and leaf calli of Beletech variety (A and B), petiole and leaf calli of Awassa-83 variety (C and D) on medium supplemented with 1 mg/l BAP after six weeks of culture.



Fig. 8. Shoot multiplication medium supplemented with 2 mg/l BAP resulted in degeneration of shoots to callus

5.4. Effect of BAP and GA₃ on shoot multiplication

Medium supplemented with BAP in combination with GA₃ was also tested for shoot multiplication. Among the entire media, the medium supplemented with 1 mg/l BAP in combination with 0.5 mg/l GA₃ was the most suitable combination of growth regulator for shoot multiplication of the two sweet potato varieties. As shown in Table 7, the results of shoot multiplication are similar for both varieties and means of 1.70 and 1.50 shoots per node were observed for shoots that were derived from petiole and leaf explants, respectively. The average height of shoots was 6.78 cm for petiole derived and 6.67 cm for leaf derived shoots of the variety Beletech whereas the corresponding mean heights for the variety Awassa-83 was 5.89 cm and 5.67 cm (table 8). Two mg/l GA₃ in combination with 1 mg/l BAP resulted in dwarf shoot for shoots regenerated from petiole and leaf calli of Awassa-83 variety and degenerated shoots to calli were observed for Beletech variety (Fig 9). In general, BAP in combination with GA₃ plays fewer roles for shoot multiplication of sweet potato regenerated from petiole and leaf calli of Beletech and Awassa-83 varieties.

Table 7. Mean number of shoots per node of shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 sweet potato varieties at different concentrations of BAP in combination with GA₃, values given as mean ± SE

BAP (mg/l)	GA ₃	Mean number of shoots per node			
		Beletech		Awassa-83	
		Petiole	Leaf	Petiole	Leaf
0.00	0.00	1.33±.079 ^{ab}	1.03±.217 ^{abc}	1.13±.104 ^{bc}	1.10±.200 ^{bc}
0.5	0.5	1.30±.153 ^{ab}	1.10±.100 ^{bc}	1.30±.153 ^a	1.10±.100 ^{bc}
0.5	1.0	1.30±.153 ^{ab}	1.00±.00 ^{abc}	1.10±.100 ^{bc}	0.90±.100 ^{ab}
0.5	2.0	1.20±.200 ^{ab}	0.70±.153 ^{ab}	1.00±.211 ^{bc}	0.80±.133 ^{ab}
1.0	0.5	1.70±.153 ^a	1.50±.167 ^a	1.70±.153 ^a	1.50±.167 ^a
1.0	1.0	1.40±.213 ^a	1.00±.149 ^{abc}	1.00±.211 ^{bc}	1.10±.100 ^{bc}
1.0	2.0	1.30±.163 ^{ab}	1.10±.100 ^{bc}	1.30±.153 ^a	1.10±.100 ^{bc}
2.0	0.5	0.90±.213 ^{ab}	0.60±.163 ^{ab}	0.50±.167 ^{ab}	0.70±.153 ^{ab}
2.0	1.0	0.60±.211 ^b	0.50±.167 ^c	0.30±.153 ^c	0.50±.167 ^c

Means within each column connected by the same superscript (a-c) are not significantly different at 5% probability level, results were recorded after six weeks

Table 8. Mean height of shoots regenerated from leaf and petiole calli of Beletech and Awassa-83 of sweet potato varieties at different concentrations of BAP in combination with GA₃, values given as mean ± SE

BAP (mg/l)	GA ₃	Mean shoot height (cm)			
		Beletech		Awassa-83	
		Petiole	Leaf	Petiole	Leaf
0.00	0.00	9.60±.211 ^a	8.50±.200 ^a	8.40±.267 ^a	8.50±.224 ^a
0.5	0.5	2.90±.100 ^d	2.70±.153 ^e	3.50±.167 ^a	3.20±.200 ^e
0.5	1.0	4.50±.167 ^c	4.30±.153 ^d	4.80±.249 ^d	4.50±.167 ^d
0.5	2.0	4.60±.307 ^c	4.50±.224 ^d	5.10±.348 ^{bc}	5.00±.365 ^{bc}
1.0	0.5	6.78±.222 ^b	6.67±.236 ^c	5.89±.351 ^c	5.67±.333 ^c
1.0	1.0	2.90±.277 ^d	2.70±.213 ^e	3.10±.150 ^e	2.90±.100 ^e
1.0	2.0	7.40±.163 ^b	7.40±.163 ^b	7.50±.167 ^b	7.50±.167 ^b
2.0	0.5	2.70±.213 ^d	2.50±.224 ^e	3.10±.180 ^e	2.80±.200 ^e
2.0	1.0	2.70±.213 ^d	2.40±.163 ^e	3.00±.149 ^e	2.70±.153 ^e

Means within each column connected by the same superscript (a-e) are not significantly different at 5% probability level, results were recorded after six weeks

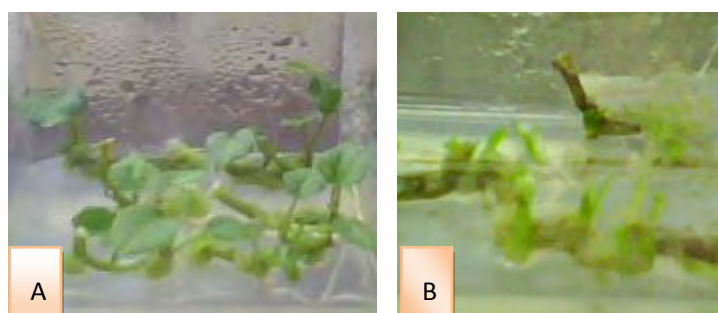


Fig. 9. Dwarf shoots of Awassa-83 (A) and degenerated shoots of Beletech variety to callus (B) after culturing on BAP and GA₃ combinations

5.5. Rooting and acclimatization

5.5.1. Rooting

After one month of culture, all rooting medium supplemented with different concentrations of IBA resulted in 100% rooting with different root length, root hairs, root thickness and number of roots per plantlet.

Among the different concentrations of IBA used for rooting, 0.1 mg/l that produced thick roots and root hairs was the most suitable concentration for shoots regenerated both from petiole and leaf calli of Awassa-83 variety. It gave mean number of 6.07 and 4.83 roots for plantlets regenerated from petiole and leaf calli of this variety, respectively. The mean root length was 8.70 cm and 10.40 cm for plantlets derives from petiole and leaf calli, respectively. However, for shoots regenerated from petiole and leaf calli of Beletech variety, rooting medium without IBA was the most suitable with mean number of roots of 8.10 and 6.80, respectively and mean root length of 10.00 cm and 10.10 cm, respectively (Fig 10 and Table 9).

Table 9. Effect of different IBA (mg/l) concentrations on number of main roots of shoots regenerated from petiole and leaf calli of Beletech and Awassa-83 sweet potato varieties, values given as mean \pm SE

IBA	Mean number of roots per plantlet				Root length (cm)			
	Beletech		Awassa-83		Beletech		Awassa-83	
	Petiole	Leaf	Petiole	Leaf	Petiole	Leaf	Leaf	Petiole
0.0	8.10 \pm .227 ^a	6.80 \pm .370 ^a	8.03 \pm .206 ^a	6.97 \pm .286 ^a	10.00 \pm .39 ^a	10.10 \pm .58 ^a	10.60 \pm .40 ^a	11.10 \pm .407 ^a
0.1	6.00 \pm .192 ^b	5.30 \pm .245 ^b	6.07 \pm .172 ^b	4.83 \pm .17 ^b	8.50 \pm .269 ^b	9.70 \pm .587 ^a	10.40 \pm .47 ^a	8.70 \pm .423 ^b
0.5	3.97 \pm .122 ^c	3.80 \pm .130 ^c	3.73 \pm .135 ^c	3.40 \pm .091 ^c	6.90 \pm .567 ^c	7.70 \pm .389 ^b	8.80 \pm .327 ^b	6.60 \pm .34 ^c

Means within each column connected by the same superscript (a-c) are not significantly different at 5% probability level, results were recorded after one month

5.5.2. Acclimatization

Thirty rooted shoots regenerated from each petiole and leaf calli of the two varieties were taken from Magenta GA-7 and the agar was washed using tap water and transferred to pots containing 1:2:1 ratio of red soil, sand and compost, respectively (Fig 10). Before transferred to glasshouse, the pots were covered with transparent plastic bags. After about one week, the plastic bags were removed. After about one month of acclimatization, 27 (90%) and 30 (100%) for shoots regenerated from petiole and leaf calli of Beletech variety respectively and 24 (80%) and 27 (90%) for shoots obtained from petiole and leaf calli of Awassa-83 variety respectively were survived (Fig 11).

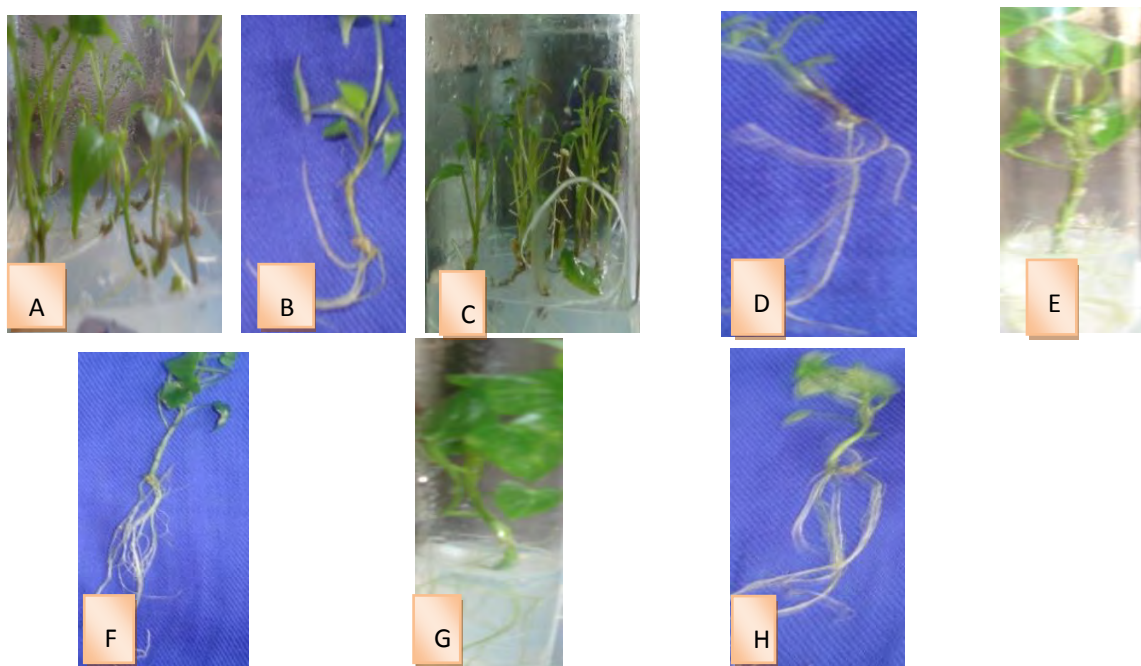


Fig. 10. Rooting of shoots regenerated from calli derived from petiole and leaf of Beletech and Awassa-83 varieties: Shoots regenerated from petiole calli of Beletech variety in rooting medium (A); rooted shoot of Beletech to be potted (B); shoots regenerated from leaf calli of Beletech variety in rooting medium (C); rooted shoot of Beletech to be potted (D); shoots regenerated from petiole calli of Awassa-83 variety in rooting medium (E); rooted shoot of Awassa-83 to be potted (F); shoots regenerated from leaf calli of Awassa-83 variety in rooting medium (G); rooted shoot of Awassa-83 to be

potted (H)



Fig. 11. Acclimatized plantlets of Beletech and Awassa-83 varieties in the glasshouse: Plantlets covered with plastic bags (A); established plantlets after one week (B); acclimatized and well established shoots regenerated from petiole and leaf calli of Beletech variety (C and D); shoots regenerated from petiole and leaf calli of Awassa-83 variety (E and F) after about one month in glasshouse.

6. Discussion

6.1. Effect of 2,4-D and kinetin on callus induction

Different concentrations of 2,4-D in combination with kinetin were tested for callus induction of petiole and leaf explants of Beletech and Awassa-83 sweet potato varieties. The presence of 2,4-D and kinetin in the callus induction medium was necessary. This is because the treatment lacking these growth regulators failed to induce callus. However, only small concentrations were required for better callus induction. Amiri *et al.* (2010) reported that low concentrations of 2,4-D and kinetin (0.25 or 0.5) mg/l gave better calli for datura (*Datura stramonium*. L) leaf explants. In contrary, Brasileiro *et al.* (1999) reported 2,4-D didn't form callus (*Lycopersicon esculentum*). Rashid *et al.* (2009) reported that kinetin negatively affects callus induction but 2,4-D up to 2 mg/l increases the production of callus of wheat (*Triticum aestivum*) seed.

In the present study, the addition of low concentration (0.05 mg/l 2,4-D and 0.5 mg/l kinetin) concentration resulted in maximum (90, 83, 93 and 87%) of callus induction from petiole and leaf explants of Beletech and Awassa-83 variety, respectively (Table 1). This shows that low concentration of 2,4-D and kinetin is less toxic to explants. Similar result was stated by Tileye Feyissa *et al.* (2005) that was done on leaf explants of *Hagenia abyssinica* and Sato *et al.* (1999) that was done on callus induction of sweet potato from petiole and leaf explants. But inconsistency with Triqui *et al.* (2008) that was done on somatic embryogenesis of sweet potato from lateral bud.

However, when the concentrations of 2,4-D and kinetin were equal (i.e. 0.5 mg/l), 77 % and 73 % callus induction was obtained from petiole and leaf explants of Beletech, respectively and 83 % and 80 % from petiole and leaf explants of Awassa-83, respectively. This shows that equal concentrations of the two growth regulators promote calli induction. The percentage of callus induction also significantly varied between the type explants. Petiole explants induced more calli than leaf explant. This indicates that

callus induction depends on the source of explants. Callus induction was faster when the explants were wounded as compared to unwounded explants. This might be wounded area traps more nutrients so that callus induction would be faster than unwounded explants. This work was also consistent with the work of Tileye Feyissa *et al.* (2005).

6.2. Effect of BAP on shoot regeneration

Three weeks old calli were transferred on a medium supplemented with different concentrations of BAP (0.00, 0.5, 1 and 2) mg/l for shoot regeneration. After one month of transfer, shoot formation was observed only from those calli induced on a medium containing 0.05 mg/l 2,4-D in combination with 0.5 mg/l, 1 mg/l and 1.5 mg/l kinetin. The calli formed on other combinations of the two growth regulators didn't regenerate shoot. This shows that the callus induction medium indirectly influences shoot regeneration and that 2,4-D is toxic when it exceeding 0.05 mg/l, for shoot regeneration on media containing different concentrations of BAP. The concentration of kinetin included in the callus induction medium also affected the frequency of shoot regeneration. This implies the importance of low concentration of kinetin for shoot regeneration. The high frequencies (80 and 77 %) of shoots regenerated on medium without growth regulators from leaf calli of Beletech and Awassa-83 varieties. Shows the less importance of BAP for leaf calli of the two (Beletech and Awassa-83) varieties to regenerate shoots. However, from petiole calli, 1 mg/l BAP regenerated 70 % and 71% for Beletech and Awassa-83 varieties, respectively. This also suggests the importance of BAP to regenerate shoots from petiole calli. This shows the dependency of BAP on source of explant in plant regeneration. Sato *et al.* (1999) mentioned that the petiole calli of Genki sweet potato variety that had been cultured on the regeneration medium supplemented with 3.0 mg/l BAP gave a higher regeneration frequency than those cultured on the hormone free medium. However, higher frequencies were obtained by using hormone free medium from leaf calli. This difference may be related to the endogenous levels of hormone in various explants.

At 2 mg/l BAP, only callus formation was observed. This is because high concentration of BAP resulted in callus. Different results were obtained from different reports regarding the effect of BAP on shoot regeneration from species related to sweet potato and others. Otani and Shimada (1988) showed that shoot regeneration from leaf calli of *Ipomoea trichocarpa* was induced only on the medium supplemented with more than 2 mg/l BAP, and the one with 10 mg/l was the most effective. Rashid *et al.* (2009) also mentioned that shoot regeneration on medium supplemented with 2 mg/l BAP resulted in maximum percentage of shoots from seed calli of wheat (*Triticum aestivum*). In contrast, Otani *et al.* (1996) mentioned that BAP inhibited shoot regeneration in tissue cultures of sweet potato. They observed that the higher frequency of shoot regeneration was obtained on the hormone free medium and the percentage of shoot regeneration was reduced with an increase in BAP concentration.

In the present study, the addition of BAP to regeneration medium was less effective for shoot regeneration from leaf calli of Beletech and Awassa-83 but more effective for petiole calli of both varieties.

6.3. Shoot multiplication

Shoot segments containing three nodes of Beletech and Awassa-83 varieties were cultured on medium supplemented with BAP alone and in combination with GA₃ for shoot multiplication (Tables 5 and 7). No significant differences were observed between shoot of the varieties on medium supplemented with 1 mg/l BAP. Two shoots per node were the maximum mean number of shoots recorded at this concentration. This shows that the optimum concentration of BAP for shoot multiplication for these varieties as it was 1 mg/l. At 2 mg/l BAP, only degenerated shoots to calli were observed. This indicates that high concentration of BAP resulted in callus instead of shoot. This agrees with the work of Rabbani *et al.* (2001) on potato and Geleta Dugassa (2009) on meristem culture of Awassa local and Awassa-83 sweet potato varieties. Berrie (1984) also reported

that synthetic cytokinin inhibit shoot elongation and multiplication at higher concentrations.

Medium supplemented with 1 mg/l BAP in combination with 0.5 mg/l GA₃ was the most suitable medium among BAP and GA₃ combinations. But as compared to BAP alone, the medium supplemented with combination of the two growth regulators has less number of shoot per node. As the concentration of GA₃ increases at constant BAP, degenerated shoot to calli were observed than shoot. Robbins (1972) also described that increasing the concentration of GA₃ gave more callus than shoot.

6.4. Rooting and acclimatization

In the present study, all rooting medium was resulted in 100% rooting. This implies the less difficulty of rooting in sweet potato even with and/or with less concentration of auxin. The other reason could be the ability of sweet potato to propagate through vegetative means. For shoots regenerated from petiole and leaf calli of Beletech, growth regulators free medium was found to be the best with mean number of roots 8.10 and 6.80 and root length of 10.00 cm and 10.10 cm, respectively. For Awassa-83, 0.1 mg/l IBA with thick roots and root hairs was the best for shoots regenerated from petiole and leaf calli with mean number of roots 6.07 cm and 4.83 cm per shoot and mean length of 8.70 cm and 10.40 cm, respectively. This implies variety reliance of IBA but not differences of explants. Root hairs were observed on Awassa-83. This shows that IBA plays role in root hair formation. This work is consistent with Neja Jemal and Geleta Dugassa (2009) on rooting of shoots derived from meristem culture of sweet potato and also with the work of Lity and Conoer (1978) reported IBA significantly increased the number of roots produced in sweet potato varieties. High concentration of IBA (0.5 mg/l) plays fewer roles in rooting. This is because *in vitro* rooting is achieved by using low concentration of auxin or growth regulators free rooting medium (Nitzche and Wenzel, 1977).

The difference in survival rate of 90% and 100% for shoots regenerated from petiole and leaf calli of Beletech and 80 % and 90 % for shoots regenerated from petiole and leaf calli of Awassa-83 might be due to differences in adaptation to the new environment (*ex vitro*).

7. Conclusion

- The highest percentage of callus induction was 90% and 83% from Beletech petiole and leaf explants and 93% and 87% from Awassa-83 petiole and leaf explants respectively on MS medium supplemented with 0.05 mg/l 2,4-D in combination with 0.5 mg/l kinetin.
- The highest percentage of shoot regeneration (70 and 80%) was obtained from petiole and leaf calli of Beletech variety on medium supplemented with 1 mg/l BAP and growth regulators free MS medium, respectively. From Awassa-83 petiole and leaf calli, 71% and 77% of shoots were regenerated on medium supplemented with 1 mg/l BAP and growth regulators free, respectively.
- The best concentration of IBA for rooting was growth regulators free MS medium for shoots regenerated from petiole and leaf calli of Beletech variety and 0.1 mg/l was the best concentration for shoots regenerated from petiole and leaf calli of Awassa-83 variety.
- Among all acclimatized plantlets, maximum percentage of survival (100%) was obtained from shoots regenerated from leaf calli of Beletech variety.

8. Recommendations

- Other plant growth regulators such as TDZ, NAA for shoot regeneration and callus induction should be tested.
- Different strengths of MS media and liquid media should be tested for callus induction and shoot regeneration.
- Shoot regeneration through homogenization of different explants should be tested.
- Agronomic performance of sweet potato derived from tissue culture regenerated plantlets should be tested.

9. References

- Abo El-Abbas, F., Ishak, J. and El-Bedewy, R. (1998). Control of sweet potato viruses. Annual CIP program report. Lima, Peru.
- Alicai, T., Fenby, N.S., Gibson, R.W., Adipala, E., Vetten, H.J., Foster, G.D. and Seal, S.E. (1999). Occurrence of two serotypes of sweet potato chlorotic stunt virus in East Africa and their associated differences in coat protein and HSP70 homologue gene sequences. *Plant Pathology*. **48**: 718-726.
- Amiri, S., Kazemitabar, K.S, Ranjbar, A. G. and Azadbakht , M. (2010). *In vitro* propagation and whole plant regeneration from callus in datura (*Datura stramonium*). *Afr. J. Biotechnol.* **10**: 442-448.
- Assefa Tofu, Teshome Anshebo, Engida Tsegaye and Tesfaye Tadesse (2007). Summary of progress on orange-fleshed sweet potato research and development in Ethiopia. A paper presented on Proceedings of the 13th ISTRC Symposium, pp.728-731.
- Austin, D.F., 1987. The taxonomy, evolution and genetic diversity of sweet potato and related absence of viruses in most symptomless field grown in Uganda. *Ann.Appl.Biol.***130**: 481-490.
- Bashaasha, B., Mwanga, R.O.M., Ocitti, P., Obwoya, C. and Ewell, P.T. (1995). Sweet potato in the farming and food systems of Uganda: A farm survey report. CIP, Sub-Saharan Africa Region, Nairobi, Kenya/National Agricultural Research Organization, Kampla, Uganda. 63.
- Berrie, A. M. (1984). Germination and growth regulators. **In:** *Micropropagation of Cassava (Manihot esculantum) Using Different Concentrations of BAP*. *J. Eng and App. Sci.* **2**:1229-1231.

- Bioethics Nuffield Council (2004). The use of GM crops in developing countries. Case study 5: Improved resistance to viruses in sweet potato.
- Brasileiro, A.C.R, Willadino, L., Carvalheira, G.G and Guerra, M. (1999). Callus induction and plant regeneration of tomato (*Lycopersicon esculentum* CV. IPA 5) via anther culture. *Cienc. Rural.* **29**:435-440.
- Byamukama, E., Mpembe, I., Kayongo, J., Adolph, B. and Gibson, R. (2004). Establishing impact of participatory research in the adoption of new technologies in sweet potato research in Uganda. Presentation at: International Society for Tropical Root Crops- Africa Branch 9th Triennial Symposium, 31 October 5- November 2004, Mombasa, Kenya.
- Carey, E.F., Gichuki, S.T., Mwanga, R.O.M., Kasule, S., Fuentes, S., Macharia, C. and Gibson, R.W. (1998). Sweet potato viruses in Uganda and Kenya: Results of a survey. Pages 457-461. **In:** *Proceeding of the Sixth Triennial Symposium of the International Society of Tropical Root Crops-Africa Branch (ISTRC-AB) on Root Crops and Poverty Alleviation.* 22-28 October 1995, Lilongwe, Malawi.
- Cavalcante, J.M., Sihachakr, D., Allot, M. Tizroutine, S., Mussio, I., Servaes, A. and Ducreux, G. (1994). Isozyme modifications and plant regeneration through somatic embryogenesis in sweet potato (*Ipomoea batatas* (L.) Lam.). *Plant Cell Rep.* **13**: 437-441.
- Chavri, F., Robertson, A.I. and Verduin, B.J.M. (1997). Survey and characterization of viruses in sweet potato from Zimbabwe. *Plant Disease.* **81**:1115-1122.
- Chen, L., Bhagsari, A. and Carter, J. (2006). Effects of medium composition and culture duration on *in vitro* morphogenesis of sweet potato. *Bilologia Plantarum.* **50**: 114-117.

- Chugh, A. and Khurana, P. (2003). Herbicide-resistant transgenic of bread wheat (*T. aestivum*) and emmer wheat (*T. dicoccum*) by particle bombardment and *Agrobacterium*-mediated approaches. *Current Science*. **8**:78-83.
- Clerk, G.C. (1960). A vein clearing virus of sweet potato in Ghana. *Plant Disease Reporter*. **44**: 931-933.
- Cohen, J., Franck, A., Vetten, H.J., Lesemann, D.E. and Loebenstein, G. (1992). Purification and properties of closterovirus-like particles associated with a whitefly-transmitted disease of sweet potato. *Annals of Applied Biology*. **121**: 257-268.
- De Candolle, A. (1959). *The Origin of Cultivated Plants*. New York: Hafner.
- Dong, C.Y. (1987). Effects of growth regulators on sweet potato. A report by ARC, pp.1-5.
- Duke, J.A. (1983). Hand book of energy book, *Ipomoea batatas* (L.) Lam.
URL:http://www.hort.purdue.edu/newcrop/duke_energy/ipomoea_batatas.html.
- Ewell, P. T. (2002). Sweet potato production in Sub-Saharan Africa: Patterns and key issues. CIP, Nairobi, Kenya.
- FAO (1997). FAO database.<http://faostat.fao.org/site567/default>.
- FAO (2007). FAO database.<http://faostat.fao.org/site776/default>.
- FAOSTAT (2006). FAO database.<http://faostat.fao.org/site567/default.aspx>.
- FAOSTAT (2000). PRODSTAT. Priorities and Strategies for Resource Allocation during 1998-2000 and Centre Proposals and TAC Recommendations, June 2000. CIP

web site.

- Gad, N. and Kandil, H. (2008). Response of sweet potato (*Ipomoea batatas* L.) plants to different levels of cobalt. INSI net publication. *Aust. J. Basic App. Sci.* **2**: 949-955.
- Geddes, A.M.W. (1990). The relative improvement of crop pests in Sub-Saharan Africa. Nri, Kent, UK: Natural Resources Institute Bulletin no.36.
- Geleta Dugassa (2009). *In vitro* production of virus free sweet potato (*Ipomoea batatas* (L.) Lam) by meristem culture and thermotherapy. M.Sc.thesis. Addis Ababa University.
- George, E. (1993). *Plant Propagation by Tissue Culture: Part 1, The Technology*, 2th edn. Exegenetics, Editing, UK.
- Gibson, R.W., Kaitisha, G.C., Randrianaivoarivony, J.M. and Vetten, H.J. (1998). Identification of the East African strain of sweet potato chlorotic stunt virus as a major component of sweet potato virus disease in Southern Africa. *Plant Disease*. **82**: 10-63.
- Gibson, R.W., Mwanga, R.O.M., Kasule, S., Mpembe, I. and Carey, E.E. (1997). Apparent absence of viruses in most symptomless field-grown sweet potato in Uganda. *Annal. Appl. Biol.* **130**: 481-490.
- Gichuki, S.T. Carey, E.E., Mwanga, R.O.M., Kapinga, R., Ndolo, P.J and Kamau, J.W. (1998). Evaluation of sweet potato seedling progenies in Kenya. Page 461-465. Proceedings of the 6th Triennial Symposium of the International Society for Tropical Root Crop- Africa Branch.
- Gong, Y.F, Gao, F. and Zhang, P.B. (1998). Advances of *in vitro* culture of sweet potato

in China. *Crop Research*. **2**: 46–48.

González, R.G., Sánchez, D.S. Campos, J.M. Vázquez, E.P., Guerra, A.L., Quesada, Z.Z., Morán, R. and González, M.G. (1999). Plant regeneration from leaf and stem explants from two sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars. *Biotecnología Aplicada*. **16**: 11-14.

Gosukonda, R.M., Porobodessai, A., Blay, E. and Prakash, C.S. (1995) Thidiazuron-induced adventitious shoot regeneration of sweet potato (*Ipomoea batatas*). *In Vitro Cellular and Developmental Biology*. **31**: 65–71.

Hahn, S.K. (1979). Resistance of sweet potato to insects. *Hort. science*. **10**:95-106.

Hahn, S.K., Terry, E.R and Leuschner, K. (1981). Resistance of sweet potato to virus complex. *Hort. Science*. **16**:252-256.

Hakluyt, R. (1851). Discovery and Conquest of Terra Florida by Don Ferdinando De Soto. Hakluyt Society, No. 9. New York: Burt Franklin.

Hironori, M., Ogasawara, F. Sato, K., Higo, H. and Minobe, Y. (2007). Isolation of a regulatory gene of anthocyanin biosynthesis in tuberous roots of purple-fleshed sweet potato. *Plant Physiology*. **143**: 1252-1268.

Hollings, N., Stone, O.M. and Block, K.R. (1976). Purification and properties of sweet potato mild mottle, a white fly borne virus from sweet potato in East Africa. *Annals of Applied Biology*. **82**: 511-518.

Huaman, Z. (1996). Botany, origin, evolution and biodiversity of the sweet potato. **In** : *Sweet Potato Germplasm Management Training Manual* (CIP, 1996).

International Service for the Acquisition of Agri-biotech Applications (ISAAA) (2006). *Transferring sweet potatoes with resistance to feathery mottle virus* (ISAAA

- 1996 Annual Report: Shared Solutions to Shared Problems: Reviewing the ISAAA Biotechnology Fellowships). Ithaca, NY: Author.
- Jarret, R.L. and Gawel, N. (1991). Chemical and environmental growth of sweet potato (*Ipomoea batatas* (L.) Lam.) *In Vitro. Plant Cell, Tissue and Organ Culture*. **25**: 153-159.
- Karyeija, R.F., Gibson, R.W. and Valkonen, J.P.T. (1998). The significance of sweet potato feathery mottle virus in subsistence sweet potato production in Africa. *Plant Disease*. **82**:4-15.
- Karyeija, R.F., Kreuze, J.F., Gibson, R.W. and Valkonen, J.P.T. (2000a). Two serotypes of sweet potato feathery mottle virus in Uganda and their interaction with resistant sweet potato cultivars. *Phytopathol*. **90**: 1250-1255.
- Karyeija, R.F., Kreuze, J.F., Gibson, R.W. and Valkonen, J.P.T. (2000b). Synergistic interactions of a potyvirus and aphloem-limited crinivirus in sweet potato plants. *Virology*. **269**: 26-36.
- Liu, K., Kubota, C. and Kitaya, Y. (2001). Sweet potato technology for saving the global issues on food, energy, natural resources and environment in the 21th century. *Environment Control in Biologist*. **34**: 105-114.
- Lity, R. E. and Conoer, R. A. (1978). *In vitro* propagation of sweet potato. *Hort. Sci*. **13**: 659-660.
- Lou, R., Shimada, T. and Rarkris, T. (2006). Efficient embryogenic callus formation in sweet potato (*Ipomoea batatas* L. Lam.). *Breed. Sci*. **46**: 257-260.
- Lowe, J.M, Newel, C.A. and Buitron, F. (1992). Development of plant regeneration and transformation systems in sweet potato (*Ipomoea batatas*). *Phytophatol*. **28**: 121.

- Martin, F.W. (1970). Self and inter specific incompatibility in the Convolvulaceae. *Bot. Gaz.* **131**: 139-144.
- Martin, F.W. and Jones, A. (1971). Flowering and fertility changes in six generations of open-pollinated sweet potato. *Am. J. Hortic. Sci.* **96**: 493-495.
- Martin, F.W. (1988). Sweet potato, ECHO technical note, USA. URL:<http://www.echonet.org>.
- Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**: 473-497.
- Neja Jemal (2009). Effectiveness of meristem culture and chemotherapy on the production of virus free sweet potato (*Ipomoea batatas* (L.) Lam.). M.Sc.thesis. Addis Ababa University.
- Newell, C.A., Lowe, J.A., Merryweather, A., Rooke, L.M. and Hamilton, W.D.O. (1995). Transformation of Sweet potato (*Ipomoea batatas* (L.) Lam.) with *Agrobacterium tumefaciens*. *Plant Sci. J.* **107**: 215-227.
- Nitzche, M.W. and Wenzel, G. (1977). *Haploid in Plant Breeding*. Berlin, Hamburg, Parey, pp. 85-90.
- Njeru, R.W.M., Mburu, M.W.K., Cheramgon, E., Gibson, R., Obudho.W. and Yobera, D. (2004). Studies on the physiology effects of virus on sweet potato in Kenya. *Annals of Applied Biology* **145**: 71-76.
- Nutritional Institute (1995). National survey for assessment of vitamin A status in Egypt in collaboration with Triqui, Z.A. Guedra, A Chlyah V and.Sihachakr, 2007. Effect on genotype, gelling agent and auxin on induction of somatic embryogenesis in sweet potato c.r.biologist.unecef.

- Odame, H.P., Mbote, K. and Wafula, D. (2002). The role of innovation in policy and institutional change: Influence of modern biotechnology on institutional and policy change in Kenya. IELRC. Environment Team, IDS, Sussex.
- Oggema, J.N., Ouma, J.P. and Kinyua, M.G. (2007). Response of five locally adapted sweet potato (*Ipomoea batatas* (L.) Lam.) cultivars to *in vitro* plant regeneration via direct and indirect embryogenesis. *Asian Journal of Plant Sciences* **6**: 617-622.
- Otani, M. and Shimada, T. (1988). Plant regeneration from leaf calli of *Ipomoea trichocarpa* Ell. *Japan J. Breed.* **38**: 205-211.
- Otani, M., Mii, M. and Shimada, T. (1996). High frequency plant regeneration from leaf calli in sweet potato cv. Chugoku 25. *Plant Tiss. Cult. Lett.* **13**: 23-27.
- Otani, M., Wakita, Y. and Shimada, T. (2003). Production of herbicide resistant sweet potato (*Ipomoea batatas* (L.) Lam.) plants by *Agrobacterium tumefaciens*-mediated transformation. *Breed. Sci.* **53**: 145-148.
- Rabbani, A., Askari, B., Abbasi, N. A., Bhatti, M. and Quraishi, A. (2001). Effect of growth regulators on *in vitro* multiplication of potato. *Int. J. Agri. Biol.* **3**:181-182.
- Rashid, U., Ali, S., Ali, M. G., Ayub, N. and Masood, S. M. (2009). Establishment of an efficient callus induction and plant regeneration system in Pakistani wheat (*Triticum aestivum*) cultivars. *Electronic Journal of Biotechnology*.**12**:1-12.
- Robbins, W. J. (1972). Cultivation of excised root and stem tips under sterile condition. *Bot. Gaz.* **73**: 376-390.
- Rossel, G., Kriegner, A. and Zhang, D.P. (2001). The sweet potato: its origin and dispersal. *Am. Anthropol.* **74**:342–365.

- Sato, M., Wang, J.S., and Satoru, T. (1999). High frequency plant regeneration from leaf and petiole explants cultures of sweet potato. *Mem. Fac. Agr. Kagoshima Univ.* **35**: 1-5.
- Scott, G.J., Rosegrant, M.W. and Ringer, C. (2000). Roots and tubers for the 21st century: trends, projection and policy options, 2020 brief No. 66. International Crop Research Institute for the Semi-Arid Tropics, India.
- Sheffield, F.M.L. (1953). Virus diseases of sweet potato in parts of Africa. *Emp J.Exper. Agri.* **21**: 184-189.
- Sihachakr, D. and Ducreux, G. (1993). Regeneration of plants from protoplasts of sweet potato (*Ipomoea batatas* L. Lam.). **In:** Y.P.S. Bajaj (Ed.), *Plant Protoplasts and Genetic Engineering IV*, in Biotechnology, Agriculture and Forestry, 23, Springer-Verlag, Berlin, Heidelberg, pp: 43-59.
- Sihachakr, D. Honda, H. and Yamaguchi, K. (1997). Efficient *Agrobacterium tumefaciens*-mediated transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) from stem explants using a two-step kanamycinhygromycin. *In Vitro Cellular and Developmental Biology-Plant* article. **40**: 359-365.
- Technical Center for Agricultural and Rural Cooperation (CTA) (2007). Making sweet potato chips and flour. CTA practical guide series No.6, pp.1-6.
- Tewe, O.O., Ojeniyi, F.E. and Abu, O.A. (2003). *Sweet Potato Production, Utilization, and Marketing in Nigeria*, Kales Publisher, Nigeria.
- Tileye Feyissa, Welander, M. and Legesse Negash (2005). *In vitro* regeneration of *Hagenia abyssinica* (Bruce) J.F. Gmel. (Rosaceae) from leaf explants. *Plant Cell Reports.* **24**: 392-400.

Triqui, A.E.Z., Abdelkarim, G., Averil, C., Hassane, C. and Vongthip, S. (2008). Effect of genotype, gelling agent and auxin on the induction of somatic embryogenesis in sweet potato (*Ipomoea batatas* (L) Lam.). *Comptes Rendus Biologies*. **331**:198-205.

URL:[http// www.self.com/food/blogs/nutritional diet/sweet potato.gif](http://www.self.com/food/blogs/nutritional%20diet/sweet%20potato.gif)

URL:[http//www.nlm.nih.gov](http://www.nlm.nih.gov).

URL:[http// en.wikipedia.org](http://en.wikipedia.org)

U.S. Department of Agriculture (2001). Agriculture Research Service Nutrient Database for Standard Reference.

Van Bruggen, A.H.C. (1984). Sweet potato stem blight caused by *alternaria* sp: A new disease in Ethiopia. *Neth. J. Pl. Path.* **90**:155-164.

Wang, J.S., Sato, M. and Taura, S. (1999). High frequency plant regeneration in leaf and petiole explants cultures of sweet potato. *Mem. Fac. Agric. Kagoshima Univ.* **35**: 1-5.

Wheatley, C.C. and Song, B. (2000). Sweet potato starch in China: Current status and future prospects. **In:** *Cassava, Starch and Starch Derivatives*. R.H. Howeler, C.G. Oates, and G.M. O'Brien (eds). Proceedings of the International Symposium held in Nanning, Guangxi, China Nov. 11-15 1996. Centro Internacional de Agricultura Tropical (CIAT), 201-215.

Winter, S.A., Purac, F., Leggett, E.A., Frison, H.W., Rossel and Hamilton, R.I. (1992). Partial characterization and molecular cloning of a closterovirus from sweet potato infected with the sweet potato virus disease complex from Nigeria. *Phytopathology*. **82**: 869-875.

Woolfe, J.A. (1992). *Sweet Potato an Untapped Food Resource*. Cambridge University Press, New York, 324-340.

World Bank (1995). World development report.

Zheng, Q., Dessai, A.P. and Prakash, C.S. (1996). Rapid and repetitive plant regeneration in sweet potato via somatic embryogenesis. *Plant Cell Rep.* **15**: 381-385.

Zuger, R. (2003). *Impact study of the adoption of new varieties of sweet potatoes released by INIA in Peru: Case of the Canete valley*. Lima, Peru: CIP.

Appendix 1. Average constituents of the sweet potato: values per 100 g (USDA, 2001)

	Units	Raw sweet potato	Cooked, baked in skin	Cooked, boiled without skin
Water	g	72.84	72.84	72.84
Energy	kcal	105	103	105
Protein	g	1.65	1.72	1.65
Lipid (fat)	g	0.30	0.11	0.30
Carbohydrate	g	24.28	24.27	24.28
Fiber	g	3.0	3.0	1.8
Pantothenic acid B ₅	mg	0.591	0.646	0.532
Vitamin B ₆	mg	0.257	0.241	0.244
Folate	mcg	14	23	11
Vitamin A	mcg	2,006	2,182	1,705
Vitamin E	mg	0.280	0.280	0.280

Appendix 2. Full MS basal medium stock solution composition

Components	Concentration (g/l)
Micronutrients	
Fe –Na-EDTA	4.00
ZnSO ₄ .7H ₂ O	0.860
H ₃ BO ₃	0.620
*MnSO ₄ .H ₂ O	1.69
*MnSO ₄ .4H ₂ O	2.23
CuSO ₂ .5H ₂ O	0.025
KI	0.083
Na ₂ MoO ₄ .2H ₂ O	0.025
CoCl ₂ .6H ₂ O	0.025
Macronutrient (g/l)	
NH ₄ NO ₃	16.5
KNO ₃	19
CaCl ₂ 2H ₂ O	4.4
MgSO ₄ .7H ₂ O	3.7
KH ₂ PO ₄	1.7
Vitamin (g/l)	
Nicotinic acid (NaOH)	1.00
Glycin(glycocoll)	0.2
Nicotinic acid(NaOH)	0.05
Pyridoxin(B6)	0.05
Thiamin(B1)	0.01

* are alternatives

Declaration

I, the undersigned, declare that this thesis is my original work. It has never been submitted for a degree in any other university and that all sources of materials used for the thesis have been fully acknowledged.

Name: Tasew Getu

Signature: _____

Date: _____

This paper has been submitted for examination with our approval as University advisor.

Tileye Feyissa (PhD)