

**Protocol Development for Micropropagation of the Widely Cultivated
Ethiopian Yam (*Dioscorea spp.*, Bulcha variety)**

By: Birhan Demisse Agidew



A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfilment of the Requirements for the Degree of

Master's of Science in Applied Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

December, 2019

**Protocol Development for Micropropagation of the Widely Cultivated
Ethiopian Yam (*Dioscorea spp.*, Bulcha variety)**

By: Birhan Demisse Agidew

Advisors: Dr. Mulugeta Kebede

Mr. Obsi Dessalegn

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfilment of the Requirements for the Degree of

Master's of Science in Applied Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

December, 2019

Table of Content

ACKNOWLEDGEMENT	I
LIST OF TABLES	II
LIST OF FIGURES	III
LIST OF APPENDICES	IV
List of Acronyms and Abbreviations	V
Abstract	VI
1. INTRODUCTION	1
1.1. Back ground of the study	1
1.2. Objectives of the study	3
1.2.1. General Objective	3
1.2.2. Specific Objectives	3
2. LITERATURE REVIEW	4
2.1. Botany of Yam	4
2.2. Yam Origin and Distribution	5
2.3. Yam in Ethiopia	6
2.4. Importance of Yam.....	7
2.4.1. Medicinal Significance of Yam.....	8
2.4.2. Nutritional Value of Yam	9
2.5. Bioactive Compounds Present in YAM.....	10
2.6. Propagation of Yam	11
2.6.1. Conventional Propagation Techniques of Yam.....	11
2.6.2. Plant tissue culture and its application	13
3. Material and Methods	15

3.1. Study Area.....	15
3.2 .Plant Materials.....	15
3.3. Stock Solution and Media Preparation.....	17
3.3.1. Stock solution preparation	17
3.3.2. Plant growth regulators stock solution preparation	17
3.3.3. Culture medium preparation	17
3.4. Experiments and Experimental Designs	18
3.4.1. Explants Preparation and Sterilization	18
3.4.2. Initiation and culture of explants in culture media	19
3.4.3. Effect of different concentrations of BAP on shoot multiplications	19
3.4.4. Effect of NAA and IBA Combinations on <i>in vitro</i> Rooting.....	19
3.5. Acclimatization	20
3.6. Data analysis	20
4. Result and discussion	21
4.1. Explants Preparation and Sterilization	21
4.2. Effect of different concentrations and combinations of BAP and NAA on shoot initiation	24
4.3. Effect of different concentrations of BAP on shoot multiplications.....	28
4.4. Effect of NAA and IBA Combinations on <i>in vitro</i> Rooting	31
4.5. Acclimatization	36
5. CONCLUSIONS	37
6. RECOMMENDATIONS.....	38
7. REFERENCES.....	39
8. APPENDICES	51

Approval of Board of Examiners

We, the undersigned members of the Board of Examiners of the final open defense by Birhan Demisse have read and evaluated his thesis entitled “Protocol Development for micro propagation of the widely cultivated Ethiopian Yam (*Dioscorea spp.*, Bulcha variety)” and examined the candidate. Therefore, this is to certify that the thesis has been accepted in partial fulfillment for the requirement of the Degree of Master’s of science in Applied Biology (Biotechnology).

_____	_____	_____
Advisor	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Department Chairperson	Signature	Date

Declaration

I hereby declare that this M.Sc. thesis entitled “Protocol Development for micro propagation of the widely cultivated Ethiopian Yam (*Dioscorea spp.*, Bulcha variety)” is my original work and that all sources of materials used for this thesis have been duly acknowledged.

Name: _____

Signature: _____

This MSc thesis has been submitted for examination with my approval as thesis advisor.

Name: _____

Signature: _____

Date of Submission: _____

Advisor's Approval Sheet

To: Department of Applied Biology

Subject: Thesis Submission

This is to certify that the thesis entitled “**Protocol Development for micro propagation of the widely cultivated Ethiopian Yam (*Dioscorea spp.*, Bulcha variety)**” submitted in partial fulfillment of the requirements for the degree of Master's of Science in Applied Biology (Biotechnology), Graduate Program to the Department of Applied Biology and has been carried out by **Birhan Demisse ID No:A/PR15459/10** under my supervision. Therefore, I recommend that the student has fulfilled the requirements and hence hereby she can submit the thesis to the department.

Birhan Demisse

Student Name

Signature

Date

Dr. Mulugeta Kebede

Major Advisor

Signature

Date

Mr. Obsi Dessalegn

Co-Advisor

Signature

Date

ACKNOWLEDGEMENT

First and foremost, I would like to thank Almighty God for his wisdom and protection throughout my life. Secondly, I would like to express my sincere and heartfelt gratitude to my advisors Dr. Mulugeta Kebede, and Mr.Obssi Dessalegn for their encouragement, guidance and support that made the successful completion of this work. My sincere thanks also goes to Adama Science and Technology University, Department of Applied Biology for admitting me to school of graduate studies and National Agricultural Biotechnology Research Center (NABRC) for the permission to access to laboratory facilities and enabling working environment. Specially plant tissue culture staff members who helped me in one way or the other in their technical advice and made my stay pleasant. I am thankful to Hawasa Agricultural Research Center specially Dr.Atnafua Bekele for providing me yam tubers.

My thanks are also extended to Mr.Birhanu Mangistu for his technical advice and the role he played in the statistical data analysis. I have also special acknowledgement to Adaba Tilahun for suggesting to me the idea of this work and other constructive clues. This work would not have been completed without the support and encouragement of my Husband Getahun Worku for His dedication and financial support that brought this work to existence. The encouragement, love and material support from my husband has made anything possible. I am greatly indebted to acknowledge my sister Elisabeth Demise and her husband Bekele Mekonine for their kind approach and walking along with me during the ups and downs. All my friends who provided me important advice help and encouragement but fail to name all of them, I want to say thank you so much. May God bless you all.

LIST OF TABLES

Table 1: Interaction effect of Sodium hypochlorite concentrations and its time of exposure on sterilization.....	21
Table 2: Effects of BAP and NAA on <i>in vitro</i> shoot induction rate of <i>Dioscorea alata</i> from nodal explants.	24
Table 3: Shoot multiplication in nodal explants of <i>Dioscorea alata</i>	28
Table 4: Influence of different combination of NAA and IBA on in vitro rooting of <i>Dioscorea alata</i>	33

LIST OF FIGURES

Figure 1: Plant material source and mother stock establishment in green house.	16
Figure 2: Response of nodal explants of Bulcha varieties on initiation medium with different combinations of BAP and NAA..	Error! Bookmark not defined.
Figure 3: Effect of BAP on shoot multiplication medium.	Error! Bookmark not defined.
Figure 4: <i>In vitro</i> rooting of Bulcha variety shoots on full strength MS medium containing combination of NAA and IBA.....	33
Figure 5: Acclimatization in greenhouse	36

LIST OF APPENDICES

Appendix 1: Analysis of variance for effect of different concentration of NaOCl for different exposure time	Error! Bookmark not defined.
Appendix 2 : Analysis of variance for effect of different concentration of BAP in combination with NAA on percent of explants showing shoot initiation.....	Error! Bookmark not defined.
Appendix 3 : Analysis of variance for number and length of roots in different concentrations of IBA and NAA in combination.	Error! Bookmark not defined.
Appendix 4: Murashige and Skoog MS (1962) media components.....	56

List of Acronyms and Abbreviations

BAP	6-Benzyl Amino Purine
IBA	Indol-3-Butyric Acid
MS	Murashige and Skoog
NAA	α -Naphthalene Acetic Acid
PGR	Plant Growth Regulator
HARC	Holetta Agricultural Research Center
FAO	Food and Agricultural Organization
CRD	Completely Randomized Design
AC	Activated Charcoal
ANOVA	Analysis of Variance
IBC	Institute of Biodiversity Conservation
DDW	Double Distilled Water
NABRC	National Agricultural Biotechnology Research Center
PVP	Polyvinylpyrrolidone
NaOCl	Sodium hypo-chlorite

Abstract

Yam (Dioscorea species) is a monocotyledonous tuber forming tropical vine, which belongs to the order Liliiflorae, family Dioscoreaceae, and genus Dioscorea. Yam is well known for its medicinal use as well as nutritional values. Conventional propagation of yam (Dioscorea spp.) is limited due to low propagation rates. Hence, in vitro propagation is the best alternative to overcome such limitations of conventional propagation. The present study reports a high and cost effective micropropagation protocol for Ethiopian yam (Dioscorea alata, Bulcha variety) and used for high and efficient plantlet regeneration from nodal explants. Explants were sterilized using different concentrations of NaOCl for different time exposure. MS culture media supplemented with different types and concentration of auxins and cytokinins were used for culture initiation, shoot multiplication and root induction. Sodium hypochlorite (NaOCl) at a concentration of 1% at exposure time of 15minutes gave the highest percentages (100%) of survived explants. The combination of 0.5 BAP with 1mg/l NAA was found to be an optimum concentration for shoot induction. BAP at 1.5 mg/l was found to be the optimum concentration for shoot multiplication, yielding 7.28 ± 0.07 shoots number per explant with a shoot length of 8.72 ± 0.43 cm. MS medium with 2.0 mg/l NAA with 1.5 mg/l IBA gave the highest rooting percentage and gave optimum root number (10.6 ± 0.44) and root length (13.80 ± 0.44). Finally, for acclimatization, in vitro multiplied plantlets were transferred to greenhouse for hardening off. In vitro raised plantlets were acclimatized and recorded 90% of survival rate on soil medium with combination ratio of 1:2:1 top soil, sand soil and compost, respectively. Finally use of the already developed micropropagation protocol for large scale production of the Bulcha varieties and distributing to farmers to increase yam tuber production in Ethiopia should be given due attention.

Key words: *Dioscorea, In Vitro Propagation, MS Medium, Nodal Vine, Plant Growth Regulators, Yam.*

1. INTRODUCTION

1.1. Background of the study

Yam (*Dioscorea* spp.) is a monocotyledonous tuber forming tropical vine, which belongs to the order *Liliflorae*, family *Dioscoreaceae*, and genus *Dioscorea* (Course, 1976). There are about 600 species of *Dioscorea*, among of which; white yam (*Dioscorea rotundata*), water yam (*Dioscorea alata*), aerial yam (*Dioscorea bulbifera*), yellow yam (*Dioscorea cayenensis*), trifoliolate yam (*Dioscorea dumentorum*) and Chinese yam (*Dioscoreae sculenta*) are economically the most important species (Onwueme, 1978). *Dioscorea rotundata* and *Dioscorea cayenensis* are indigenous to West Africa while *Dioscorea alata* and *Dioscoreae sculenta* are native to Asia. Globally *Dioscorea* is grown in an area of about 7.76 million hectares with production of 68.13 million tons; average yield being 29.68 tons/ha in 2014 .For instance, West African yam zone accounts for over 90% of the 4.59 million hectares of yam cultivation which is estimated at about 20-25million tons per year (Izekor and Olumese, 2010). From east African countries, Ethiopia and Sudan are major yam producers (Musa *et al.*, 2011).

It is also reported that Ethiopia is the center of origin for one of *Dioscorea* species (*D.abbyssinica*) (Zeven and Wet, 1982). Hence, these species typically spread more or less in Metekel, Sidama, Wolayta, Gambella, Maji, Jimma and North Omo in South and South west of the country (Umeta *et al.*, 2005). Yam is an important source of carbohydrates, fibers, and small amounts of fats and can be processed into various end-product forms (Jaleel *et al.*, 2007). It is a flexible vegetable that can be roasted, fried, grilled, boiled and baked, (Albrecht and Mccarthy, 2006). Tubers are the main economic importance of all the *Dioscorea* species (Lebot, 2009). According to Lebot (2009) and Kole (2011) those yam storage organs whether underground or aerial tubers are source of proteins and vitamins. Moreover, it is deliberated as a medicinal herb. Since the genus is rich in steroidal saponins that are used as a source of biologically active compounds in pharmaceutical industries (Wang *et al.*, 2006). Tubers of some species of *Dioscorea* are sources of important chemical called Diosgenin which is noticeably essential for synthesis sex hormones and corticosteroids that are commonly used as anti-inflammatory and contraceptive drugs (Zuluaga *et al.*, 2007).

Eventhough yam has an important contribution to food security and hunger reducing programs particularly in the developing countries, research and documentation for this important staple food crop is still limited (Sangoyomi, 2004) and its production has shown fluctuation since 2007 (FAO,2012). The main reason for this is, the propagation of yam by seeds using conventional methods is slow and not adequate for rapid multiplication of this species (Nweke *et al.*, 1991). In addition, pests and diseases are accounted to be the constraints of yam production (Atiri *et al.*, 2003). Therefore, there is a need to develop *in vitro* propagation method for its application in the production of disease free plant materials under controlled conditions in small spaces with reduced labor requirement (Vaillant *et al.*, 2005; Abraham, 2009). Arditti (2009) reported that *in vitro* propagation method provides large scale proliferation of high quality planting materials. It offers many advantages over conventional methods like mass propagation and production of pathogen free planting materials (Obsi *et al.*, 2015). It can minimize the susceptibility of the plant to diseases those transmitted at the time of tuber cutting to propagate (Alizadeh *et al.*, 1998).

A few studies have been reported on some economically important *Dioscorea* species using nodal segments with limited auxin and cytokinin types, concentrations and combinations (Twyford and Mantell, 1996; Wheatley *et al.*, 2005; Borges *et al.*, 2005; Jova *et al.*,2011; Das *et al.*, 2013; and Obsi *et al.*, 2015). But each variety requires its own regeneration protocol (Gonzalez *et al.*, 1999). Therefore, the present investigation was made to optimiz a protocol for micro-propagation of Yam (*D. alata*, Bulcha variety) using different types, concentrations, and combinations of auxin and cytokinin in order to increase the rapid production of disease free and excess plant materials. This will have its own contribution to improve the food security and health related problems in Ethiopia, being one of the developing countries suffering from food insecurity and health related problems, by producing the healthy yam seedlings with relatively short period of time.

1.2. Objectives of the study

1.2.1. General Objective

General objective of this study was to optimize a protocol for micropropagation of Yam (*Dioscorea alata*), Bulcha variety, that is widely cultivated in Ethiopia.

1.2.2. Specific Objectives

- To establish the best sterilization protocol for in *vitro* culture of *D.alata*.
- To determine the optimum concentration of BAP and NAA combinations for shoot initiation.
- To determine the optimum concentration of BAP for shoot multiplication.
- To determine the optimum concentration of IBA and NAA combinations for root formations.
- To evaluate acclimatization and survival rates of plantlet in greenhouse condition.

2. LITERATURE REVIEW

2.1. Botany of yam

Yam tubers are enlarged storage organs containing food reserves in the form of starch, and vary greatly in size. They are round, cylindrical, oval or flattened (Toyohara *et al.*, 2000) and are usually produced underground. Yam can grow up to about 2.4 m long and infatuated with weight up to 45Kg (Kay, 1987). It is a drought tolerant tuber crop which is capable of producing a good yield under water scarcity conditions (Asiedu and Sartie, 2010). Depending on the variety, the tuber flesh may be various shades of white, yellow, purple, or pink, and the skin can be white to dark brown. Some species of yam, such as *D. bulbifera*, produce aerial tubers. Yam shoots are climbing and vine-like thus usually requiring support from either neighboring plants or stakes on which they twine clockwise or anti-clockwise depending on the species (Johnson, 2003). Most *Dioscorea* species have simple, acuminate leaf borne on long petioles, but in some species, they are lobed or palmate with pointed tips. They are climbers and climb by twining. The direction of twining of the vine (anti-clockwise or clockwise) is a characteristic unusual to the particular sections within the genus *Dioscorea* (Johnson, 2003).

The wings present in some species, such as *D. alata*, support the twining habit. The flowers are basically dioeciously, with male and female flowers borne separately or on separate plants. The male or female flowers are borne on axillary spikes in the leaf axils. The male flowers are sessile, glabrous, and spherical and are borne axially or terminally. These flowers consist of calyx of three sepals and corolla of three petals, arranged regularly and almost similar in size and appearance, with three or six stamens (Onwueme, 1978). Fruits are mostly capsules. The seed in each capsule is small and has wings that vary in shape in different species (Onwueme, 1978). The seeds are flat and light and the wings help in wind dispersion. Some species, such as *D. alata*, *D. bulbifera*, *D. pentaphylla*, *D. puberablume*, have bulbs in the axils. Bulbs are specifically adapted for vegetative propagation (Coursey, 1967). They are very smaller than the underground tubers. *Dioscorea* own low fibrous root systems, normally un-branched and intense in the top layer of the soil, and in fact penetrate up to 1m depth (Onwueme, 1978).

2.2. Yam Origin and Distribution

Yam belongs to the largest genus *Dioscorea* in the family *Dioscoreaceae*. It is assumed that the family is among the original angiosperms that believed to be originated from Asia. It is also supposed that, in the late 1450s, yams have been carried by Malaysians from Asia and across the Savannah to West Africa from Madagascar and then reached to Caribbean across Atlantic (Johnson, 2003). It is believed that the production of yam species from West Africa to America took place in the 16th century (Coursey, 1967). In addition, evidences revealed for the origin and then distribution of the various yam species to have originated in three areas of the tropics and subtropics (West Africa, South America and Asia). In fact, the various *Dioscorea* species followed a different evolution in three continents separated by the formation of the Atlantic Ocean and aridity of the Middle East (Hahn, 1995). As a result, West Africa, Southeast Asia, and Tropical America are the three isolated centers of this major species (Alexander and coursey, 1969).

They also considered as independent centers of yam domestication, and represent a considerable diversity (Asiedu *et al.*, 1997). Among the selected *Dioscorea* species, *D.alata* which originated in Southeast Asia more specifically, in tropical Myanmar and Thailand considered as most important species economically (Orkwor *et al.*, 1998) and most diversified and extensively distributed species in present time. In addition, it is dispersed all over the tropics, Southeast Asia, Papua New Guinea, Indonesia, Malaysia, Philippines, South Pacific Islands, Vanuatu, West Africa, Brazil, Caribbean, South America, Central America, Florida, Escambia, Gadsden, Leon, Levy, and Charlotte (Lebot *et al.*, 1998). *D. bulbifera* is the other most studied popular *Dioscorea* species. The indigenous range of this species is widely distributed in much of Asia, tropical Africa, and Northern Australia (Terauchi *et al.*, 1991). Another species of it is also native to South Asia and spread in Central and South America, Africa, Australia, Louisiana, Texas, Hawaii, Puerto Rico, Polynesia, Florida, and West Africa, (Langl and Burks, 1998). *D.hispida* Dennst, one of *D. bulbifera* species, is also distributed in tropical and sub-tropical regions, Philippines, China, Taiwan, Malaya, New Guinea, Malaysia, Fujian, Bhutan Indonesia, Bangladesh, Sikkim, Thailand, Cambodia, Laos, Myanmar, Vietnam, and Africa (Sharma and Bastakoti, 2009). Moreover, *D.pubera* Blume is native to the Indo-China region and it is distributed throughout the temperate, tropical America, China, wet regions of Himalayas, Central Nepal, Western Malaysia, and Bhutan (Coursey, 1967).

2.3. Yam in Ethiopia

In Ethiopia, there are numerous indigenous cultivated root and tuber crops. These crops have an important place in the diet of the population (IBC, 2008). Edossa Etissa, (1998) revealed that Ethiopia is the seventh largest yam producing country in Africa with about 62,000 hectares of land under yam cultivation. The total annual production of yam was estimated about 339,740 metric tons from an area of about 1,104,300 Sq.km in 2011 (FAOSTAT, 2013). Among the most important tuber crops in the country, Yams (*Dioscorea* spp.) are grown in complex cropping systems together with cereals, and other root and tuber crops (Westphal, 1975). It is reported as it possessed different local names in different parts of the country such as Boye, by Dauro people; Kocho or Wocheno, Oromo; Bohe, Wolayita; Kuso, Yem; and Boina, Sidama (EdosaEtisa, 1996). Another study in 2002, reported that around 23 indigenous yam kinds belonging to at least four *Dioscorea* species were described in Sheko, South east Ethiopia (Hildebrand *et al.* ,2002).

There are several methods for yam food preparation in the different growing regions of the country. For instance, in the south west of Ethiopia, Sheko districts, cooking methods are different based on gender men prepare a yam, either in the field or at home. First, he cleans the tuber and roasts up the wild yam that he found in the field as a full meal if he is single, or prepare it as a late afternoon snack for his wife and children. In another way, women prepare yams by steaming. To get a family meal, a woman will harvest one or two large stake yams and peel them (Elisabeth, 2003). Even if there are several species of *Dioscorea* grow in different parts of Ethiopia, all are not edible. Both wild and cultivated are found in the country that indicated the wide diversity of the species in the country (Mie“ge and Sebsebe Demissew, 1997). As a result, the importance of yam for local subsistence and its indigenous knowledge, as well as priorities for conservation and improvement of the crop was highlighted. As study showed that, both wild and cultivated, *Dioscorea* species, are found in the country which indicates that Yam is widely distributed in the country and is grown by subsistence farmers in the Southern, Southwestern and Western parts of the country (Mie“ge and Sebsebe Demissew, 1997). Even though Yam is a traditional crop that has long been cultivated in Wolayita and Gamo-Gofa zones as co-staple with enset, cereals, and other root and tuber crops, the extent and distribution of the available inter and intraspecific diversity is poorly investigated (Westphal,1975).

2.4. Importance of Yam

In the world, there are about 50-60 species of *Dioscorea* which is known to be cultivated or at least gathered for food or pharmaceutical purposes. However, 12 species were used as food (Coursey, 1967). The most important of these are: White Guinea yam (*D. rotundata*), Water yam or Greater yam (*D. alata*), Yellow yam (*D. cayenensis*), Lesser yam (*D. esculenta*), Bitter yam or Trifoliate yam (*D. dumetorum*), Aerial potato yam (*D. bulbifera*), *D. trifida* (Cush-cush yam), *D. opposite*, also known as *D. batatas*, *D. nummularia* Lam, *D. pentaphylla*, and *D. hispida* (Craufurd *et al.*, 2001). In developing countries, *Dioscorea* is the main food crop, especially in the tropics and subtropics. Yam is propagated in Africa for food, to generate income, celebrate fertility and marriage ceremonies, and in Ethiopia, it is good for distinguished guests and Meskel holiday ceremonies.

The tuber part of the yam crop is the most costly in the market among tuber crops, and thus it generates income for low income farmers. Although yam is cultivated for different purpose for its edible tubers, potential reservoir of protein in some extent and mainly carbohydrates of food security and industrial value, the flora is now a day's also a renewable source of high value compounds (Price *et al.*, 2016).

Yam tuber is said to contain some pharmacologically active substances such as dioscorine, saponins and sapogenin. Dioscorine which is the major alkaloid in yam is medicinally a heart stimulant (Eka, 1985). In addition, it has been reported that yam is a good source of industrial starch whose quality varies with the species (Shehu *et al.*, 2010). Yams has high moisture, dry matter, starch, and potassium contents. It also contains high potassium and manganese and low in saturated fat, sodium and cholesterol (Walsh, 2003). The high potassium and low sodium content of yam produces good potassium-sodium balance in the human body and so protect against osteoporosis and heart disease (Walsh, 2003). Slow breakdown of carbohydrates and gradually release of glucose into the blood stream means that they will provide more sustained form of energy, and give better protection against obesity and diabetes (Brand-Miller *et al.*, 2003). It is used for blood pressure regulation to overcome hypertension (Liu *et al.*, 2009). It also decreases the danger of diabetics and fatness (Apriantia *et al.*, 2009). Moreover, it decreases menstrual pains and it is a source of antioxidant (Lubag *et al.*, 2008).

2.4.1. Medicinal Significance of Yam

Yams are often used for medicinal purposes for the sapogenins, aglycons of yam saponins are important mostly due to the fact of their steroid structure (Okwu and Ndu, 2006). According to Crabbe (1979), yams are antecedents for the chemosynthesis of start to influence tablets (with estrogen and progesterone) as well as equivalent hormones and corticosteroids. There are a number of reports on hand on nearby claims on *Dioscorea* species worldwide. Local human beings in some forest areas of Southern Thailand, which are located in the tropical rain wooded area of Southeast Asia, use *Dioscorea species* to treat warts (Maneenoon *et al.*, 2008). *D. prazeri* is used as cleaning soap and shampoo to kill lice (Maneenoon *et al.*, 2008).

Dioscorea is used in curing gastritis (Kadiri *et al.*, 2014). Tubers of *D. hamiltonii* are used as physique refrigerant during the summer season and used to treat diarrhea. *D. bulbifera* is also used against tuberculosis and raw tuber of *D. pentaphylla* against diphtheria in cattle (Sharma and Bastakoti, 2009). Besides, tubers of *D. oppositifolia* are used in the cure of swellings, and snake bites and Juice of *D. wallichii* is used in the therapy of Jaundice (Sinha and Lakra, 2005). The toxic alkaloid dioscorine and its dehydro-derivatives were in the previous used in poison-making, such as, hunting arrow-poison, crook poison etc. in particular parts of the world. *D. dometorum* species also contain Dioscorine in which is responsible for the hypoglycaemic exercise of the tubers (Iwu *et al.*, 1990).

2.4.2. Nutritional Value of Yam

Yams are the main beneficial plants in the diet of several residents of tropical region. The nutritional value of yam attests its significance to human being. It is the main source of carbohydrate, minerals of calcium, phosphorus, iron and vitamins such as riboflavin, thiamin and vitamins B and C (Coursey, 1967). This plant is basically carbohydrate and is one of the cheapest sources of carbohydrate to man (Kochlar, 1981). Edible roots and tubers not only enrich the diet due to the presence of starch and energy supplemented metabolite skin them, but also possess medicinal properties due to the presence of diverse secondary metabolites. As it is confirmed by studies, *Dioscorea*, is superior to many others as an important medico-food used by about 300 million people throughout the world (Arnau *et al.*, 2010). In fact, they are one of the major sources of energy food for a lot of people in the Tropics (Nayaboga *et al.*, 2014). Lev and shriver (1998) reported as *Dioscorea* species rank as the world's fourth most important root and tuber crops after potatoes, cassava, and sweet potatoes in terms of dietary nutrients.

Many of the tubers of *Dioscorea* are bitter in taste, and people use traditional skills to remove bitterness which includes boiling/steaming and/or baking over coals after either cleaning (bulbs) or cleaning and peeling (tubers) (Bhandari and Kawabata, 2005). Martin (1994) documented that the tubers of several toxic varieties of *D.bulbifera*, are made tastier by the above said techniques. Aborigines also use their tubers as snacks, and in roasted, powdered, and other forms (Kumar *et al.*, 2012). Yam is an important source of carbohydrate to the people of the tropical and subtropical Africa, Central and Southern America, parts of Asia, the Caribbean and Pacific Islands (Coursey, 1967). According to the 2005 report by FAO, Africa accounts for nearly 96% of the world's yam production. Almost all of the African output is confined to West Africa. In Ghana; daily consumption is expected in 0.5–1.0g per person (Purseglove, 1989).

2.5. Bioactive Compounds Present in YAM

A bioactive compound is just a substance that has a biological bustle, related to its capability to modulate one or supplementary metabolic process, which offers the promotion of improved health conditions. Plant extract are readily available, non-phytotoxic and are recyclable thus been friendly to man and his environment (Akueshi *et al.*, 2002). According to Okwu (2004), phytochemicals are natural bioactive compounds present in plants, dietary fiber to care for against diseases. Research works have shown the phytochemicals working in conjunction with nutrients found in food plants may help to slow down the aging process and reduce the threat of many diseases together with heart disease, stroke, urinary tract infections, cataracts and osteoporosis in humans.

Apart from food, yams are mainly used for medicinal purpose. The major phytochemicals characteristics of yams are the existence of dioscorine alkaloid, diosgenin and saponin, although dioscorine and diosgenin are obviously considered as toxic and which is removed by washing, boiling and cooking (Eka, 1998). The tuber and other parts of *Dioscorea* species possess different types of bioactive compounds. Therefore, its parts are used against different diseases. These bioactive compounds also imitate the indigenous therapeutic values among many races of aboriginals of the world (Nayaboga *et al.*, 2014). All these issues make them highly valuable pharmacological agents and good sources for the separation and formulation of new compounds that can fight against different types of diseases. Martin (1969) reported that diosgenin which is the primary active ingredient in *Dioscorea* species and structurally comparable to cholesterol. It is metabolized in the liver and eliminated via the bile after oral administration (Caven and Dyornik, 1979). Asha and Nair (2005) described that *D. deltoidea*, is the major species exploited in India for diosgenin production from rhizomes.

2.6. Propagation of Yam

2.6.1. Conventional Propagation Techniques of Yam

The yam crop halts in the field for 8 to 10 months and the tubers are ready to grow after another 2 to 3 months after harvest when dormancy is broken. The traditional methods which are employed in producing planting material have poor quality (Nweke *et al.*, 1991; Aighewi, 1998). Farmers have been saving seed tubers; select and plant their good seed tubers and sell any leftover which are usually of poor quality. Besides, their methods of production are normally typified by little multiplication ratios (Alvarez and Hahn, 1984) and through unequal and prolonged sprouting periods when planted (Otoo *et al.*, 1987). Mother yam is usually set from latent rooting or preserved plants and needs more time to produce efficient amounts of yam. A variety of techniques have been employed to produce commercial amounts of planting stock over a short period of time (Winkler, 1974). Convectional propagation is slow and only a limited number of plants can be grown from a stock plant due to the seasonal reaction and inability to rooting (Crassweller, 2008).

Double Harvesting or Milking

This is a system in which the identical plant of premature variety is harvested twice. The major harvest is made of tubers destined for food while the shoot of the mother plant is still green. This is usually done between 4 and 5 months after shoot emergence. The mound is unlocked from one side, the tuber separated from the base of the vine while keeping most roots intact and the mound is re-moulded. The plant initiates production of a new tuber from the base of the vine, which often bulks into an amorphous shape that can be detached and planted separately at harvest. The second harvest is done at the end of the season when the plant senesces (1–3 months after the first harvest) for regenerated tubers to be used solely as seed (Onwueme, 1973). Degras (1993) noted that while tubers from the first harvest were often infested with nematodes (*Scutellonemabradys* and *Pratylenchus coffeae*), those of the second harvest were either somewhat or not at all infected. This makes regenerated tubers better planting material than normal sets from single harvests, however, the means is labor intensive and only suitable for early maturing varieties. The farmer has to make a critical decision of when doing the first harvest so as to have significant tubers for the ware yam market with no compromising the amount of seed tubers to get the second harvest as the same photosynthetic apparatus produces both.

Cut sets

At planting time, yam sets can be gained by cutting the larger ware size tubers into tops, middles and bottoms in which each weighing 200 to 500 g. The sets are planted after cut surfaces are dry; generally following a day or two. Hence, only the top portion would have a sprout and it is favored since it establishes better and faster. However, this method accounts for harsh nematode damaged and decreased the growing zone of the tuber (Degras, 1993). After plantings shoots from the middle and bottom portions take longer to emerge as they are not planted through pre-formed sprouting points. Based on the variety and agro-ecology, tubers are milked or harvested from the cut sets after seven to 12 months. As a result sets that sprout earlier have a longer growth period since the crop is harvested after the dry season sets in and plants senesce.

2.6.2. Plant tissue culture and its application

Plant tissue culture can be defined as the culture of all types of plant cells, tissues and organs under aseptic condition to produce the clones of plants (Singh and Shetty, 2011). The controlled condition provides the culture an environment conducive for their growth and multiplication. These conditions include proper supply of nutrients, pH, adequate temperature and proper gaseous and liquid environment (Neumann *et al.*, 2009). Plant tissue culture is an integral part of molecular approaches to plant improvement and act as an intermediary whereby advances made by the molecular biologists in gene isolation and modification are transferred to plant cells (Singh and Shetty, 2011).

Plant tissue culture technology is being widely used for large scale plant multiplication. Small pieces of tissue (explants) can be used to produce hundreds and thousands of plants in continuous process. *In vitro* techniques were initially developed to demonstrate the totipotency of plant cell. Totipotency is the ability of plant cell to perform all the functions of development which are characteristic of zygote i.e. It is ability to develop into a complete plant. Plant tissue culture is a direct result of development of methods for the regeneration of plants from individual cells. This is of much importance today and has benefits: In a single experiment, regeneration enables hundreds of plants to be produced; It is possible to isolate virus free cells that can be used to produce virus free crops, which will give an increased yield per acre (Sobti and Panchauri, 2009).

Tiny replicas of single parent are produced by growing small part of plant explants aseptically on a nutritional medium in a test tube or in appropriate container. It happens in absence of microorganisms and presence of a balanced diet of chemicals. Plants multiplied by tissue culture generally have identical genetic make ups, they will, thus, prove to be true clones. Tissue culture techniques can be used for most plants, provided that right chemical solution and processes are used. Generally, via tissue culture procedures, diseases transmitted from parent to offspring can be eliminated. This also helps in saving of time and space. Plant tissue culture techniques are being used for the following purposes: To improve stress, salt, cold and chemical tolerance and to increase the nutrient contents in various crops; for production of virus, pesticide and herbicide resistant plants; to bypass the growing whole plants to get desired products (Sobti and Panchauri, 2009).

2.6.2.1 . Micropropagation and its Advantage

Micropropagation is the application of tissue culture technique to the propagation of plants starting with very small parts grown aseptically in test tube or other suitable containers (Sidhu, 2010). And allows the production of large number of plants from small pieces of the mother plant in relatively short period of time and limited space. It is an aseptic process which requires sophisticated laboratory procedures and special skills (Hartman *et al.*, 2004).

Micro propagation confers distinct advantages not possible with conventional propagation method. It is possible to multiply single explants into several thousands in less than a year. Actively dividing cultures are continuous sources of plantlet without seasonal interruption. It has high commercial potential due to the speed of propagation, clonal propagation, germplasm conservation, genetic transformation and its high quality and ability to produce disease-free plants (Tileye Feyissa *et al.*, 2005; Hartman *et al.*, 2004).

3. Materials and Methods

3.1. Study Area

The laboratory activities and experiments were conducted at National Agricultural Biotechnology Research Center (NABRC), Plant Tissue Culture Laboratory at Holeta in 2019.

3.2 .Plant Materials

The material we have used was the popular variety of yam (*Dioscorea alata*,) Bulcha which was released by Bako Agricultural Research enter in 2012. This variety showed superior performance, particularly in terms of productivity and tolerance of disease across the year and out yielded than other tested yam varieties on farmers fields. It yields 46.6 tones per hectare (Bikela *et al.*, 2015). Mature *Dioscorea* tubers were obtained from Hawasa Agricultural Research Center located in Southern Nations and Nationalities regional state. Each tuber was divided into fragments (Figure1) and cleaned under running tap water. Fragments were germinated in plastic pots filled with sterilized soil and mother plant was established in greenhouse at NABRC (Figure1) at average temperature of $25 \pm 2^{\circ}\text{C}$. The tuber set in pots were watered twice a week and allowed to grow for two months until bud sprout was produced. Two months later, the apex was differentiated into stem includes 10-16 nodes. The plants were staked with sticks and node cuttings were cut from stems for sterilization.



A



B



C

Figure1: Plant material source and mother stock establishment in Green house: (A), plastic pots filled with sterilized soil; (B), tubers and fragments of tuber; (C), Mother Stock plant under greenhouse condition.

3.3. Stock Solution and Media Preparation

3.3.1. Stock solution preparation

Murashige and Skoog (MS) basal medium was used throughout this research activity. Initially, full strength stock solutions of macronutrients, micronutrients and vitamins and other organic supplements were separately prepared (Appendix 4). To do so, appropriate amount of each nutrient was weighted in grams per liter and dissolved in double distilled water, consecutively in such a way that the next nutrient was added after the first one was completely dissolved. After all the components were completely dissolved using magnetic stirrer, the solution was poured into plastic bottles and stored at +4⁰C until used, for maximum of four weeks.

3.3.2. Plant growth regulators stock solution preparation

Plant growth regulators such as 6-Benzyl Amino-purine (BAP), Indol-Butyric Acid (IBA) and α -Naphthalene Acetic Acid (NAA) were used in this study. All of the plant growth regulators stock solution were prepared by weighing and dissolving the powder in double distilled water at a ratio of 1:1 (1mg/ml) and dissolved by 2-3 drops of NaOH based on the requirement of the plant growth regulators. Then, the volume was adjusted by adding double distilled water and stirred using magnetic stirrer. Upon complete dissolution, the solution of each PGR was poured into labeled 50 ml plastic bottles. Finally, growth regulator stock solutions were stored in a refrigerator at +4⁰C for short term use.

3.3.3. Culture medium preparation

MS nutrient medium with its full macro, micro, vitamins and iron source compositions was used to prepare stock solution. 50 ml of MS1 and 5 ml of MS2, MS3, MS4, MS5 and MS6 stocks were added into one liter beaker. Then, 30 g/l of sucrose and 1g/l activated charcoal were weighed and dissolved in double distilled water in all cases. The shoot initiation medium containing different concentrations of BAP (0.25, 0.5, 0.75 and 1mg/l) and different concentrations of NAA (0.5, 1 and 1.5 mg/l) in combination were used. The shoot multiplication medium prepared using different concentrations of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) was used. The rooting media containing IBA (0.5, 1.0, 1.5, and 2 mg/l) in combination with NAA (0.5, 1.0, 1.5, 2 and 2.5 mg/l) in full MS medium were prepared.

After added all the appropriate amount of sucrose, activated charcoal, plant growth regulators BAP, NAA and IBA at required concentrations, the mixture was stirred using a magnetic stirrer, and the volume was adjusted using double distilled water (DDW). The pH of the medium was adjusted with HCl and NaOH solution between 5.7 to 5.8. After adjusting the pH, 2.3g/l agar powder was added. Then gently mixed medium was boiled in microwave oven until the agar was melted. Then 30 ml of the prepared medium was dispensed into culture jars. The culture jars were covered with caps immediately after dispensing the medium and autoclaved at a temperature of 121⁰C and pressure of 105 KPa for 15 minutes. Immediately after autoclaving, the medium was taken and kept in culture room until used.

3.4. Experiments and Experimental Designs

All experiments were laid in a Completely Randomized Design (CRD) with factorial treatment combinations, having five replications per treatment and three explants per jar under each replicate. Before laying the multiplication and rooting experiments, sufficient explants were made to multiply till the desired numbers of explants are gained at all times. Explants were cultured on a PGR-free medium before their use for an experiment; to avoid any sort of carry-over effects from the previous culture medium they were retained. Controls were set for each experiment with zero concentration of growth regulators.

3.4.1. Explants Preparation and Sterilization

The nodal segments were used as a source of explants and collected from actively growing shoots from 1-2 months old established mother plant in the green house and transported to the laboratory. The nodal vine cuttings (6-10cm) were washed with detergent solution (liquid soap) and rinsed several times with running tap water. Then the nodal vine cuttings /explants were transferred to the laminar flow hood cabinet and surface sterilized in 70% ethanol for 3 minutes. Then the treated explants were rinsed repeatedly four times with sterilized distilled water and surface sterilized further using different percent (0.5%, 1%, and 1.5%) of NaOCl (sodium hypo-chlorite) disinfectant solutions plus a few drops of tween-20 (2-3 drops) for varying exposure times (5, 10 and 15 minutes), with continuous agitation. Finally, the nodal cut vines were transferred in to double distilled water and washed severally (at least 3 times) till all traces of the sterilant were off, and soaked with sterile blotting paper. Sterilized nodal explants were further reduced down 1cm size

by removing all the dead and affected tissue prior to culture. All steps were performed in a laminar flow cabinet to maintain aseptic condition, and three explants were cultured per glass jar with five replications.

3.4.2. Initiation and culture of explants in culture media

Aseptic nodal cultures were transferred on MS media fortified with different concentrations of BAP in combination with NAA for two weeks to determine optimum medium for shoot induction. The media used contained macro and micro salts according to Murashige and Skoog, sucrose and agar. In addition, NAA (0.5, 1, and 1.5mg/l) and BAP (0.25, 0.5, 0.75 and 1mg/l) were added to the MS medium with 1.0 g/l activated charcoal (AC). The sterilized explants which were dissected and reduced into 1cm single nodal segments were implanted on to the solid Murashige and Skoogs medium. It was then sealed, labeled and kept inside the growth room with 16 hours photoperiod (8 hours dark) and 2500 lux light intensity from cool white florescent 60 watt bulbs, at average temperature of $25 \pm 2^{\circ}\text{C}$. The growths of the nodal cultures were observed daily. The subculture was done on the 15th days of culture. Data on percentages of explants showing shoot initiation were collected after two weeks of culture.

3.4.3. Effect of different concentrations of BAP on shoot multiplications

After bud initiation, for further growth, cultured plants were transferred to the media supplemented with BAP. Five concentrations of BAP (0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) were used to investigate its effect on shoot multiplication. The mean of shoot length in cm, shoot number and leaf number were recorded after 30 days of culture.

3.4.4. NAA and IBA Combinations on *in vitro* Rooting

In this experiment, the effect of two auxin types and their concentration on rooting of *in vitro* shoots were studied. MS medium supplemented with five concentrations of NAA (0.5, 1, 1.5, 2 and 2.5 mg/l) and four concentration of IBA (0.5, 1, 1.5 and 2 mg/l) were tested with 1g/l AC. Mean of root number and root length were collected after 40 days of culture.

3.5. Acclimatization

For acclimatization, plantlets with well-developed root and leaf systems were washed with tap water to remove adhering media and agar attached on the roots of plantlets. Rooted shoots were transplanted to plastic pots filled with top soil, sand soil and compost in the ratio of 1:2:1. The plastic pots were placed in greenhouse where pot trays were covered with a white plastic bag to maintain humidity. The plantlets were transferred to greenhouse for gradual acclimatization and after a week the transparent plastic bags were removed. Finally, the data of survival percentage was recorded after four weeks of removal of plastic covering bags.

3.6. Data analysis

Statistical analysis of quantitative data were carried out by SAS software version 9.3. To detect the significance of differences among treatments below the probability level of 0.05, analysis of variance (ANOVA) was also made using SAS software. A difference at probability level of $p < 0.05$ was considered significant for analyses. Least Significance Difference (LSD) was used for mean separation at $p < 0.05$ probability level.

4. Result and discussion

4.1. Explants Preparation and Sterilization

The explants collected from open field or green house conditions infested with fungi and bacteria (Odutayo *et al.*, 2007). Therefore, establishing aseptic conditions in such circumstances are challenging (Cassells *et al.*, 2005). In this study, surface sterilization executed with sodium hypochlorite and ethanol is sufficient to disinfect *Dioscorea* plants from microbes effectively. The effect of different concentration of NaOCl at different exposure time on sterilization was stated in Table 1.

The percentages of contamination and survival rate in cultures were recorded. Minimum contamination and maximum survival response were found *in vitro* cultures that were surface sterilized with 1% of NaOCl for 15 minutes (the optimum concentration and time). The percentages of contamination and survival rate in cultures were recorded as, 0% and 100% respectively. After 7 days of culture, 0 % tissue death was recorded with unaffected growth rate. NaOCl is believed a very effective sterilizing agent and is used frequently for surface sterilization of plants for *in vitro* culture (Talei *et al.*, 2011). These results are confirmatory with earlier works (Oyebanji *et al.*, 2009) which have reported sodium hypochlorite as good sterilizing agents for many other plants. In addition, Ogero *et al.* (2012) reported that ethanol and NaOCl were good sterilizing agents for the plants like, peach, soft rush, oil palm, and others.

Table1: Interaction effect of Sodium hypochlorite concentrations and its time of exposure on sterilization of *Dioscorea species* (*Dioscorea alata*).

Sodium hypochlorite Concentration (%)	Time of exposure (minute)	Contamination (%)	Clean culture (%)	Tissue Survival rate (%)
0.5	5	100 ^a	0 ^h	0 ^h
0.5	10	93.33 ^b	6.67 ^g	13.33 ^g
0.5	15	86.66 ^c	13.34 ^f	26.66 ^f
1	5	80 ^d	20 ^e	53.33 ^c
1	10	60 ^e	40 ^d	86.66 ^b
1	15	0 ^h	100 ^a	100 ^a
1.5	5	13.33 ^f	86.67 ^c	46.66 ^d
1.5	10	6.66 ^g	93.34 ^b	33.33 ^e
1.5	15	0 ^h	100 ^a	0 ^h
CV		7.02	6.03	5.89

Note: Means with the same letter in the same column are not significantly different, CV=Coefficient of Variation.

When explants were sterilized for 15 minute with increased concentration of NaOCl (1.5 %), high efficiency of sterilization was achieved, but there were negative effects on survival rate, as well as on growth and development of explants. Sodium hypochlorite treatment for 10 minutes at 1% was also effective surface sterilizer with 86.66% survival rate and 60.00% of contamination (Table1). The data analysis revealed that the contamination percentage was dramatically decreased as the exposure time increased within the same level of sodium hypochlorite solution (Table1). However, disinfection with 1.5% sodium hypochlorite solution for 15 minute also resulted in least contamination (0%) and highest clean explants (100%) but no live tissues. Comparing the interaction effect posed by the chemical and the duration of disinfection against the aim of sterilization, disinfection of Bulcha variety with 1% sodium hypochlorite solution for 15 minutes was the most effective sterilization treatment (Appendix1). This resulted in the highest survival percentage (100%) without any contamination.

Alam *et al.* (2016) further reported that the requirements for sterilization are different and depend on the tissues and the type of the explants used for micropropagation. Many sterilization procedures based on sodium hypochlorite solution and 70% ethanol rinse are usually considered good sterilizing procedures for the *in vitro* culture of many plants (Aasim *et al.*, 2013). The present finding shows that 1% Sodium hypochlorite in combination with a few drops of Tween-20 for 15 minutes is the best of all sterilization procedures tested, with the lowest contamination and highest survival response of the variety.

The increase of sodium hypochlorite concentration with long exposure time showed a negative effect on plantlets, resulting in brownish color with a lower survival rate (0%). The occurrence of high contamination rate of culture at a relatively lower concentration and shorter exposure time treatment combinations was possibly due to the insufficiency of sterilant concentration and exposure duration to remove or kill the contaminant agents mainly fungi and bacteria. The high concentration of NaOCl and long exposure duration of explants in the sterilant solution resulted in better removal of microbes due to the concentration of which is negatively correlated with bactericidal activity, perhaps in part due to lethal DNA damage (Dukan *et al.*, 1999). The effect of the sterillant chemical could also alter the nature, the shape and function of microbial enzymes (George *et al.*, 2008). However, the increase in sterilant concentration and exposure time above certain optimum limit (1% and 15 minute) cause loss of explants because of the oxidant chemical ingredient killing the plant tissue as well.

4.2. Effect of different concentrations and combinations of BAP and NAA on shoot initiation

Aseptic nodal cultures were transferred to MS media fortified with different concentrations of BAP in combination with NAA for two weeks to determine optimum medium for shoot induction of *Dioscorea* spp of Bulcha variety. The analysis of variance obtained from the data (Table 2) showed that the interaction effect of BAP and NAA (BAP*NAA) on rate of shoot induction was found to be highly significant ($P < 0.0001$) (Appendix 2). The response of nodal explants cultured on different shoot initiation media is presented in Table 2. The result showed that cultured medium lack of growth regulators (control) failed to stimulate the bud break response in the cultured explants. Even when the cultures were maintained further than the normal observation period, 0.5 mg /l BAP combine with 1mg /l NAA result in the highest response in which an average of 100 % explant response with a mean shoot length of 4.42 ± 0.02 cm was obtained (Table 2, Figure 2). The second best shoot induction (86%) was obtained in the MS medium supplemented with 0.25 mg /l BAP combined with 1 mg /l NAA with a mean shoot length of 3.55 ± 0.04 cm (Table 2; Figure 2). From all treatments the minimum rate of shoot induction (20%) was observed on MS containing 1mg /l BAP combine with 1.5mg /l NAA where percent of shoot induction is significantly lower than that of the first two combinations of plant growth regulators.

In such cultures shoots were stunted with a mean shoot length of 0.9 ± 0.06 cm (Table 2; Figure 2). Shoot bud development capacity of nodal cultures increased with the increase in concentration of NAA and BAP from zero to 1 mg/l and 0.5mg/l respectively and reduced with further addition of both PGRS (Table 2). The present result of the shoot induction rate is compatible with the report of Fotso *et al*, (2013) who considered 0.5mg/l of BAP with 1.0 mg/l of NAA as the best-supplemented medium for nodal cuttings derived shoot regeneration. The dependence of cultured explants on bud break response has already been established (George and Sherrington, 1984). This has also been recently reported in the case of micropropagation of other *Dioscorea species* like *Dioscorea composita* (Alizadeh *et al.*, 1998), *Dioscorea floribunda* (Borthakur and Singh, 2002), *Dioscorea batatas* (Koda and Kikuta, 1991) and *Dioscorea abyssinica* (Martine and Cappadocia, 1991).

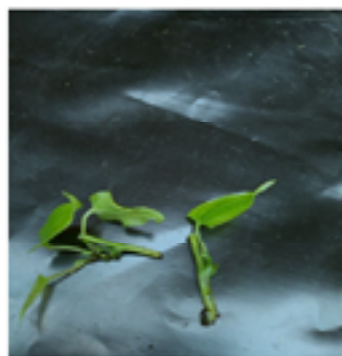
Table 2: Effects of BAP and NAA on *in vitro* shoot induction rate of *Dioscorea species* (*Dioscorea alata*), from nodal explants cultured on MS.

Plant growth regulators (mg/l)		Shoot induction (%)	Shoot length(cm) Mean±S.D
BAP (mg/l)	NAA (mg/l)		
0	0	0.00 ^l	0.00±0.34 ^j
0.25	0.5	26.66 ^j	0.97±0.01 ^{hi}
0.25	1	86 ^b	3.55±0.04 ^b
0.25	1.5	53 ^f	1.46±0.03 ^e
0.5	0.5	59.99 ^e	1.74±0.01 ^d
0.5	1	100 ^a	4.42±0.02 ^a
0.5	1.5	32.66 ⁱ	1.12±0.19 ^{gh}
0.75	0.5	46 ^g	1.36±0.40 ^{ef}
0.75	1	73 ^c	2.91±0.02 ^c
0.75	1.5	40 ^h	1.27±0.00 ^{fg}
1	0.5	33.33 ⁱ	1.15±0.02 ^g
1	1	66.66 ^d	1.84±0.03 ^d
1	1.5	20 ^k	0.9±0.06 ⁱ
CV		7.29	6.94

Note: BAP=Benzyl Amino Purine. NAA= α -Naphthalene Acetic Acid, Means with the same letter in the same column are not significantly different at $p < 0.005$, CV= Coefficient of Variation.



A



B



C

Figure 1: Response of Nodal explants of *Dioscorea alata* on initiation medium with different combinations of BAP and NAA. MS medium with 0.5 mg/l BAP and 1mg/l NAA (A), MS medium with 0.25mg/l BAP and 1 mg/l NAA (B), MS medium with 1mg/l BAP and 1.5 mg/l NAA (C).

In this work, cultured *Dioscorea spp* in solid MS medium, similarly *in vitro* propagation of other yam species were tried by Martine and Cappadocia (1992) using the solid medium. Nodal explants started to develop micro leaves after two week of culturing, though they were cultured on a medium with activated charcoal. Better growth responses of plant tissues have been associated with the addition of AC as it removes inhibitory substances from the media produced either on autoclaving (Weatherhea *et al.*, 1978) or by the tissue itself (Fridborg *et al.*, 1978). The effects of auxins and cytokinins on shoot induction of various plants are reported by many workers (Ahuja *et al.*, 1982 , Alizadeh *et al.*, 1998).

From this study, it was observed that after two weeks of growth on the initiation medium, cultured nodal segments gave varying responses to all of the culture media compositions except the control (without plant growth regulators) (Table 2). George *et al* (2008) reported that, for shoot initiation cytokinins do not act alone unless combined with auxins. Such an effect was observed in the present study, the combination of 0.5mg/l BAP and 1mg/l NAA produced very high response of shoot bud induction. Moreover, the addition of 1g/l activated charcoal and dark treatment is highly important for the adsorption and reduction of phenolic compounds released from the cultured explants which otherwise inhibit growth. As indicated by Fridborg *et al*, (1978), dark treatment of initiated shoots reduces the release of phenolic compounds from the explants. Moreover, transfer of the cultured shoots to new fresh medium is important to reduce the risk of death of explants.

4.3. Effect of different concentrations of BAP on shoot multiplications

Effects of different concentrations of BAP are shown in Table 3. Induction of shoot in all treatments cultured on MS media supplemented with different concentration of BAP in present study was similar with the report of Mahesh *et al*,(2010). Results indicated that MS media supplemented with BAP (1.5mg/l) induced maximum number of shoot per explant (7.28 ± 0.07) with highest shoot length of 8.72 ± 0.43 cm and 7.12 ± 0.22 number of leaves (Table 3;Figure 3).

Table 1: Shoot multiplication in nodal explants of *Dioscorea species* (*Dioscorea alata*) cultured on semisolid MS medium supplemented with various concentration of BAP.

BAP (mg/l)	Number of leaves Mean $\pm$ S.D	Number of Shoot Mean $\pm$ S.D	Shoot Height (cm) Mean $\pm$ S.D
0	2.09 ± 0.09^f	3.05 ± 0.37^e	2.53 ± 0.14^e
0.5	3.38 ± 0.18^d	4.54 ± 0.28^c	4.06 ± 0.26^c
1	5.01 ± 0.35^b	6.3 ± 0.45^b	6.12 ± 0.52^b
1.5	7.12 ± 0.22^a	7.28 ± 0.07^a	8.72 ± 0.43^a
2	4.40 ± 0.19^c	3.64 ± 0.25^d	3.44 ± 0.44^d
2.5	2.51 ± 0.35^e	2.12 ± 0.08^f	1.97 ± 0.12^f
CV	5.90	6.65	7.80

Note: BAP=Benzyl Amino Purine. Means with the same letter in the same column are not significantly different, CV= Coefficient of Variation.

In addition, present result was matched with the finding of Poornima and Ravishankar, (2007); Fay, (1992) and Chu *et al.*, (2002). It was observed that for shoot proliferation cytokinin required optimal quantity and the appearance of stems on the different control medium concludes where BAP appears to be the best growth regulator adapted to axillary propagation and the importance of cytokines for the development of plant aerial organs. Zrýd (1988) reported effect of cytokinins on the aerial parts of plants *in vitro*. This result confirms the reports of Saleil *et al.*, (1990) and Mantell and Hugo (1989) that the nature and the amount of the cytokinin had determined significantly, variations of growth on various genotypes of *Dioscorea* spp. But, the report of Manoharan *et al.*, (2016) is contradictory with this result because they investigated that MS media with 0.4 mg/l BAP was the optimum concentration to shoot proliferation. The contradiction might be occurred due to the difference in type of media and variety. Increasing 0.5 to 1.00 mg/l BAP showed a significant increase from 4.54 ± 0.2 to 6.3 ± 0.45 shoots/ explant, from 4.06 ± 0.26 to 6.12 ± 0.52 cm shoot length and from 3.38 ± 0.18 to 5.01 ± 0.35 leaves / shoot (Table 3). MS media with 1mg/l BAP gave the second highest shoot number (6.3 ± 0.45) with shoot length of 6.12 ± 0.52 cm and highest leaf number 5.01 ± 0.35 (Table3; Figure 3).

This finding is harmonious with the report of Susmita and Shukla (2014) , Borges *et al* (2004) and Belarmino and Gonzales (2008). Therefore, MS media with 1.5 mg/l BAP could be taken as the optimum concentration for this variety, because it provides the maximum value for the shoot number, shoot length, and leaf number. On the other hand, minimum shoot number (2.12 ± 0.08) with minimum shoot length (1.97 ± 0.12 cm) on MS medium with 2.5 mg/l BAP, and minimum leaf number (2.51 ± 0.35) were recorded on MS media with 2.5 mg/l BAP (maximum concentration) (Table3; Figure 3). Increasing BAP above 1.5 mg/l resulted in shoots with reduced number of shoots with stunted features, which are generally unusable due to high dosage of hormone that disorders the metabolism of the shoot, inhibits cell division and multiplication. Therefore, according to Gopitha *et al* ,(2010) low concentration of cytokinin promotes shoot multiplication and elongation. Finding of Ammirato (1982) and Ezeibekwe *et al.* (2009) reported that BAP greater than 2.00 mg/l resulted best in shoot proliferation. The finding showed that increasing leaf number, number and length of shoot with increase of BAP until 1.5mg/l . And either increasing or decreasing the BAP concentration from 1.5mg/l leads to decline for the growth of leaf number, shoot height and shoot number Particularly, BAP with 1.5 mg/l was the favorable concentration of cytokinin for shoot proliferation for this yam variety.



A



B



C

Figure 2: Effect of BAP on shoot multiplication, MS medium with 1.5mg/l BAP (A), MS medium with 1 mg/l BAP (B), MS medium with 2.5 mg/l BAP (C).

4.4. Effect of NAA and IBA Combinations on *in vitro* Rooting

Well-developed elongated shoots were excised from the shoot clump and transferred to MS medium containing NAA and IBA. The rooting responses of shoots on different media showed the difference, in mean number of roots and mean root length over a period of four weeks (Figure 4; Table 4). Roots were not spontaneously induced during culture initiation and shoot multiplication. The analysis of variance revealed that root number and root length varied significantly with MS medium supplemented with NAA and IBA. The interaction effects of NAA and IBA were highly significant ($p < 0.0001$) for number of root/shoot and average root length (Appendix 3). In the present study combination of NAA and IBA were used and best rooting was observed with 2 mg /l NAA with 1.5 mg/l IBA. Transferring the cultured shoots on rooting medium to dark was better for root initiation. George (1996) also reported that use of activated charcoal in rooting medium enhances rooting.

According to Kumar and Nair (1979), the production of plantlets with abundant rooting *in vitro* is very important for the successful establishment of regenerated plantlets in soil. NAA, IBA, and IAA are the main auxin hormones and rooting occurs in the presence of these hormones. Some authors also support the present finding and reported that the combination of auxin NAA and IBA were needed for better production of roots in *Dioscorea prazeri* (Thankappan and Patell, 2011), *Dioscorea nipponica* (Chen *et al.*, 2007), *Dioscorea esculenta* (Belarmino and Rosario, 1991) and Obsi *et al.* (2015). But Bahar *et al* (2010), suggested that NAA or IBA alone used to profuse rooting on *Dioscorea oppositifolia* and report that NAA more effective than IBA. Rooting was better in the culture which had a combination of 2mg/l NAA with 1.5 mg/l IBA about 10.6 ± 0.44 number of roots were responded with an average number of 13.80 ± 0.44 cm root length per plantlets (Figure 4). Relatively this result is in agreement with the result of Behera *et al.* (2008) who noted the same result on different species of *Dioscorea* on higher concentration 2mg/l NAA and 2 mg/l IBA in half-strength MS basal medium induced highest rooting .



A



B



C



D

Figure 4: *In vitro* rooting of *Dioscorea alata* shoots on full strength MS medium containing combination of NAA and IBA. Root induction on 2mg/l NAA with 1.5 mg/l IBA(A), Root induction on 2 mg/l NAA with 1 mg/l IBA (B), Root induction on 0.5 mg/l NAA with 0.5 mg/l IBA (C), Root induction on hormone free media (D).

Table 4: Influence of different combination of NAA and IBA on rooting of *in vitro* generated shoot lets of *Dioscorea species* (*Dioscorea alata*).

NAA (mg/l)	IBA (mg/l)	Root Height(cm) (Mean±S.D)	Root Number (Mean±S.D)
0	0	1.00±0.01 ^o	1.00±0.29 ^p
0.5	0.5	1.04±0.21 ^o	1.4±0.31 ^{op}
0.5	1	2.70±0.68 ^{km}	3.24±0.43 ^m
0.5	1.5	3.56±0.38 ^l	4±0.22 ^{kl}
0.5	2	3.22±0.40 ^{lm}	3.64±0.03 ^{lm}
1	0.5	4.90±0.54 ^{ij}	4.6±0.06 ^j
1	1	5.38±0.05 ^{hi}	4.76±0.06 ^{ij}
1	1.5	7.49±0.34 ^e	6.94±0.22 ^d
1	2	6.50±0.18 ^f	5.52±1.7 ^{gh}
1.5	0.5	6.95±0.03 ^f	6.7±0.22 ^{de}
1.5	1	8.07±0.74 ^d	7.68±0.23 ^c
1.5	1.5	9.11±0.11 ^c	8.19±0.54 ^{cb}
1.5	2	6.95±0.02 ^f	6.2±0.23 ^{ef}
2	0.5	6.50±0.15 ^f	5.94±0.04 ^{fg}
2	1	11.50±0.21 ^b	8.67±1.13 ^b
2	1.5	13.80±0.44 ^a	10.6±0.44 ^a
2	2	5.94±0.04 ^g	5.26±0.32 ^{hi}
2.5	0.5	4.50±0.41 ^j	4.26±0.18 ^{jk}
2.5	1	3.04±0.69 ^{lm}	3.39±0.23 ^m
2.5	1.5	1.94±0.30 ⁿ	2.6±0.35 ⁿ
2.5	2	1.46±0.10 ⁿ	1.6±0.54 ^o
CV		9.19	7.60

Note: IBA=Indol-3-Butyric Acid, NAA=α -Naphthalene Acetic Acid. Means with the same letter in the same column are not significantly different, CV= Coefficient of Variation.

The second-highest percentage of rooting was at 1mg/l of IBA and 2mg/l of NAA about 8.67 ± 1.13 number of roots/shoot responded with 11.50 ± 0.21 cm average root length per plantlet (Figure 4). In addition, rooting at lower level of NAA and IBA (0.5 mg/l) treatments showed not well elongated and minimum root number with minimum root length totally with poor root growth and with dwarf growth of shoots (Figure 4), in the cultured shoots during the four weeks of observation period. Produced 1.4 ± 0.31 number of roots/shoot with 1.04 ± 0.21 cm average root length per plantlet (Table 4 ;Figure 4). However, higher concentration of NAA and IBA at all concentration tested responded well (Figure 4) and required only short days for root initiation.

Very small amount of root with minimum length in case of shoot planted on auxin free medium Produced average of 1.00 ± 0.01 number of roots responded with 1.00 ± 0.029 cm average root length (Figure 4). These might be due to the ability of yam variety to propagate through vegetative means and although it is possible that there was endogenous auxin concentration in the explanted organ. From NAA at 2.5mg/l combination with 2mg/l of IBA, 1.6 ± 0.54 number of roots/shoot responded with 1.46 ± 0.10 cm average root length per plantlet (Table 4). By increasing the concentration of IBA and NAA from optimum concentration (1.5 and 2 mg/l respectively), the percentage of rooted shoots decreased continuously.

This result Contradicted the conclusion of Forsyth and Stadan (1981), concerning their report of rooting relies on MS media with 5mg/l IBA. Low concentration of IBA and NAA promotes root induction and elongation than the extremely higher concentration that inhibited rooting. The findings of this experiment is similar to a report of Mao *et al*, (1995), who reported that higher concentration of NAA, IBA or IAA induces root rather than the lower concentration of IAA, NAA and IBA. Additionally, these results could be explained on the bases that auxin-induced number of responses which involved cell division, cell enlargement, protein and nucleic acids synthesis. These are naturally accompanying of auxin-induced growth and changes in plant cell and increase the apical dominance as there are essential and rapid processes involved in growth and elongation (Wilkins, 1989).

4.5. Acclimatization

Among transferred newly formed plantlets, satisfactory shoot and roots were successfully acclimatized and cultivated in greenhouse (Figure 5). About 90% of plantlets were survived and established as healthy plant. And successfully established from *in vitro* experiments of Bulcha variety. The present finding of 90% survival rate is similar with finding of Behera *et al.*, (2008); Behera *et al.* (2009) and Behera *et al.* (2010) who reported 90% survival rate of different *Dioscorea* species in *ex vitro* establishment. Obsi *et al.* (2015) also reported that 86% of survival rate of the plantlets within 3-4 months after acclimatization and the young plant grow vigorously in greenhouse. It is clear that leaving rooted plantlets on the rooting medium for longer period of time (optimum period) increased the efficiency of roots which led to the increase of the survival percentages of acclimatized plantlets.



Figure 5: Acclimatization in greenhouse:(A) plastic pots filled with sterilized soil ,(B) first week of acclimatization (as covered),(C) 15 days after covering plastic bag has been removed,(D) 30 days after covering plastic bag has been removed.

5. CONCLUSIONS

Sterilization of selected explants is a key step for removing any sorts of microbial contaminants in order to attain successful *in vitro* establishment of plant tissue culture. From different concentration of sterilant used for sterilizing yam nodal explants, one percent sodium hypochlorite with 15 minute exposure time was optimum for nodal explants of *D. alata*. Optimum sterilant concentration and exposure time is crucial for micropropagation of *D. alata*. The maximum percentage of shoot induction (100%) with $4.42 \pm 0.02\%$ shoot length was observed on MS medium supplemented with 0.5mg/l of BAP and 1mg/l of NAA for nodal explants. MS medium containing 1.5mg/l of BAP also produced $7.28 \pm 0.07\%$ shoot number with good morphological appearance. Best rooting percentage was achieved on full strength MS basal media containing 1.5 mg/l of IBA in combination with 2.0 mg/l of NAA which resulted in mean values of 13.80 ± 0.44 cm root length with 10.6 ± 0.44 root number, followed by MS basal media containing 2.0 mg/l of NAA and 1mg/l IBA which resulted in 8.67 ± 0.13 root number, and 11.50 ± 0.21 cm root length.

Those plantlets well performed *in vitro* showing 90% survival efficiency after acclimatization on soil mixture by ratio of 1:2:1 top soil, sand soil and compost, respectively. This protocol provided large-scale production of *D. alata* cultivar, genetic improvement and high disease free yield of the crop using nodal explants sources. The *in vitro* propagation protocols developed in the present study thus can be effectively utilized for commercial cultivation and domestication of this valuable species as well as for further biotechnological and molecular research as the species has quick growing capacity. Results of this study indicated that large scale propagation of Bulcha variety by tissue culture methods is economically feasible and several plantlets can be regenerated from single nodal explants.

6. RECOMMENDATIONS

Based on the results of this study, the following recommendations were forwarded:

- Explants other than nodal segments culture for *in vitro* propagation of Bulcha variety should be considered to investigate if they can offer better and efficient responses.
- As this protocol was tested on a single cultivar, further studies using other genotypes is required so as to have a reliable protocol for *in vitro* propagation of yam.
- Further studies on the performance of the tested cytokinins in combination with other type of hormone (cytokinins and auxins) should be carried out for better result on shoot multiplication.
- The effect of different concentrations of auxins other than NAA and IBA on induction of roots should be studied further.
- It is also recommended to practice other techniques like culturing of explants in liquid medium for mass propagation of Bulcha variety.
- The effect of other phenolic compound adsorbing chemicals like polyvinylpyrrolidone (PVP) on initiation potential of the explants should be investigated in combination with activated charcoal.
- Use of the already developed micro propagation protocol for large scale production of the Bulcha varieties and distributing to farmers to increase yam tuber production in Ethiopia should be given due attention.
- The current study focused on micropropagation of Bulcha variety, so that it can serve as alternative source in solving shortage of the planting materials and its contribution towards ensuring food security in the country.

7. REFERENCES

- Aasim, M., Khawar, K. M. and Ozcan, S. (2013). *Production of herbicide resistant* Addis Ababa, Ethiopia.
- Afolabi, A.S and Ogbadu, G.H. (2009). Simple, effective and economical explant-surface sterilization protocol for cowpea, rice and sorghum seeds. *African Journal of Agricultural Research*, **8**:5395-5399.
- Aighewi, B.A. (1998). Seed yam (*Dioscorea rotundata* Poir.) production and quality in selected yam zones of Nigeria. Ph.D. thesis, University of Ibadan, Ibadan, Nigeria. Pp 252.
- Akueshi, C.O., Kadir, C.O., Akueshi, E.U., Agina, S.E. and Ngurukwem, C. (2002). Antimicrobial potential of *H. sauveoleus* Poit (lamiacea). *Nigerian Journal of Botany*, **15**:37-41.
- Albrecht, M. A. and McCarthy, B. C. (2006). Seed germination and dormancy in the medicinal woodland herbs *Collinsonia canadensis* L. (Lamiaceae) and *Dioscorea villosa* L. (Dioscoreaceae). *Flora*, **201**: 24-31.
- Alexander, J., and Coursey, D. G. (1969). "The origins of yam cultivation," in *The Domestication and Exploitation of Plants and Animals. Proceedings of a Meeting of the Research Seminar in Archaeology and Related Subjects Held at the Institute of Archaeology, London University*, eds P. J. Ucko and G. H. Dimbleby (Gerald: Duckworth & Co. Ltd.), 405–425.
- Alvarez, M. N., & Hahn, S. K. (1984) Seed yam production. In E. R. Terry, E. V. Doku, O. B.
- Alizadeh, S., Mantell, S. H. and Viana, A. M., (1998). *In vitro* culture and microtuber induction in the steroidal yam *Dioscorea* composite Hemsl. *Plant Cell Tissue Organ Cult*, **53**:107-112.
- Ammirato, P.V., 1982. Growth and morphogenesis in cultures of the monocot yam, *Dioscorea*. In *Plant tissue culture proceedings, 5th International Congress of Plant Tissue and Cell Culture held at Tokyo and Lake Yamanake, Japan, July 11-16, 1982/edited by Akio Fujiwara*. Tokyo: Japanese Association for *Plant Tissue Culture*.
- Aprianita A, Purwandari U, Watson B, Vasiljevic T (2009). Physicochemical properties of flours and starches from selected commercial tubers available in Australia. *International Food Research J.* **16**: 507-52.
- Arditti, J. (2009). History of orchid propagation: a mirror of the history of biotechnology. *Plant Biotechnology Reports*, **3**: 1–56.

- Arnau, G., Abraham, K., Sheela, M. N., Chair, H., Sartie, A., and Asiedu, R. (2010). “Yams” in *Root and Tuber Crops, Handbook of Plant Breeding*, ed J. E. Bradshaw (Singapore: Springer Science Business Media, LLC), 127–148.
- Asha, K. I., and Nair, G. M.(2005). Screening of *Dioscorea* species for diosgenin from southern Ghats of India. *Indian J. Plant Genet.Resour*, **18**: 227–230.
- Asiedu, R. and Sartie, A.2010. Crops that feed the world 1.Yams.*Food Security*, **2(4)**: pp.305-315.
- Asiedu, R., Wanyera, N. M., Nag, S. Y. C., and Nag, N. Q. (1997). *Yams, CGIAR Centres*. Cambridge: Cambridge University Press.
- Atiri, G. I., Winter, S. and Alabi, O. J. 2003. Yam. In: Virus and virus-like diseases of Major Crops in Developing Countries. Leobenstein, G. and Thottappilly, G. eds. Kluwer, Acad Press. Dordrecht, the Netherlands. Pp: 249-267.
- Ayensu, E. S., and Coursey, D. G. (1972). Guinea yams: the botany, ethnobotany, use and possible future of yams in West Africa. *Econ. Bot*,**26**: 301–318.
- Balogun, M.O., &Gueye, B.(2013).Status and prospects of biotechnology applications to conservation, propagation and genetic improvement of yam. In K.G. Ramawat, & J-M Merillon (Eds.), *Bulbous Plants: Biotechnology* pp: 92–112.
- Barna KS, Wakhlu AK .(1988). Axillary shoot induction and Plant regeneration in *Plantagoovata*Forsak. *Plant Cell Tissue Organ.Cult*, **15**: 167-169.
- Behera KK, Sahoo S, Prusti A .(2009).Regeneration of plantlet of water yam (*Dioscorea oppositifolia*L.) through *in vitro* culture of nodal segments. *Not. Bot. Hort. Agrobot. Cluj*, **37**:94-102.
- Behera, K. K., S. Sahoo and A. B. Prusti.(2008).Effect of plant growth regulator on *invitro* micropropagation of Bitter Yam (*Dioscorea hispida*Dennst.). *Inter National Journal of Integrative Biology*, **4(1)**:50-54.
- Behera, K.K., Sahoo, S.S. and Prusti, A. 2010. Micropropagation of greater yam (*Dioscoreaalata* L. cv. Hatikhujia) through vine nodes.*J. Root Crops*, **36(1)**: 27-32.
- Belarmino, M. and A. G. Rosario del .(1991). Callus induction and Organogenesis in *Dioscorea species*. *Japan. J. Breed*,**41**:561-569.
- Benmahioul, B., Dorion, N., Kaid-Harche, M. and Daguin, F. 2012.Micropropagation and *ex vitro* rooting of pistachio (*Pistaciavera* L.).*Plant Cell, Tissue and Organ Culture (PCTOC)*, **108(2)**:353-358.

- Bhandari, M. R., and Kawabata, J. (2005). Bitterness and toxicity in wild Yam (*Dioscorea* spp.) tubers of Nepal plant foods. *Hum. Nutr*, **60**: 129–135.
- Bikila, A, Abraham, N, Girma, Ch., Tilahun, W., Negash, T., Lelise, A., Daniel, M., and Meseret, N. (2015). Registration of Bulcha and Lalo Yam Varieties, *East African Journal of Sciences*, Vol, **9 (1)**: 65-68.
- Borthakur, M. and R. S. Singh. (2002). Direct Plantlet Regeneration from Male Inflorescences of Medicinal Yam (*Dioscorea floribunda* Mart. & Gal.). *In Vitro Cell. Dev. Biol. Plant*, **38**: 183-185.
- Borges, M., Ceiro, W., Meneses, S., Aguilera, N., Vázquez, J., Infante, Z. and Fonseca, M. 2004. Regeneration and multiplication of *Dioscorea alata* germplasm maintained *in vitro*. *Plant Cell, Tissue and Organ Culture*, **76(1)**: 87-90.
- Brand-Miller, J., Burani, J. and Foster-Powell, K. (2003). The New Glucose Revolution - Pocket Guide to the Top 100 Low GI Foods. Marlowe and Company. Pp. 158.
- Burkill, I. H. (1960). The organography and the evolution of *Dioscoreaceae*, the family of the yams. *J. Linn. Soc. (Botany)* **56**: 319–412.
- Cassells, A.C. and Doyle, B.M. (2005). Pathogen and biological contamination Management.
- Caven, M. N., and Dvornik, D. (1979). Effect of diosgenin on lipid metabolism in rats. *J. Lipid Res.* **20**: 162–174.
- Chu, E.P. and Ribeiro, R.D.C.L.F. 2002. Growth and carbohydrate changes in shoot cultures of *Plant Cell, Tissue and Organ Culture*, **70(3)**: 241-249.
- Coursey DG, Yams. *Dioscorea* spp. (*Dioscoreaceae*) In: Simmonds ED (ed) Evolution of crop plants. Longman. London, **1976**: 70-74.
- Crabbe P. (1979). Some aspects of steroid research based on natural product from plant origin. *Bull. Soc. Chim. Belg*, **88**: 5-7. Eka OU. (1998). Roots and Tuber crops, in nutritional quality of plant foods. Osagie A, Eka OA (Eds) Postharvest res. Unit Publ. Univ. Benin. pp 1-31.
- Crassweller, R. 2008. Fruit Production for the Home Gardener: A Comprehensive Guide.
- Craufurd PO, Summerfield RJ, Asiedu R, Prasad V. (2001). Dormancy in Yams. *Exp. Agric*, **37**: 75-109.
- Degras, L. (1993). In C. René (Ed.), The yam: A tropical crop. London: Macmillan Press Ltd. 409.
- Dukan S., Belkin S., Touati D. 1999. Reactive oxygen species are partially involved in the bactericidal action of hypochlorous acid. *Arch. Biochem. Biophys*, **367**: 311–316.

- EdossaEtissa.(1996). Root and tuber crops potential as food crops in the humid areas of Ethiopia.*Newsletter of Agricultural Research* **11**:9-11.
- EdossaEtissa.(1998). Yams: exploration, collection and evaluation.
- Eka, O. U. (1985). The Chemical Composition of Yam Tubers, In: *Advances in Yam Research*, pp. 61-83.
- Eka, O.U. (1998). Roots and Tuber Crops, in nutritional quality of plant foods. Post Harvest Resources. Unit Publication University of Benin pp 31.
- Elisabeth, A., H. (2003). Motives and opportunities for domestication: an ethnoarchaeological study in southwest Ethiopia. *Journal of Anthropological Archaeology* **22** (2003) :358–375.
- Ezeibekwe, I.O., Ezenwaka, C.L., Mbagwu, F.N. and Unamba, C.I.N. 2009. Effects of combination of different levels of Auxin (NAA) and Cytokinin on in vitro propagation of *Dioscorea rotundata* L. (White Yam).*Journal of Molecular Genetics*, **1(2-4)**: 18-22.
- F.A.O. report .(1998). FAO Annual Report.Food and Agriculture Organisation Production Year Book. FAO Rome.
- FAO .(2012). Food and Agricultural Organization of the United Nations Production Year.
- FAO (Food & Agriculture Organization of the United Nations). (1998). *The State of the World's Plan Genetic Resources for Food and Agriculture, Annual Report*. Rome: FAO.
- FAO.(2011). Ethiopia, yam production quantity for the year of 2008. Available online: <http://www.factfish.com/statistic> (Accessed: October, 2013).
- FAOSTAT.(2013).Quoted in 2010-2013 Factfish, Germany.
- Fay, M.F.1992. Conservation of rare and endangered plants using in vitro methods.*In Vitro,Cellula &Developmental Biology-Plant*, **28(1)**: 1-4.
- Forsyth, C. and Van Staden, J.(1981). An Improved Method of *in vitro* Propagation of *Dioscorea bulbifera*.*Plant Cell, Tissue and Organ Culture*, **1(1)**: 275-281.
- Fridborg, G., M. Pedersen, C. E. L and Strom and J. Eriksson .(1978). The effect of activated charcoal on tissue culture, adsorption of metabolites inhibiting morphogenesis. *Physiol.Plant*.**43**:104-106.
- George, E. F., Hall, M. A. and De Clerk, G. J. 2008 .Plant propagation by Tissue culture.
- Gopitha, K., Bhavani, L. and Senthilmanickam, J.2010. Effect of the different auxins and cytokinins in callus induction shoot root regeneration in sugarcane.*Int.J.Pharma.Biot.Sci*.**1(3)**:1-34.

- Hahn, S. K. (1995). "Yams, *Dioscorea* spp. (*Dioscoreaceae*)," in *Evolution of Crops Plants*, eds J.Smartt and N. W. Simmonds (London: Longman Group Limited), 112–120.
- Hahn, S. K., Osiru, D. S. O., Akoroda, M. O., and Otoo, J. A. (1987). Yam production and its future prospects. *Outlook Agric*, **16**: 105–110.
- Hartmann, H. T., Kaster, D. E., Davies, F. T. and Geneve, R. L. (2004). Plant Propagation: Principles and Practices. 6th ed. Prentice Hall of India Private Limited, New Delhi, India, pp. 770.
- Hildebrand E, Demissew S, Wilkin P .(2002). Local and regional disappearance in species of *Dioscorea* L. (Yams) in southwest Ethiopia. In: Stepp JR, Wynd- ham FS, Zarger RR (eds) *Ethnobiology and biocultural diversity. Proceedings of the 7th international congress of ethnobiology*. University of Georgia Press, USA, pp 678–695.
- IBC, (2008). Ethiopia; Second Country Report on the State of PGRFA to FAO. International Institute for Tropical Agriculture (IITA).(2009).
- Izekor, O. B. and Olumese, M.I.(2010).Determinants of yam production and profitability in Edo State, Nigeria.*Afr. J. General Agric*, **6**: 205-210.
- Jaleel, C.A., Gopi, R., Manivannan, P., Kishorekumar, A., Gomathinayagam, M. and Vam, R.P., 2007.Changes in biochemical constituents and induction of early sprouting by triadimefon treatment in white yam (*Dioscorea rotundata* Poir.) tubers during storage.*Journal of Zhejiang University Science B*, **8**(4):pp.283-288.
- Johnson, D. (2003). Yams for food; *Dioscorea* spp. Paper presented at the 2002 Spring.
- Jova MC, Kosky RG, Cuellar EE .(2011). Effect of liquid media culture systemson yam plant growth (*Dioscorea alata* L.'PacalaDuclos').*Biotechnol.Agron. Soc. Environ*,**15**:515-521.
- Kadiri, M., Ojewumi, A. W., Aniola, T. O., and Musa, A. H. (2014).Ethno-botanical survey of plants commonly used for ceremonial activities among Yoruba tribe of south West Nigeria.*Direct Res. J. Health Pharmacol*, **2**:1–5.
- Kay, D. (1987). Root Crops Tropical Development and Research Institute. London. Lape, I.M. and Treche, S. (1994). Nutritional quality of yam (*Dioscorea dumetorum* and *D. rotundata*) flours for growing rats. *J. Sci. Food Agric*, **66**(4): 447-455.
- Kochlar S.L. (1981). Tropical Crops, a Text Book of Economic Botany. Mcmillan Int. 469 pp
- Koda Y, Kikuta Y .(1991). Possible involvement of jasmonic acid in tuberization of yam plants. *Plant Cell Physiol*,**32**:629-633.

- Kohmura H, Araki H, Imoto M. (1995). Micropropagation of ‘yamatoimo’ Chinese yam (*Dioscorea opposita* Thun.) from immature leaves. *Plant Cell Tissue Organ Cult*, **40**:271-276.
- Kole, C., 2011. Wild crop relatives: Genomic and breeding resources: Cereals (1). Springer Science and Business Media.
- Kumar, S., Jena, P. K., and Tripathy, P. K. (2012). Study of wild edible plants among tribal groups of Similipal Biosphere Reserve forest, Odisha, India; with special reference to *Dioscorea* species. *Int. J. Biol. Technol*, **3**:11–19.
- Kumar CRM, Nair, GM. (1979). Notes on the effects of planting material and levels of farmyard manure on the yield of *D. alata*. *Journal of root crop*, **5**:67-68.
- Langl and, K. A., and Burks, C. K. (1998). *Identification and Biology of Non-native Plants in Florida's Natural Areas*. Gainesville, FL: University of Florida.
- Lebot, V., Trilles, B., Noyer, J. L., and Modesto, J. (1998). Genetic relationships between *Dioscorea alata* L. cultivars. *Genet. Resour. Crop Evol*, **45**:499–509.
- Lebot, V. 2009. Tropical root and tuber crops Cassava, sweet potato, yams and aroids. *Crop Production Science in Horticulture Series*, 17, MPG books group.
- Lev, L. S., and Shriver, A. L. (1998). “A trend analysis of yam production, area, yield, and trade. (1961-1996).” in *Ligname, Plante Seculaire et Culture Davenir. Actes du Seminaire International CIRAD-INRA-ORSTOM-CORAF* (Montpellier: CIRAD), 8–10.
- Liu, Y., Lin, Y., Liu, D., Han, C., Chen, C. and Fan, M. (2009). Effects of different types of Yam (*Dioscorea alata*) products on blood pressure of spontaneously hypertensive rats. *Bioscience Biotechnology and Biochemistry*, **73**: 1371 - 1376.
- Lubag AJM, Laurena AC, Tecson-Mendoza EM. Antioxidants of purple and white greater yam (*Dioscorea alata* L.) varieties: identification of anthocyanidin alatanin with high antioxidant potential. *Philipp. J. Sci.* 2008. **137**:61–67.
- Mahesh R, Muthuchelian K, Maridass, Raju G. (2010). *In vitro* propagation of wild yam, *Dioscorea wightii* through nodal cultures. *Int. J. Biol. Technol*, **1**:111-113.
- Maina S.M., Emongor Q., Sharma K.K., Gichuki S.T., Gathaara M., de Villiers S.M. 2010. Surface sterilant effect on the regeneration efficiency from cotyledon explants of groundnut (*Arachis hypogea* L.) varieties adapted to eastern and Southern Africa. *African J. Biotechnol*, **9**: 2866–2871.

- Maneenoon, K., Sirirugsa, P., and Sridith, K. (2008). Ethnobotany of *Dioscorea* L. (*Dioscoreaceae*), a major food plant of the Sakai tribe at Banthad Range, Peninsular, Thailand. *Ethnobot. Res. Appl.*, **6**:385–394.
- Mantell SH, Hugo SA .(1989). Effects of photoperiod, mineral medium strength, inorganic ammonium, sucrose and cytokinin on root, shoot and microtuber development in shoot cultures of *Dioscorea alata* L. and *D. bulbifera* L. yams. Plant cell, tissue and organ culture. pp. 23-37.
- Mantell SH, Hugo SA (1989). Effects of photoperiod, mineral medium strength, inorganic ammonium, sucrose and cytokinin on root, shoot and microtuber development in shoot cultures of *Dioscorea alata* L. and *D. bulbifera* L. Yams. Plant Cell Tissue Organ Cult. **16**: 23-37.
- Manoharan, R., Tripathi, J.N. and Tripathi, L., 2016. Plant regeneration from axillary bud derived callus in white yam (*Dioscorea rotundata*). *Plant Cell, Tissue and Organ Culture (PCTOC)*, **126(3)**: 481-497.
- Mao AH, Wetten A, Fay M, Caligari PDS .(1995). *In vitro* propagation of *Clerodendrum colebrookianum* Walp, a potential natural antihypertension medicinal plant. Plant Cell Rep, **14**: 493-496.
- Martine J, Cappadocia M. (1991) .*In vitro* tuberization in *Dioscorea alata* L. ‘Brazo fuerte’ and ‘Florido’ and *D. abyssinica* Hoch. Plant Cell Tissue Organ Cult, **26**:147-152.
- Martine J, Cappadocia M. (1991). *In vitro* tuberization in *Dioscorea alata* L. ‘Brazo fuerte’ and ‘Florido’ and *D. abyssinica* Hoch. Plant Cell Tissue Organ Cult, **26**:147-152.
- Martin, F. W. (1969). The species of *Dioscorea* containing sapogenin. *Econom. Bot.*, **23**:373–379.
- Miche L., Balandreau J. 2001. Effects of rice seed surface sterilization with hypochlorite on inoculated *Burkholderia vietnamiensis*. *Appl. Environ. Microbio.*, **67**: 3046–3052.
- Miège, J., and Sebsebe Demissew .(1997). *Dioscoreaceae*. In: *Flora of Ethiopia and Eritrea* (Edwards, S., Sebsebe Demissew and Hedberg, I., eds.). A joint publication of the National Herbarium, Biology Department, AAU, Ethiopia and the Department of Systematic Botany, Uppsala University, Sweden.
- Mignouna, Djana, Akinola, A., Suleman, I., Nweke, F. (2014). Yam: A cash crop in West Africa, S., Acholo, M. N., McNamara, N. and Oliver, R. (2000). Control of storage insects as a means of limiting yam tuber fungal rots. *J. Stored Prod. Res.*, **36**: 37-45.
- Murashige, T and Skoog, F .(1962). A revised medium for rapid growth and bioassays with

- tobacco tissue cultures. *Physiologia plantarum* **15**: 473-479. Musa, Y. H., Onu, J., Vosanka, I. P. and Anonguku, I. (2011). Production efficiency of yam in Zing Local Government area of Taraba State, Nigeria. *J. Hort. Forest*, **3**: 372-378.
- Nakagawara S., Goto T., Nara M., Ozawa Y., Hotta K., Arata, Y. 1998. Spectroscopic characterization and the pH dependence of bactericidal activity of the aqueous chlorine solution. *Anal. Sci*, **14**: 691–698.
- Nashriyah, M., Athiqah, M. Y. N., Amin, H. S., Norhayati, N., Azhar, A. W. M., and Khairil, M. (2011). Ethnobotany and distribution of wild edible tubers in Pulau Redang and nearby islands of Tereengganu, Malaysia. *Int. J. Biol. Vert. Agric. Food Eng*, **5**: 110–113.
- Nayaboga, E., Tripathi, J. N., Manoharan, R., and Tripathi, L. (2014). Agrobacterium-mediated genetic transformation of yam (*Dioscorea rotundata*): an important tool for functional study of genes and crop improvement. *Front. Plant Sci*, **5**: 463.
- Neumann, K.H., Kumar, A. and Iman, J. (2009). *Plant Cell and Tissue Culture: A Tool in Biotechnology*. University of Rajasthan Press, Jaipur, pp. 4-6.
- Nweke, F.I., Ugwu, B.O., Asadu, C.L.A. & Ay, P. (1991). Production cost in the yam based cropping systems of southeastern Nigeria. RCPM Research Monograph No. 6. International Institute of Tropical Agriculture, Ibadan, Nigeria. 29pp.
- Obsi, D., Bantte, K. and Diro, M., 2015. Effect of Different Combinations of Plant Growth Regulators on *in vitro* Propagation of Yam (*Dioscorea Species*). *Journal of Applied Biotechnology*, **3**(2), p.20.
- Odior, A. O. and Oyawale, F. A. (2012). Analysis of production capacity of a yam flour producing firm using a mathematical model. *J. Eng. Appl. Sci*, **7**: 820-824.
- Odu, B.O., Hughes, J.D.A., Shoyinka, S.A., Dongo, L.N. (1999). Isolation, characterisation and identification of a potyvirus from *Dioscorea alata* (water yam) in Nigeria. *Annals of Applied Biology* **139**: 65 - 71.
- Odutayo, O.I., Amusa, N.A., Okutade, O.O. and Ogunsanwo, Y.R. (2007). Sources of diversity. Pudoc, Wageningen, The Netherlands. Pp. 261.
- Ogero, O., Mburugu, G., Mwangi, M., Ngugi, M. and Ombori, O. (2012). Low Cost Tissue Culture Technology in the Regeneration of Sweet Potato (*Ipomoea batatas* L). *Res. J. Biol*, **2**(2): 51-58.

- Oke, O. L. (1990). Roots, Tubers, Plantain and Bananas in Human Nutrition, FAO Food and Nutrition Series No. 24.
- Okwu, D.E. (2004). Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *Journal of Sustainable African Environment*, **6**:30-34.
- Okwu, D.E. and Ndu, C.U. (2006). Evaluation of the Phytonutrients, Mineral and Vitamin Contents of some Varieties of Yam (*Dioscorea* spp). *International Journal of Molecular and Advanced Sciences* **2(2)**:199-203.
- Olaoye, J. O. and Oyewole, S. N. (2012). Optimization of some "poundo" yam production parameters. *Agric. Eng. Int. CIGR J*, **14**: Manuscript No. 2063.
- Onwueme, I. C. (1973). The tropical tuber crop: Yams, cassava, sweet potato, cocoyam. English language book society and John. Chichester: Wiley & Sons.
- Onwueme, I. C. (1978). *The Tropical Root Crops: Yams, Cassava, Sweet Potato and Cocoyams*. Chichester: John Wiley and Sons Ltd.
- Orkwor, G. C., Asiedu, R., and Ekanayaka, I. J. (1998). *Food Yams: Advances in Research*. Ibadan: International Institute of Tropical Agriculture/NRCRI.
- Otoo, J. A., Osiru, D. S. O., Ng, S. Y., & Hahn, S. K. (1987). Improved technology for seed yam production. Ibadan: International Institute of Tropical Agriculture. 56 p.
- Oyebanji, O.B., (2009) Nweke, O., Odebunmi, O., Galadima, N.B., Idris, M.S., Nnodi, U.N., *Plymouth Student Scientist*, **4**: 432-449.
- Poornima, G.N. and Ravishankar, R.V., 2007. *In vitro* propagation of wild yams, *Dioscorea oppositifolia* (Linn) and *Dioscorea pentaphylla* (Linn). *African Journal of Biotechnology*, **6**(20).
- Price, E.J., Wilkin, P., Sarasan, V. and Fraser, P.D. (2016). Metabolite profiling of *Dioscorea* (yam) species reveals under-utilized biodiversity and renewable sources for high-value compounds. *Scientific reports* **6**:29136, DOI: 10.1038/srep29136.rica. YIIFSWA Working Paper No. 3. IITA, Ibadan.
- Pursglove J.W. (1989). Tropical Crops Monocotyledons. Longmans London. 607pp.
- Robinson, J., 2006. The Oxford Companion to yam. Oxford University Press. Third Edition pp:183-184.
- Saleil V, Degras L, Jonard R. (1990). Obtention des plantes indemnes du virus de la mosaïque de l'igname (YMV) par culture *in vitro* des apex chez l'igname américaine *Dioscorea trifida* L. *Elsevier/INRA*, **10**: 605-615.

- Sangoyomi, T.E.(2004). *Post harvest fungal deterioration of yam (Dioscorea spp) and its control*.(Unpublished) Ph.D. Thesis University of Ibadan, Nigeria. 179pp
- Sengupta J, Mitra GC, Sharma AK (1984) Organogenesis and tuberization in cultures of *Dioscorea floribunda*. *Plant Cell Tissue Organ Cult*,**3**:325-331.
- Sharma, L. N., and Bastakoti, R. (2009). Ethnobotany of *Dioscorea* L. with emphasis on food value in Chepang communities in Dhading District, central Nepal.*Botanica Orientalis. J. Plant Sci*,**6**:12–17.
- Sharma, L. N., and Bastakoti, R. (2009).Ethnobotany of *Dioscorea* L. with emphasis on food value in Chepang communities in Dhading District, central Nepal.*Botanica Orientalis. J. Plant Sci*,**6**:12–17.
- Shehu, J. F., Iyortyer, J. T., Mshelia, S. I. and Jongur, A. A. U. (2010). Determinants of yam production and technical efficiency among yam farmers in Benue State, Nigeria.*J. Soc. Sci*,**24**: 143-148.
- Sidhu, Y. (2010). In *vitro* micro propagation of medicinal plants by tissue culture.
- Singh, G. and Shetty, S. (2011). Impact of tissue culture on agriculture in India. *Biotechnol. Bioinf. Bioeng*, **3**: 279-288.
- Sinha, R., and Lakra, V. (2005). Wild tribal food plants of Orissa. *Indian J.Tradit. Know*,**4**:246–252.
- Sobti, R.C. and Panchauri, S.S. (2009). *Essentials of Biotechnology*. DryaGanj Press.
- Susmita, S. and Shukla, S.K. 2014.*In vitro* regeneration of *Dioscorea hispida* through nodalexplants-A rich source of starch.*GSTF International Journal on Bioformatics & Biotechnology(JBio)*, **3**(1), p.34.
- Talei, D., Saad, M.S., Yusop, M.K., Mihdzar, A.K. and Valdiani, A. (2011). Effect of different surface sterilizers on seed germination and con-tamination of King of Bitters (*Andrographis paniculata* Nees.) *American Eurasian Journal of Agriculture and Environmental Sciences*, **10**: 639-643.
- Terauchi, R., Terachi, T., and Tsunewaki, K. (1991). Intraspecific variation of chloroplast DNA in *Dioscorea bulbifera* L. *Theor. Appl. Genet*,**81**: 461–470.
- Tileye Feyissa, Welander, M. and Legesse Negash .(2005). Micropropagation of *Hagenia abyssinica*: a multipurpose tree. *Plant Cell Tiss. Org*, **80**:119-127.
- Tschannen AB, Escher F, Stamp P. (2005). Post-harvest treatment of seed tubers with gibberellic acid and field performance of yam (*Dioscorea cayenensis - rotundata*) in Ivory Coast. *Expl. Agric*, **41**:175-186.

- Twyford CT, Mantell SH .(1996). Production of somatic embryos and plantlets from root cells of Greater Yam. *Plant Cell Tissue Organ Cult*,**46**:17-26.
- Umeta, M., West, C. E. and Fufa, H. (2005).Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia..*J. Food Composition and Analysis*, **18**: 803-817.
- Vaillant, V., Bade, P. and Constant, C., 2005. Photoperiod affects the growth and development of yam plantlets obtained by *in vitro* propagation. *Biologiaplantarum*, **49(3)**:355-359.
- Weatherhead MA, Burdon L, Henshaw GG. (1978). Some effects of activated charcoal as an additive to plant tissue culture media. *Z. Pflanzenphysiol*,**89**: 147-149.
- Walsh, S. (2003). *Plant Based Nutrition and Health*. Vegan Society Ltd. pp 240.
- Wang, S., Gao, W., Chen, H. and Xiao, P.2006. Studies on the morphological, thermal and crystalli properties of starches separated from medicinal plants. *Journal of food engineering*, **76(3)**:420-426.
- Westphal E. (1975). *Agricultural systems in Ethiopia*.Center for Agriculture Publishing and Documentation, Wageningen, the Netherlands, p 278.
- Wilkins, M.B., 1989. *Advanced Plant Physiology*. The Bath Press, Avon, pp: 13-15.
- Winkler, A.J., Cook, J.A., Kliwer, W.M. and Lider, L.A. 1974. Berkeley, CA: University of California Press.
- Yam, T. W., &Arditti, J. (2009). History of orchid propagation: a mirror of the history of biotechnology. *Plant Biotechnology Reports*, **3**:1–56.
- Yan H, Yang L, Li Yangrui. (2011). Improved growth and quality of *Dioscorea fordii*Prainet Burk and *Dioscorea alata*plantlets using temporary immersion system. *Afr. J. Biotechnol*, **10**: 19444-19448.
- Zeven, A.C. and De Wet, J.M.J. (1982). *Dictionary of cultivated plants and their regions*.
- ZrýdJP.(1988).Culturesdescellules,tissusetorganesvégétaux.Fondementsthéoriquesetutilisationsprati ques. Presses.
- Zuluaga, M., Baena, Y., Mora, C. and D'Leo'n, L. 2007. Physicochemical characterization and application of yam (*Dioscoreacayenensis-rotundata*) starch as a pharmaceutical excipient. *Starch* **59**:307–317.

8. APPENDICES

Appendix 1: Analysis of variance for effect of different concentration of NaOCl for different exposure time on percent of explants showing contamination, clean culture and tissue survival.

Source	DF	Contamination	Clean culture	Tissue Survival
Naocl	2	28222.13***	28222.11***	18666.66***
Time	2	4962.99***	4963.14***	518.48***
Naocl*time	4	2074.08***	2074.03***	3073.59***
CV		7.02	6.03	5.89

Appendix 2: Analysis of variance for effect of different concentration of BAP in combination with NAA on percent of explants showing shoot initiation and shoot height.

Source	DF	Shoot height	Percent of explants response
BAP	3	3.26***	1699.39***
NAA	2	25.03***	10081.94***
BAP*NAA	6	1.73***	1394.09***
CV		6.94	7.29

Appendix 3 : ANOVA for number and length of roots in different concentrations of IBA and NAA in combination.

Source	DF	Root Height	Root Number
IBA	4	48.62***	30.50***
NAA	3	243.22***	104.37***
IBA*NAA	12	14.61***	12.54***
CV		9.19	7.60

Appendix 1: Murashige and Skoog MS(1962) media components

Code	Nutrients	Stock solution(gm)		Volume of stock for 1Lfull MS media
MS1	Ammonium nitrate(NH ₄ NO ₃)	33.0	In 1000 ml	50 ml
	Potassium nitrate(KNO ₃)	38		
MS2	Magnesium sulphate(MgSO ₄ .7H ₂ O)	18.07	In 500 ml	5 ml
	Manganese sulphate(MnSO ₄ .H ₂ O)	1.69		
	Zinc Sulphate(ZnSO ₄ .7H ₂ O)	0.86		
	Copper Sulphate(CuSO ₄ .5H ₂ O)	0.0025		
MS3	Calsium Chloride(CaCl ₂ .2H ₂ O)	33.22	In 500 ml	5 ml
	Potassium Iodide(KI)	0.083		
	Cobalt Chloride(CoCl.6H ₂ O)	0.0025		
MS4	Potassium dibasic phosphate(KH ₂ PO ₄)	17	In 500 ml	5 ml
	Boric acid(H ₃ BO ₃)	0.62		
	Sodium molbdate(Na ₂ MoO ₄ .2H ₂ O)	0.025		
MS5	NaEDTA	3.726	In 500 ml	5 ml
	Iron sulphate(FeSO ₄ .7H ₂ O)	2.78		
MS6	Myo-inositol	10.0	In 500 ml	5 ml
	Glycine	0.2		
	ThiamineHCl	0.01		
	Pyridoxine HCl	0.05		
	Nicotine acid	0.05		