IN VITRO PROPAGATION OF TWO IRISH POTATO (*Solanum tuberosum* L.) VARITIES THROUGH LATERAL BUD CULTURE

M.Sc. THESIS

MEKONNEN WOCHORE DOGISO

November, 2016

HARAMAYA UNIVERSITY

In Vitro Propagation of two Irish Potato (*Solanum tuberosum* L.) Varieties through Lateral Bud Culture

A Thesis submitted to the Department of Biology,
Post Graduate Program Directorate
HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN BIOTECHNOLOGY

By
Mekonnen Wochore Dogiso

November, 2016
Haramaya University, Haramaya

HARAMAYA UNIVERSITY
POST GRADUATE PROGRAM DIRECTORATE

As research advisors, we herby certify that we have read and evaluated this thesis prepared under our guidance by Mekonnen Wochore entitled: "*In Vitro* Propagation of two Irish Potatoes (*Solanum tuberosum* L.) Varieties through Lateral bud culture" and we recommend that it be submitted as fulfilling the thesis requirement.

Meseret Chimdessa (Ph.D)	_____	_____
Major Advisor	Signature	Date
Manikandan Muthuswamy (Ph.D)	_____	_____
Co-Advisor	Signature	Date

As member of Examining Board of the Final M.Sc. Open Defense, we certify that we have read and evaluated the thesis prepared by: Mekonnen Wochore, and recommended that the thesis be accepted as fulfilling the requirement for the degree of: Master of Science in Biotechnology.

Misrak_Kebede_ (Ph.D)_	_____	_____
Chairman	Signature	Date
Yohans_Petros_ (Ph.D)__	_____	_____
Internal Examiner	Signature	Date
Kifle_Dagne__(Ph.D)___	_____	_____
External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy to the Council of graduate Studies (CGS) through the Graduate Council of the Department of Biology.

DEDICATION

I dedicate this thesis to my beloved father Ato Wochore Dogiso and my mother W/o Mame Abeche who always nurse and support me in my academic endeavors.

STATEMENT OF THE AUTHOR

By my signature below, First I declare that this thesis entitled **'*In vitro* propagation of two Irish Potato (*Solanum tuberosum* L.) varieties through lateral bud culture'** is my own work and that all sources of materials used for this thesis have been duly acknowledged. Second, I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree from Post Graduate Program Directorate at Haramay University and is reserved in the University Library to be made available to borrowers under the rules of the Library.

Brief quotations from this thesis are allowed without special permission provided that accurate and complete acknowledgement of the source is made. Requests for permission for extended quotations from, or reproduction of, this thesis in whole or in part may be granted by the Head of Biology Department or the Dean of the Post Graduate Program Directorate when in his or her judgment the proposed use of the material is for a scholarly interest. In all other instances, however, permission must be obtained from the Author.

Name of candidate: Mekonnen Wochore Dogiso Signature: _____

Place: Haramaya University, Haramaya

Date of submission: November, 2016

BIOGRAPHICAL SKETCH OF THE AUTHOR

The author was born on September 5, 1972 from his father Ato. Wochore Dogiso and his mother W/o Mame Abeche at Durgi Kebele, Temebaro Wereda, Southern Nations Nationalities and peoples Reginal state, Ethiopia. He attended his elementary and junior secondary classes (0-8) at Durgi Primary School from September 1977 – Jun 1987 E.C. After successfully passing his grade eight exam he joined Hawassa Senior and Comprehensive Secondary School (Tabor) and attended his high school education from September 1988-Jun1991 E.C. Then studied at Hawassa College of Teacher Education in year Sept 1992- Jun 1994 E.C and obtained diploma in Biology,in July 1995 E.C. After graduation, he was employed as a Biology teacher at Arbaminch Zone Zala woreda Education Bureau. He joined Arbaminch University in Sept 2000, and graduated with a B.Ed degree in Biology. In sept 2006, he joined the Post Graduate Program Directorate of Haramaya University to study for a Master of Science Degree in Biotechnology.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my special gratitude and heartfelt thanks to the Almighty God for blessing my efforts, giving me the strength, determination and discipline to complete my study.

I would like to extend my sincere appreciation to my major advisor, Dr. Meseret Chimdessa, for his encouragement, constructive suggestions and moral support from the very beginning in executing the research work and in the write-up of the thesis. I take this opportunity to extend my special gratitude and indebtedness to my co-advisor, Dr. Manikandan Muthuswamy, for his inspiration, through full guidance and suggestions which to the successful completion of this research work. My sincere gratitude also goes to all laboratory staff of Areka Agricultural Research Institute, the Biotechnology Laboratory staff in particular, Mr. Genene Gezahegn, who shared his research experience with me, and, Mrs. Tigist Markos, Mrs. Bethelehem Natnael, Miss, Zinash Yilma, Miss, Aynalem Waza, and Miss, Almaz Fekadu for their cooperation and assistance during the laboratory work and for giving me the constructive idea during my laboratory work. who shared their research experience with me and I also wish to express my special thanks to my friend Mr. Dejene Zenabu who have directly or indirectly helped and shared information with me in finishing my thesis. Last but not least, I acknowledge the Department of Biology of Haramaya university for providing me with all of the required support.

ABBREVIATIONS AND ACRONYMS

AARC	Areka Agricultural Research Center
ANOVA	Analysis of Variance
BA /BAP	Benzyl Adenine /6-benzylaminopurine
CBD	Complete Block Design
CRD	Completely Randomized Design
CSA	Central Statistical Agency
DDW	Double Distilled Water
EIAR	Ethiopian Institute of Agricultural Research
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization Statistics
IAA	Indole-3-acetic acid
IBA	Indol-3-Butyric acid
KN	Kinetin
LSD	Least Significance Difference
M.a.s.l	Meter above Sea Level
MS	Murashige and Skoog
NAA	α-naphthalene Acetic Acid
NaOcl	Sodium hypochlorite
PGR	Plant Growth Regulators
PPFD	Photosynthesis photon flux density
Psi	Pounds per Square inch
RMTs	Rapid Multiplication Techniques
SAS	Statistical Analysis system
SNNPRS	Southern Nations Nationalities and Peoples Regional State
TC	Tissue culture
V/V	Volume by Volume
W/V	Weight by Volume

TABLE OF CONTENTS

TABLE OF CONTETS [CONTINUED]

TABLE OF CONTETS [CONTINUED]

LIST OF TABLES

Page

LIST OF TABLES IN THE APPENDIX

LIST OF FIGURES IN THE APPENDIX

IN VITRO PROPAGATION OF TWO IRISH POTATOES (*Solanum tuberosum* L.) VARITIES THROUGH LATERAL BUD CULTURE

ABSTRACT

Irish Potato (Solanum tuberosum L.) is one of the world's most economically important tuber crops belonging to the family Solanaceae. It is a versatile vegetable that is eaten all around the year. The objective of this study was to establish a protocol for in vitro micropropagation of Gudene and Belete Irish potato varieties from lateral bud explants. For shoot induction lateral buds of Gudene and Belete were cultured on MS basal medium that contained 30 g/l sucrose, 6 g/l agar, vitamins and supplemented with BAP in 1, 2, 3, 4 mg/l concentrations combined with different levels (0.5, 1, 1.5, 2 mg/l) of IAA. Shoot multiplication was done by culturing micro-shoots on full strength MS basal medium supplemented with BAP (0.5, 1, 1.5, 2 mg/l) and KN (0.5, 1, 1.5, 2 mg/l) in sole or combination. Rooting of the multiplied shoots was done on full strength MS basal medium supplemented with different concentrations of IBA (0.25, 0.5 0.75, 1 mg/l) and NAA (0.25, 0.5 0.75, 1 mg/l) in sole or combination. In all cases, MS basal medium without PGRs was used as control. The rooted plantlets were then acclimatized under greenhouse conditions by transplanting the plantlets on moist red soil, sand soil and compost or their mixture in 2:1:1 ratio. Results showed that shoot initiation, shoot multiplication and root formation responses were significantly ($P<0.05$) higher under all levels and combinations of hormones when compared with control treatments. There was significant difference between PGRs treatments in all measured parameters with 91.67% and 87.5% survived proliferating cultures seen for Gudenie and Belete varieties, respectively on shoot initiation MS basal medium supplemented with combination of 2.0 mg/l BAP and 1.0 mg/l IAA. Compared to the control, both BAP and KN alone or in combination showed significant positive effect on shoot multiplication of both varieties of Irish potato at all concentration levels. For Gudenie variety, 0.5mg/l BAP and 2mg/l KN combination had higher response than at other treatments. Number of days to shoot emergence was also found to be shorter at this level of hormonal combination than other treatments. In the case of Belete variety, 2mg/l KN alone treatment had similar effects as that of 0.5mg/l BAP and 2mg/l KN. Compared to the control, both IBA and NAA alone or in combination showed significant positive effect on root formation of shoots of both varieties. In both varieties, agronomic traits had significantly higher values at 1mg/l IBA to 0.25mg/l NAA combination treatment than other treatments. Number of days to root emergence was also found to be shorter at this level of hormonal combination than other treatments. The acclimatization experiment showed that plantlets of both varieties survived better on the sterilized mixture of moist red soil, sand and compost soil in a ratio of 2:1:1. Comparison of the performance of both varieties showed that Gudenie showed superior performance compared to Belete variety with respect to all measured agronomic traits, suggesting that varietal difference for the difference in the response to the PGRs.

Key words: *auxin, cytokinin, growth regulators, In vitro propagation, MS medium, Solanum tuberosum .*

1. INTRODUCTION

Irish potato *(Solanum tuberosum L.)* is one of the world's most economically important tuber crops belonging to the family Solanaceae. It is a versatile vegetable that is eaten all around the year. It is considered to be the fourth major food crop of the world following rice, wheat and maize (Mustafa *et al*., 2002). It contains about 79% water, 18% starch, 2% protein, 1% vitamins, minerals and many trace elements (Ahmad *et al*., 2011), though it is best known for its carbohydrate contents. The annual diet of an average global citizen in the first decade of the 21st century included about 33kg of potato (FAO, 2008). Moreover, it has use as an industrial raw material apart from its daily consumption by humans (Hoque, 2010).

Irish Potato is one of the most important and widely grown food crops in the world. The cultivated forms originated from a narrow genetic base, but 160 wild species are recognized and the global gene pool is relatively unexploited (Hawkes, 1978). Worldwide, it is grown in 180 countries. According to FAO data potato is highly produced in Asia followed by Europe, South America and North and Central America. Europe's largest producers of potato are Ukraine, Poland, Belarus, Germany, Romania, the Netherlands and France (FAOSTAT, 2012).

In Ethiopia, potato can significantly contribute to food security improvement by increasing food availability and cash income of smallholder farmers (Hirpa *et al*., 2010). It is also described that potato is regarded as a high potential food security crop because of its ability to provide a high yield of high quality product per unit input with a shorter cropping cycle (mostly <120 days) than major cereal crops like maize. The Ethiopian average yield is approximately 10.5 t ha^{-1}, which is very lower than the world's average yield of 17 t ha^{-1} (Muthoni *et al*., 2011).

Currently, potato production is expanding from highland areas to mid and lowland parts of the country due to its potential for production in a short period of time, high yield per unit area, source of food and cash crops to large number of food-insecure smallholder farmers and pastoralists in the country. Nowadays, in Ethiopia the main production season for potato represents only 22% (34,000 ha), while the off-season production is around 128,000 ha in northern, southern and central Ethiopia (Haverkort *et al*., 2012).

In Ethiopia, potato production could fill the gap in food supply during the hungry months of July to August before the grain crops are harvested. Currently, in Ethiopia, potato is grown on around 164,146 hectares, producing an estimated total tuber yield of 940,087 tons. This implies that average yield in the country reaches only 7 t/ha when the potential for small holder is around 25 t/ha (CSA, 2002). There are many factors that have been identified as the causes for this low yield in Ethiopia and most of the East African countries, but the lack of high quality seed tuber seems to explain most for the reduction of yield. Moreover, unavailability of seed tubers; lack of well adapted cultivars; the prevalence of diseases and insect pests, and drought, frost at high altitude and extreme temperature at very low altitudes are some of the problems that contribute to the reduced yield of potato (Tsoka *et al*., 2012).

Plant growth regulators are to be expected for *in vitro* regeneration of crop plants in any artificial nutrient medium under controlled conditions. Generally, cytokinin helps in shoot proliferation and auxins help in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety and culture conditions. Genetic factors are considered to be a major contributor to the *in vitro* response of cultured tissues.

In vitro regeneration and micropropagation methods are now widely used for mass multiplication of plant material, germplasem conservation and for the improvement of crops through biotechnological methods. *In vitro* multiplication of Irish potato is a more advanced technology over traditional method of Irish potato production with respect to optimal yield, uniformity, disease free planting material and true to type plants. Mass multiplication of tissue culture plants could also be done in a short time. The development of an optimal micro propagation protocol would have a major significance in the propagation of good agronomic traits of Irish potato clones for its distribution to farmers (Rabbani et al., 2001).

So far, In Ethiopia very limited efforts have been made for *in vitro* micropropagation of Irish potato. In order to meet the objectives of mass propagation within short period of time, produce disease free seed tubers etc. Efficient micropropagation protocol needs to be developed. Few researchers have reported their research on Irish potato tissue culture. For example, Hussain *et al*. (2005) investigated micropropagation of potato from lateral bud explants on MS basal medium with plant growth regulators (auxins and cytokinin). Molla *et al*. (2011) also studied the

effect of growth regulators Indole-3-Acetic Acid (IAA), Indole-3-Butyric Acid (IBA), α-Naphthalene acetic Acid (NAA), 6-Benzylaminopurine (BAP), Kinetin (KN) on direct regeneration of potato.

Moreover, Kishor and Devir (2009) reported that exogenous application of different cytokinins, viz., BAP and Kinetin,has become essential for induction of multiple shoots in many plants. Accordingly Vinterhalter *et al*. (1997) studied induction of roots in *in vitro* regenerated potato using full strength MS medium and the result showed that the number of roots produced per shoot varied with type of auxins used and their concentrations. In all of these investigations, however, only partial success was obtained. So far, Irish potatoes have usually been propagated mainly from apical meristem. There is limited work on the *in vitro* propagation of the two potato varieties (Belete and Gudene) from lateral meristem in Ethiopia. Therefore, this study attempted to develop micropropagation protocol for these potato varieties with the following objectives.

General objective

- To develop optimal *in vitro* propagation protocol for Belete and Gudene Irish potato (*Solanum Tuberosum* L.) varieties from lateral bud explants

Specific objectives

- To determine the optimal concentration of plant growth regulators [auxin and cytokinin] for Belete and Gudene Irish potato varieties shoot for initiation and multiplication
- To determine the optimal concentrations of plant growth regulators [auxin] for Belete and Gudene Irish potato varieties root induction
- To evaluate the survival rate of in vitro regenerated potato plantlets in the green house

2. LITERATURE REVIEW

2.1. Ecology and Morphological Description of Irish Potato

The Irish potato plant (*Solanum tuberosum* L.) can grow to be a large bushy plant up to 1 meter high. Potato plants are medium sized (up to 8 cm long) green leaves, smallish white flowers and tubers than grow underground. Potato plants are annuals and are dicotyledons. Even though they are annuals their tubers can sprout and grow new plants. Irish potato stems have their vascular bundles in a circular pattern and have layers of xylem and phloem of dicotyledonous plant, which belongs to the family Solanaceae. It is widely grown throughout the tropics and temperate regions of the world between latitude 40^{o} North and South of the equator and between sea level and 2300 m.a.s.l (Jana, 1982). The altitude between 1,800 to 2,500m is suitable for seed and tubers potato growing and 70% of the Ethiopian agricultural land is located at this altitude (FAO, 2008).

2.2. Origin, Taxonomy and Distribution

The Irish potato originated from South America, most likely from the central Andes in Peru. The potato was domesticated and has been grown by indigenous farming communities for over 4,000 years. Introduced into Europe in the sixteenth century, the crop subsequently was distributed throughout the world, including Asia. Irish potato belongs to one of the largest and most diverse families with about 90 genera and more than 3000 species, Knapp *et al.* (2004).

Potato belongs to the genus *Solanum,* which has around 1,500 species the largest genus within the family Solanaceae, and the genus also includes tomato (*solanum. lycopersicum*), eggplant (*solanum. melongena*), tobacco (*Nicotiana tabacum*), pepper (*Capsicum annuum*) and potato (*solanum. tuberosum*) and woody nightshade (*S. dulcamara)* (Knapp, 2008).
Solanum tuberosum is divided into two subspecies: *tuberosum* and *andigena*. The subspecies *tuberosum,* adapted to lower altitudes and longer day length, is the cultivated potato widely used in, for example, North America and Europe. The subspecies *andigena,* which is restricted to

Central and South America, prefers cultivation at high altitudes and short days for tuberization (Roa, 2010; Gopal *et al.,* 2013).

2.3. Conventional Propagation System of Irish Potato

Potato is mainly propagated via seed tubers (Mih and Atiri, 2006). Food and Agriculture Organization of the United Nations, 2008), This method is sometimes called conventional propagation and it ensures uniformity of the crop in terms of growth and yield, but results in degeneration of the crop due to virus infection, the rate of degeneration varying from place to place and from cropping season to cropping season (Kagona, 2008).

Conventional techniques of seed potato production involve the use of potatoes that are propagated by harvesting and replanting the tubers in the field. The tubers used for planting are known as "seed potatoes"(Badoni and Chauhan, 2010). However, this method of seed production has proved to be labour intensive, prone to pest and disease infestation and time consuming. Considering that vegetatively propagated crops especially potatoes are prone to both viral and bacterial diseases, the conventional production of seed potatoes favors disease build-up, which drastically reduces crop yield (El-Komy *et al.,* 2010).

If the mother potato plant becomes infected with a disease during the growing season, each of the new daughter tubers is likely to be infected as well. One of the setbacks in vegetatively propagated plants is disease infestation due to pathogen attack (Naik and Karihaloo, 2007). Plants produced through vegetative propagation are infested with bacteria, fungi, nematodes, or other pathogens such as viruses and viroids whose symptoms are hard to trace but have a significant effect on yield reduction and decrease in quality over generations (Thiart, 2004).

2.4. Production States and Importance of Irish Potato

Irish potato is the fourth most important food crop in the world after rice, wheat and maize (FAO, 2008; Nyende *et al.,* 2005). Potato is grown in more than 100 countries in the world. It is very adaptable and is cultivated both in temperate, subtropical and tropical conditions. In the

year 1991-2007, the potato production levels in the developed nations of Europe, North America and the former Soviet Union have declined from 18,313 to 15,989 million tones. In contrast, the production increased from 8,486 to 16,541 million tonnes in countries belonging to Asia, Africa and Latin America during the same period (FAO, 2008). The top three world leaders in potato production in 2011 included China (88.4 million tonnes), India (42.3million tonnes) and the Russian federation (32.7 million tonnes) FAOSTAT, 2011).

The global production of potatoes approached 370 Megatons in 2011, with Asia and Europe representing the regions of the globe with the largest areas of potato production (FAOSTAT, 2012). Nowadays the production states based on season for potato represents only 22% (34,000 ha), while the off-season production is around 128,000 ha in northern, southern and central Ethiopia (Haverkort *et al*., 2012).

In Ethiopia, potato production increased considerably through the twentieth century. However, potato cultivation declined in the early 1980s, due in part to widespread of late blight. In 1975, the area of cultivation was estimated at 30,000 ha, with an average yield of approximately 5 tons/ha. By 2001, Ethiopia's potato area had grown to 160,000 ha, with average yields of around 8 tons/ha).Potato can still grow on 70% of the 10 million ha of arable land in the country. In the last 10 years, potato productivity has progressed from 7 to 11 tons/ha. Nevertheless, the current area cropped with potato (about 0.16 million ha) is very small and the average yield is below 10 tons/ha, far below the country‘s potential. (Gebremedhin *et al*., 2001)

According to FAOSTAT (2008), the Belg season is also an important potato growing season. In the SNNPR, the main production is in the Belg season due to the low light blight infection and favorable market conditions.

2.5. Problems Associated with Irish Potato Production in Ethiopia

There are a number of production problems. The major ones are unavailability and high cost of seed tubers; lack of well adapted cultivars to the major agro-ecological zones; suboptimal agronomic practices; the prevalence of diseases and insect pests. One of the key production bottlenecks that contribute to low yield of potato in Ethiopia is the lack of healthy and quality seed tubers in the required quantity and quality (Gebremedhin, 1994).

There is no formal seed certification system operating for clean/healthy potato seed multiplication and distribution in Ethiopia. Hirpa *et al*. (2010) reported that potato seed production in Ethiopia is basically informal, which in most cases farmers recycle planting materials from previous crop harvest which results in viral and bacterial diseases accumulation. According to the authors, 98.7% of the current national seed potato requirements are supplied through the informal seed system such as seed entrance from neighboring farmers, friends, relatives, merchants, local market, and commercial markets where potato is sold for consumption.

Potato is one of the best examples of the yield-depressing effect of seed-borne pathogens in crop species. There is no cure once a plant or tuber is infected by bacterial wilt, viral diseases or some other potato diseases (Chiarappa, 1992). Stated that, the potential of potato crop has not been adequately exploited due to poor quality seed tubers and unavailability of seed tubers of improved varieties.

2.6. Plant Tissue Culture and Its Importance

Plant tissue culture is the science of growing plant cells, tissues or organs isolated from the mother plant, on artificial media. This is facilitated using liquid, semi-solid, or solid growth media in sterilized tubes or containers. The tissue culture technique is one of the important new methods of plant propagation available to growers and its use in seed production has allowed mass production of potato plants in a very short time. The system is characterized by very

flexible rapid multiplication giving a high rate of multiplication (Beukema and Van der Zaag, 1990).

Potato tissue culture is one of the important plant tissue culture applications for elimination of pathogen from planting materials. It is a procedure in which apical or axillary shoot tip growing at (0.1-0.3 mm) are cutted and allowed to grow into plantlets on artificial nutrient media under controlled conditions (Naik and Karihaloo, 2007); Badoni and Chauhan, 2010).

Plant tissue culture techniques have been employed in a large number of important potato varieties in agriculture (Hashem *et al*., 1990).Through tissue culture technique, an explant can be cultured *in vitro* with the purpose of obtaining a large number of plantlets, vis a viz, rapid propagation (Naik and Karihaloo, 2007). Rapid propagation is desirable for obtaining a large number of clones as compared to conventional methods. For instance, assuming effective generation of minimum 3 nodal cuttings by *in vitro* potato plantlet, and subculturing interval of 25 days, theoretically, 14.3 million microplants would be obtained from a single virus free plantlet in a year (Naik, 2005).

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have used plants small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis (Akin-Idowu, 2009).

The micropropagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well adapted high yielding genotypes with better disease resistance and stress tolerance capacities (Brown and Thorpe, 1995). Tissue culture production is independent on season (Thiart, 2004). According to some reports (Donnelly *et al.,* 2008), rapid propagation is also enhanced because a small amount of space is required to maintain or multiply plants and that plant materials need very little attention between subcultures, with no requirement for watering, weeding and spraying (Naik and Karihaloo, 2007).

Use of soil-less media is an alternative to reduce soil borne disease infections in production of vegetative propagated planting materials. The main bottleneck in potato seed tubers is slow multiplication rate on the field and contamination of the seed with viral and bacterial diseases. This has therefore, called for use of Rapid Multiplication Techniques (RMTs) such as *in vitro* propagation of potato to eliminate viral and bacterial infections (Abebe *et al.*, 2014).

2.7. Sterilization of Explants for In Vitro Propagation

The maintenance of aseptic or sterile conditions is essential for successful tissue culture procedures. To keep up an aseptic environment, all culture vessels, media and instruments used in handling tissues, as well as explants itself must be sterilized. The importance is to keep the air, surface and floor free of dust. All operations should be carried out in laminar airflow sterile cabinet (Chawla, 2003). Sterilization is the process of making explants contamination free before establishment of cultures. Various sterilization agents are used to decontaminate the tissues. These sterilants are also toxic to the plant tissues, hence proper concentration of sterilants, duration of exposing the explant to the various sterilants, the sequences of using these sterilants has to be standardized to minimize explants injury and achieve better survival (CPRI, 1992).

Sodium hypochlorite (NaOcl) has turned out to be a better sterilant than calcium hypochlorite (CaOcl) and Mercuric chloride ($Hgcl_2$) due to bleaching effects of the later and hence has been extensively used for potato sterilization. Wescott (1977) and Goodwin *et al.* (1980) disinfected sprouts with Sodium hypochlorite in which available chlorine was sterilized single node cuttings of different cultivars in 1% aqueous sodium hypochlorite.

2.8. In Vitro Regeneration of Irish Potato

Many researchers used different growth regulators for *in vitro* induction of micropropagation in potato (Hossain and Sultana, 1998). The regeneration of plants from tissue culture has potential not only to improve the existing cultivars, but also for the generation of novel plants in a comparatively short time compared to conventional breeding. Great progress has been made in

potato for plant regeneration in recent years. Remarkable work has been done on potato plant regeneration with explants and growth regulators (Khadiga *et al., 2009*).

2.8.1. Basal media type, Composition and Culture condition

One of the most important factors governing the growth and morphogenesis of plant tissue in culture is the composition of the culture medium. Plants in nature can synthesize their own food material. In contrast, plants growing *in vitro* are heterotrophic, so they cannot synthesize their own food material. Nutrient media obviously are an important factor in cell or tissue culture. Ingredients of culture media vary with the kind of plant and the preparation stage. In general, certain standard mixture is used for most plants but the exact formulation may need to be established by testing (Sanatombi *et al.,* 2006).

Media compositions are thus formulated considering specific requirements of a particular culture system. For example, some tissues show better response on a solid medium while others prefer a liquid medium. Several media formulations are commonly used for the majority of all cell and tissue culture work. But the most frequently used media formulations in plant tissue culture needs all essential nutrients (macronutrients and micronutrients), vitamins, amino acid or other nitrogen supplements, carbohydrate source, solidifying agents or support systems as well as plant growth regulators (Murashige and Skoog, 1962).

2.8.2. Effect of Plant Growth Regulators

There are five class of plant growth regulators; namely, auxins, cytokinins, gibberellins, abscisic acid and ethylene. Of these regulators, the two most important classes used to control organ and tissue development are auxins and cytokinins (Hartmann *et al*., 1990). Auxins are involved in cell division, stem elongation, cell wall synthesis and root induction. The only natural is occurring auxin found in plant tissue is IAA. The initiation medium supplemented with IBA as an auxin source, BAP as a cytokinin, they are used to generate shoot and root from Irish potato explants (Triqui *et al.,* 2007).

2.9. Acclimatization

Acclimatization is the process by which an individual organism adjusts to the change in its environment. It provides survival capacity for the change in temperature, water, food availability and other seasonal weather stresses. It is the prime measure of the survival rate of any tissue culture plantlets. Rooted micro shoots are removed from the culture vessel, agar, is washed completely to remove a potential source of contamination and the micro plants are transplanted into a standard pasteurized rooting or soil mix in small pot or cell in a more or less conventional manner. Initially the micro plants should be protected from dehydration in a shaded, high humidity tent (Venkataiah *et al.*, 2006).desirable

2.10. Environmental Control in Tissue Culture

Optimization of the microenvironment is a key step in micropropagation and ensures the production of good quality plantlets that have high chances of surviving in the *ex vitro* conditions in greenhouse and ultimately the natural environment. The culture vessel is a miniature greenhouse or growth chamber with tightly controlled conditions. The characteristics of the conventional *in vitro* environment include: constant temperature, high relative humidity, low photosynthesis photon flux density (PPFD), optimized concentrations of sugars, salt and plant growth regulators in the growth medium, aseptic conditions (Kozai *et al*., 1997).

2.10.1. Light

Energy is an important factor for plant growth and development in tissue culture. In particular, light intensity and photoperiod in the microenvironment affect plantlet physiological processes and growth. However, light may be required to a lesser degree in plant tissue culture systems for the regulation of photosynthesis and photo-morphogenesis. Though the light requirement for photosynthesis of *in vitro* plants is of less important compared to *ex vitro* plants, it still profoundly regulates plant development and growth activities. Light energy for photosynthesis is generally measured by photosynthesis photon flux density (PPFD) on the growing plantlet (Ibaraki and Nozaki, 2005).

Photoperiod, which was the length of the light period, was one of the critical environment that regulate plant development processes such as stem elongation, bud dormancy, leaf growth, flowering, pigment accumulation, hormone synthesis, gene expression and ion influx changes. Plants have a system of sensory photoreceptors that monitor changes in the ambient light environment (Kuhn *et al*., 2009).

The Light quality is measured by the spectral wavelength and has significant effects on plant development and morphogenesis *in vitro*. Plants respond differently to the spectral wavelength of light and the relationships become even more complex due to interactions with other factors such as temperature, photoperiod and light intensity. White light with wavelength band of 400-700 nm provided by cool fluorescent lamps is normally used for the maintenance of tissue cultured plants (Mack, 2009).

2.10.2. Temperature

Plant morphogenesis is a complex developmental process that is controlled and regulated by the interplay of multiple environmental stimuli, of which temperature is one of the most important. The biophysical and biochemical process of photosynthesis is temperature dependent and is a major determinant of the rate of growth of plants (Gomes *et al*., 2006). In nature, the perception of ambient temperature allows for the maintenance of plant homeostasis, thereby buffering against potential disruptive effects on cellular stability. However, in culture a constant temperature management is normally maintained and this may have effects on the development of *in vitro* plants (Franklin, 2009).

2.11. Techniques of Plant Tissue Culture

2.11.1. Micropropagation

Micropropagation starts with the selection of plant tissues (explant) from a healthy, vigorous mother plant .Any part of the plant (leaf, apical meristem, bud and root) can be used as explants (Cassells and Doyle, 2005).

2.11.2. Stage 0: Preparation of Donor Plant

Any plant tissue can be introduced *in vitro*. To enhance the probability of success, the mother plant should be *ex vitro* cultivated under optimal conditions to minimize contamination in the *in vitro* culture (Cassells and Doyle, 2005).

2.11.3. Stage I: Initiation Stage of shoot

In this stage an explant is surface sterilized and transferred into nutrient medium. Generally, the combined application of bactericide and fungicide products is suggested. The selection of products depends on the type of explant to be introduced. The surface sterilization of explants in chemical solutions is an important step to remove contaminants with minimal damage to plant cells. The cultures are incubated in growth chamber either under light or dark conditions according to the method of propagation (Husain and Anis, 2009).

2.11.4. Stage II: Multiplication Stage of explants

The aim of this phase is to increase the number of propagules. The number of propagules is multiplied by repeated subcultures until the desired (or planned) number of plants is attained. (Saini and Jaiwal, 2002).

2.11.5. Stage III: Rooting Stag of plantlets

The rooting stage may occur simultaneously in the same culture media used for multiplication of the explants. However, in some cases it is necessary to change media, including nutritional modification and growth regulator composition to induce rooting and the development of strong root growth.

2.11.6. Stage IV: Acclimatization Stage

At this stage, the *in vitro* propagated plants are weaned and hardened. Hardening is done gradually from high to low humidity and from low light intensity to high light intensity. The plants are then transferred to an appropriate substrate (*soil, sand and compost* etc.) and gradually hardened under greenhouse condition.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at the Biotechnology Laboratory of Areka Agricultural Research Center (AARC), in Southern Nations Nationalities and People's Regional State (SNNPRS), Wolaita Zone. It was located 300 km to the south of Addis Ababa and 3 km away from Areka town. And at an elevation of 1800 m.a.s.l. The annual rainfall of the area was 1520mm and the average maximum and minimum temperatures are 26^{o}C and 14^{o}C, respectively (Woliata zone Agricultural development department 2013 unpublished report).

3.2. Explants Source and Surface Sterilization

Two Irish potato varieties (Gudenie and Belete) were obtained from Areka Agricultural Research Center and were kept in the greenhouse to grow by watering and weeding throughout the duration of the study. These plants were used as stock supply of explants for the repeated Lateral bud cultures. Young and healthy shoot explants (1.0 to 2.0 cm long) containing lateral buds were cut using sterilized scalpel and washed with double distilled water (DDW). Actively growing shoots (young plants) were taken as they are more responsive to shoot regeneration and proliferation than shoot explants from adult forms as reported by Naghmouchi *et al*. (2008).

Explants were dipped in 70% alcohol for one minute and immediately washed with distilled water. Then, they were sterilized in the laminar air flow cabinet with 0.1% Berekina local bleach and 1% NaOcl (Sodium hypochlorite) for 2 to 8 minutes. Surface sterilized segments were washed 4 to 5 times with sterilized distilled water (Protocol used at Areka agricultural Research Center).

3.3. Stock Solution Preparation

Murashige and Skoog (1962) 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. To do so, appropriate amount of each nutrient was weighed in grams per liter (Appendix Table: 1) 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 $+4^0$C until used, for maximum of four weeks.

3.3.1. Plant Growth Regulators Stock Solution Preparation

Plant growth regulators (PGRs) were prepared in 1mg/ml concentration. The PGRs used for the study were the cytokinins; 6- benzyl amimopurine (BAP) and Kinetin (KN), and the auxins; indol-3- butyric acid (IBA), indol acetic acid (IAA) and Naphthalene acetic acid (NAA). The powdered crystal of the PGRs was first weighed and dissolved in 3-4 drops of 1N NaOH, and 1N HCl based on the type of PGR (NaOH for auxins and HCl for cytokinins). Upon complete dissolution, the solution of each PGR was poured into labeled 50 ml plastic bottles and filled with double distilled water to the required volume. Then gently stirred and stored at a temperature of $+4^0$C for short term use (a week) and -5^0 C for long term use (up to a month) until used.

3.3.2. Culture Media Preparation

In this study, the culture medium for shoot initiation, multiplication and root induction contained full strength MS basal medium (Murashige and Skoog, 1962) (Appendix Table 1) composed of 100 ml macronutrient, 10 ml micronutrient, and 10 ml vitamin per liter, 30 g/l sucrose as a source of carbon and agar (6g/l) as gelling agent and with or without (for control) PGRs, throughout the experiment. Finally, the pH of all media was adjusted to 5.8 by using 1 N NaOH

and/or 1N HCL after addition of agar. Because the agar used has shown slight increase in pH after addition to the media then autoclaved at 121^0C for 15 min.

After adjusting the pH, the gently mixed medium was boiled on a stove until the agar melted. Then, 40 ml of the prepared medium was dispensed into 10 x 6 cm size magenta GA7 culture vessels. The culture vessels were covered with caps immediately after dispensing the medium and autoclaved by steam sterilization at a temperature of 121^0C for 15 minutes. Immediately after autoclaving, the medium was taken and kept in lamina-air-flow-cabinet bench until used (a maximum of a week).

3.4. Experimental Design

The experiment was based on completely randomized (CRD) as factorial arrangement with three replications. The experiment was conducted to identify type and optimum concentration of plant growth regulator for *in vitro* shoot initiation (BAP and IAA), shoot multiplication (BAP and KN,) and to identify type and optimum concentration of plant growth regulator for *in vitro* rooting (IBA, NAA). The whole experimental activities were done at Areka Agricultural Research Center.

3.5. Shoot initiation

After sterilization, the explants were inoculated to shoot induction media consisting of the prepared MS basal medium supplemented with different combination plant growth regulators, BAP (1, 2, 3, 4 mg/l) combined with different levels (0.5, 1, 1.5, 2 mg/l) of IAA for shoot initiation. Three shoots per culture vessel with three replications for each treatment were used (Hussain *et al*., 2005). The culture vessel with cultured explants were properly sealed with parafilm, labeled and the treatments were arranged randomly on a table in three replications and placed on the growth room chamber with 16 hours photoperiod (8 hours dark) and 2700 lux light intensity at 25 ± 2^o C (Aggarwal and Barna, 2004). The white fluorescent lights were 28 cm away from the top of culture vessels. The stock solution composition, agar concentration and other physical conditions were the same for all the treatments. After a month of growth, every change in growth was carefully observed and recorded. PGR free medium was used as a control.

3.6. Shoots Multiplication

After 30 days of growth on the culture initiation medium, young and healthy micro-shoots were cultured on shoot multiplication, full strength MS basal medium(Murashige and Skoog, 1962), containing different concentrations of BAP (0.5, 1, 1.5, 2 mg/l) and KN (0.5, 1, 1.5, 2 mg/l) in sole or combination. Five shoots per culture vessel orderliness and were using sterilized forceps and used as explants. Have three replications for each treatment was used. The initiated cultures were aseptically cut off and cultured on multiplication medium. The culture vessels were properly sealed, labeled and randomly placed on the growth room chambers with the same culture conditions (temperature, photoperiod and light intensity) as that of the initiation experiment. PGR free medium was used as a control. Sub-culturing was made three times by transferring the newly multiplied micro-shoots to fresh medium of the same composition as the previous one. Sub-culturing was carried out at monthly interval. The growth response of the micro-shoots to different treatments and at different multiplication stages was carefully observed and recorded.

3.7. Rooting

Rooting of the already multiplied shoots of both varieties was conducted on full strength MS basal medium supplemented with different concentrations of IBA (0.25, 0.5 0.75, 1 mg/l) and NAA (0.25, 0.5 0.75, 1 mg/l) in sole or combination. PGR free full strength MS basal medium was used as control in all cases. Five shoots per culture vessel with three replications for each treatment were used. The cultures were transferred to the growth room chambers with 16 h photoperiod at a temperature of 25 ± 2^{o}C. All factors including photoperiod, temperature, and light intensity, pH of the medium, agar concentration and other growth conditions were the same as before. The numbers of roots produced from each shoot, length of the roots, rooting response, were recorded at weekly interval until four weeks of growth.

3.8. Acclimatization

The *in vitro* well rooted Irish potato plantlets were taken out gently from each PGR treatments of the culture media jar and washed under running tap water to remove traces of agar that prevent the absorption of nutrients from the acclimatization culture substrates by roots. The plantlets were transplanted into acclimatization plastic cell tray filled with culture substrates of moist red soil, sand soil and compost soil or their mixture in 2:1:1 ratio, respectively. The plantlets were covered with white transparent polythene plastic bag to maintain high humidity, and the plantlets were acclimatized in an open greenhouse environmental condition with in the same room by removing the polyethylene sheet and red cheese cloth and irrigated with tap water every day.

Plastic cover was partially removed after a week and completely removed after two weeks then regenerated plantlets were grown to maturity. Finally, after three weeks, percent of plantlets successfully hardened was measured by the number of survived shootlets and died shootlets from the total transferred plantlets.

3.9. Data collection

All the following necessary data in vitro plant growth variables were collected from the subsequent experiments:-

Number of clean explants: number of explants which are free from microorganisms and survived was counted after 15 days of culture from each replication and converted into percentage.

Average number of days for shoot emergence: the period of time (days) needed by each explant to initiate shoot was recorded from the first day of culturing and an average day was computed for each replication.

Mean number of nods: total number of nodes produced from single explants after four weeks of inoculations was counted and mean was computed for each replication.

Mean shoot number: total number of shoots produced from single explant after four weeks of inoculations was counted and mean was computed for each replication.

Shoot length: length of each initiated/proliferated shoot was measured in centimeters from base to the tip using ruler (cm) after four and six weeks of culturing.

Days of rooting response: Average number of days for the formation of root from each shootlet explants after the explants were cultured on full strength MS-medium supplemented with or without growth regulators in each replication of cultured shoots producing root.

Root number: This was the average number of roots regenerated per shootlets explants for each cultured explants. In order to estimate the multiplication rate of Irish potato *in vitro,* the number of roots was counted after four weeks of growing in root induction medium.

Main root length: length of induced roots was measured in centimeters, from base to tip for each root.

Shoot fresh weight: the average shoot fresh weights were recorded at the end of the propagation phases in both the shoot multiplication and root induction medium, using an electronic sensitive balance.

Root fresh weight: the average root fresh weights were recorded at the end of the propagation phases in both the shoot multiplication and root induction medium, using an electronic sensitive balance.

Shoot dry weight: the average shoot dry weights were recorded after drying in oven at 65^{o}C for 12 hours and data were taken at the end of the shoot multiplication and root induction medium phases.

Root dry weight: the average root dry weights were recorded after drying in oven at 65^{o}C for 12 hours and data were taken at the end of the shoot multiplication and root induction medium phases.

Acclimatization percentage: is the survival percentage of the *in vitro* derived plantlets in the *ex vitro* condition.

$$\text{Survival rate (\%)} = \frac{\text{number of plantlets after 15 days of acclimatization}}{\text{Total number of transferred plantlets}} \times 100$$

3.10. Statistical analysis

All the quantitative data analysis was done by using excel spread sheet, recorded for all responses were subjected to two-way Analysis of variance (ANOVA) and significant differences among treatments were determined by Fisher's Least Significance Difference (LSD) at 5% level of significance by using the SAS (Version 9.2) software package for the differentiation of the effect of treatment, genotype and treatment-genotype interaction.

4. RESULTS AND DISCUSSION

4.1. Lateral bud Survival and Shoot Initiation

In both varieties of Irish potato, survived lateral buds started to respond within 3-5 days after culturing. The responses were direct shoot induction (Appendix Figure: 5). Though the values were not significantly different,variety Gudene showed more response in terms of shoot proliferation at 2 mg/l BAP and 1 mg/l IAA combination treatment. There was no significant difference in percent shoot initiation in both varieties under the rest of hormonal treatments, suggesting that 2mg/l BAP and 1 mg/l IAA combination is optimal to result in maximum shoot initiation in this particular experiment. Previously, some other researchers (Hussain *et al*., 2005; Molla *et al*., 2011) reported better shoot induction in other potato varieties cultured on MS medium supplemented with BAP and IAA in 2mg/l to 0.5mg/l combination, and their results are somewhere in agreement with the result of the study. With the rise of BAP to IAA concentrations up to 2mg/l to 1mg/l, shoot induction appeared to increase, but decreased afterwards, beyond this cultures combination of might be due to the inhibitory effect of cytokinins at higher concentrations. Berrie (1984) reported that synthetic cytokinins are inhibitory to shoot growth at high concentrations.

Table 1. Percentage of initiated explants of the two Irish potato varieties ('Gudenie' and 'Belete') after 30 days on different growth initiation media supplemented with different growth regulator concentrations.

Variety	Hormone combination BAP (Mg/l)	IAA (Mg/l)	No of explants/tre	No of initiated explants	No of Dead Explants	Shoot Initiation percentage (%)
Gudenie	0	0	9	5.37	3.63	59.67^{cde}
	1	0.5	9	6.37	2.63	70.83^{c}
	2	1	9	8.25	0.75	91.67^{a}
	3	1.5	9	6.00	3.00	66.67^{cd}
	4	2	9	6.00	3.00	66.67^{cd}
Belete	0	0	9	5.00	4.00	55.56^{cde}
	1	0.5	9	6.75	2.25	75.00^{bc}
	2	1	9	7.87	1.13	87.50^{ab}
	3	1.5	9	6.37	2.63	70.83^{c}
	4	2	9	6.37	2.63	70.83^{c}
	LSD					13.24
	CV (%)					10.41

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different

4.2. Effect of Cytckinins on *in vitro* shoot multiplication

After second sub-culturing, spontaneous roots of the induced shoots were removed and transferred to Magenta culture vessels containing 40 ml shoot multiplication medium consisting of MS supplemented with different growth regulator concentrations and combinations (Table 2). Compared to the control, both BAP and Kn alone or in combination showed significant positive effect on shoot formation of both varieties of Irish potato at all concentration levels. Number of nodes/explant number of days to shoot emergence, number of shoot /explant, shoot fresh and dry weight significantly varied between treatments in both varieties (Table 2). For Gudenie variety, number of nodes/explant, number of shoots/explant and shoot length/explant were significantly higher at 0.5mg/l BAP and 2mg/l Kn combination than at other treatments. Number of days to shoot emergence was also found to be shorter at this level of hormonal combination than other treatments. Similar pattern had also been observed in Belete variety too, but the response of Gudenie in terms of the mentioned parameters was better than that of variety, Belete suggesting that varietal difference evokes varying response to the same PGRs. In the case of Belete variety,

0mg/l BAP to 2mg/l KN combination treatment had similar effects as that of 0.5mg/l BAP to 2mg/l KN. Observed in gudenie

In many plants, multiple shoots can be obtained from the shoot tips of axillary buds by administering BAP or KIN (Sood *et al*., 2002; Velayutham, 2003; Baskaran and Jayabalan, 2005; Hussain *et al*., 2005; Jimenez *et al*., 2006; Devi and Sharma, 2009; Bhat *et al*., 2010; Azar *et al*., 2011; Thiruvengadam *et al*., 2011). In line with this study, Sarker and Mustafa (2002) reported that some potato varieties treated with BAP showed better response in terms of shoots per explant, shoot length and number of nodes. The synergistic effect of BAP and Kn for increased shoot multiplication rate and proliferation was also reported on *Bambus tulda* and *Melocanna baccifera* (Waikhom and Louis, 2014).

Table 2: Effect of different concentrations of cytokinins (BAP and Kin) in sole and combination on the morphogenetic responses of Gudenie and Belete Irish potato varieties in *in vitro* shoot multiplication culture on MS basal medium with sucrose.

Varieties	Hormone Combination. BAP, (Mg/l)	KN (Mg/l)	No of nodes per expt.(n)	No of days to shoot emergence(n)	No of shoots per expt.(n)	Shoot Length per expt.(cm)	Shoot fresh wt. per expt.(g)	Shoot dry wt. per expt.(g)
Gudenie	0	0	0.00m	0.00^{e}	0.00^{n}	0.00^{k}	0.*05*^{i}	0.01^{f}
	0.5	0.5	6.67^{cdef}	3.33^{cd}	5.67^{ghij}	4.83^{efghi}	0.11^{efghi}	0.05^{cdef}
	1	1	8.33^{b}	3.17^{d}	7.67^{bcd}	7.18^{abc}	0.21^{abcde}	0.07^{abcde}
	1.5	1.5	5.67^{fghi}	3.67^{bcd}	4.67^{jklm}	5.25^{def}	0.13^{fghi}	0.05^{cdef}
	2	2	4.33^{jkl}	3.33^{cd}	3.67^{m}	4.17^{hij}	0.15^{bcdefghi}	0.06^{cdef}
	0.5	0	7.33^{bcd}	3.67^{bcd}	7.33^{bcde}	4.83^{efghi}	0.17^{bcdefg}	0.08^{abcd}
	1	0	8.33^{b}	3.33^{cd}	8.33^{b}	5.50^{cde}	0.13^{efghi}	0.09^{abc}
	1.5	0	4.67^{ijkl}	3.83^{abcd}	6.00^{fghi}	4.50^{fghij}	0.14^{cdefghi}	0.06^{bcdef}
	2	0	3.67^{l}	4.00^{abcd}	5.33^{hijk}	4.08^{ij}	0.25^{ab}	0.08^{abcd}
	0	0.5	4.00^{kl}	4.67^{a}	4.33^{klm}	3.67^{j}	0.23^{abcd}	0.11^{ab}
	0	1	5.67^{fghi}	4.00^{abcd}	5.00^{ijkl}	4.67^{efghi}	0.15^{bcdefghi}	0.07^{bcde}
	0	1.5	6.33^{defg}	3.83^{abcd}	6.00^{fghi}	4.67^{efghi}	0.13^{fghi}	0.06^{bcdef}
	0	2	7.00^{cde}	3.33^{cd}	6.67^{defg}	5.83^{cd}	0.12^{efghi}	0.05^{cdef}
	0.5	2	9.67^{a}	3.17^{d}	10.67^{a}	7.57^{a}	0.28^{a}	0.12^{ab}
	2	0.5	6.33^{defg}	3.33^{cd}	7.00^{cdef}	4.67^{efghi}	0.08^{ghi}	0.02^{ef}
Belete	0	0	0.00^{m}	0.00^{e}	0.00^{n}	0.00^{k}	0.07^{hi}	0.02^{ef}
	0.5	0.5	6.33^{defg}	3.83^{abcd}	5.67^{ghij}	5.33def	**0.11**^{**efghi**}	0.06^{cdef}
	1	1	6.67^{cdef}	3.67^{bcd}	7.00^{cdef}	6.83^{ab}	0.17^{bcdefg}	0.08^{abcd}
	1.5	1.5	4.67^{ijkl}	4.00^{abcd}	4.67^{jklm}	5.17^{defg}	0.12^{efghi}	0.05^{cdef}
	2	2	**3.67**^{**l**}	**4.67**^{**a**}	**4.00lm**	4.50^{fghij}	0.13^{efghi}	0.05^{cdef}
	0.5	0	6.67^{cdef}	4.33^{ab}	5.67^{ghij}	4.67^{efghi}	0.13^{defghi}	**0.04**^{**def**}
	1	0	7.3^{bcd}	3.83^{abcd}	6.67^{defg}	5.00^{defgh}	0.18^{abcdefg}	0.05^{cdef}
	1.5	0	5.00^{hijk}	**4.67**^{**a**}	5.33^{hijk}	4.33^{ghij}	0.13^{defghi}	0.05^{cdef}
	2	0	5.33^{ghij}	4.17^{abc}	5.00^{ijkl}	4.17^{hij}	0.20^{abcdef}	0.08^{abcd}
	0	0.5	6.00^{efgh}	3.67^{bcd}	5.33^{hijk}	**4.00**^{**ij**}	0.18^{abcdefg}	0.08^{abcd}
	0	1	6.33^{defg}	**4.67**^{**a**}	5.67^{ghij}	4.67^{efghi}	0.15^{bcdefghi}	0.06^{bcdef}
	0	1.5	6.67^{cdef}	3.12^{d}	7.00^{cdef}	5.33^{def}	0.13^{efghi}	0.05^{cdef}
	0	**2**	**7.67**^{**bc**}	**3.10**^{**bcd**}	**8.33**^{**b**}	**7.33**^{**ab**}	**0.24**^{**abc**}	**0.15**^{**a**}
	0.5	2	7.33^{bcd}	3.33^{cd}	8.00^{bc}	6.33^{bc}	0.17^{bcdefgh}	0.07^{abcde}
	2	0.5	5.67^{fghi}	**4.67**^{**a**}	6.33^{efgh}	4.33^{ghij}	0.14^{cdefghi}	0.10^{abc}
	LSD		1.04	0.85	1.04	0.85	0.11	0.05
	CV (%)		10.98	14.62	11.01	10.92	7.02	11.09

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at P<0.05. No. = number, expt. = explants and wt = weight.

4.3. Effect of Auxins on *in vitro* root induction

The development of healthy root system was required for the successful establishment of *in vitro* regenerated shoots to adapt to the external environments. For this, those shoots with height of 1cm and above were taken and transferred onto rooting medium that contained full strength MS supplemented with different IBA and NAA in different concentrations in sole or in combination. Compared to the control, both IBA and NAA alone or in combination showed significant positive effect on root formation of shoots of both varieties of Irish potato at all concentration levels. Number of roots/shoot, root length, number of days to rooting, root fresh and dry weight significantly varied between treatments in both varieties (Table 3). In both varieties, number of roots/shoot, root length/shoot and root fresh and dry weight had significantly higher values at 1mg/l IBA to 0.25mg/l NAA combination treatment than other treatments (Table 3).

Though both varieties performed well at 1mg/l IBA to 0.25mg/l NAA combination treatment, performance of Gudenie was better than Belete variety. This shows that, as in the case of shooting response, varietal difference evokes varying rooting response to the same PGRs. Rabbani(2001) reported that the best rooting response in plants such as *Solanum tuberosum*, was observed when IBA concentrations is at higher proportion than NAA in a combination of the two. The work of Khadiga (2009) also showed that use of (2.5mg/l) of IBA is good to improve root initiation of potato plantlets which agrees with the present finding that rooting increases with high IBA concentration in a combination of the two auxins.

Table 3: Effect of different concentrations of auxins (IBA and NAA) in sole and combination on the morphogenetic responses of Gudenie and Belete Irish potato varieties in *in vitro* root induction culture on MS basal medium with Sucrose

Varieties	Hormone Combination. IBA, (Mg/l)	NAA (Mg/l)	Days to rooting Response (n)	Root no. Per shoot. (n)	Length of Root Per shoot. (cm)	Root fresh wt. Per shoot. (g)	Root dry wt. Per shoot. (g)
Gudenie	*0*	*0*	13.66^{a}	1.33^{lm}	1.67^{l}	0.06^{ef}	0.02^{cde}
	0.25	0.25	8.00^{hijkl}	16.67^{bc}	5.83^{c}	0.13^{bcdef}	**0.03^{bcd}**
	0.5	0.5	6.67^{kl}	19.00^{b}	7.17^{b}	0.18^{abcd}	0.05^{abcd}
	0.75	0.75	9.33^{efghi}	10.00^{def}	5.17^{cde}	**0.11^{ef}**	0.06^{abcd}
	1	1	10.33^{def}	8.67^{efgh}	3.83^{fghij}	0.17^{bcde}	0.05^{abcd}
	0.25	0	11.33^{bcd}	6.33^{ghijk}	3.17^{hijk}	0.15^{bcdef}	0.06^{abcd}
	0.5	0	10.33^{def}	9.00^{efg}	3.83^{fghij}	0.12^{def}	0.05^{bcd}
	0.75	0	8.67^{fghij}	12.67^{d}	4.33^{defg}	0.14^{bcdef}	0.05^{abcd}
	1	0	6.67^{kl}	15.67^{c}	5.33^{cd}	0.12^{def}	0.05^{abcd}
	0	0.25	8.00^{hijkl}	12.67^{d}	3.50^{ghijk}	0.15^{bcdef}	0.06^{abcd}
	0	0.5	9.67^{defgh}	8.33^{efghi}	3.08^{ijk}	0.13^{bcdef}	0.05^{abcd}
	0	0.75	10.67^{cde}	7.33^{fghij}	2.83^{jk}	0.12^{cdef}	0.04^{bcd}
	0	1	**12.33^{abc}**	**4.67^{jk}**	**2.50^{kl}**	0.13^{bcdef}	0.05^{abcd}
	0.25	1	8.33^{ghijk}	15.67^{c}	4.67^{def}	0.12^{cdef}	0.04^{cde}
	1	**0.25**	**6.33^{l}**	**23.33^{a}**	**8.33^{a}**	**0.19^{abc}**	**0.08^{abcd}**
Belete	0	0	0.00^{m}	0.00^{m}	0.00^{m}	0.00^{g}	0.00^{e}
	0.25	0.25	9.33^{efghi}	12.33^{d}	4.17^{efgh}	**0.08^{f}**	0.04^{cde}
	0.5	0.5	**13.00^{ab}**	15.67^{c}	4.83^{cdef}	0.12^{cdef}	0.05^{abcd}
	0.75	0.75	10.00^{defg}	9.00^{efg}	3.50^{ghijk}	0.13^{bcdef}	0.06^{abcd}
	1	1	9.33^{efghi}	6.00^{hijk}	2.83^{jk}	0.13^{cdef}	0.05^{abcd}
	0.25	0	10.67^{cde}	4.67^{jk}	2.83^{jk}	0.12^{def}	0.04^{bcd}
	0.5	0	10.33^{def}	8.00^{efghi}	3.17^{hijk}	0.10^{ef}	0.04^{cde}
	0.75	0	9.67^{defgh}	9.00^{efg}	3.50^{ghijk}	0.09^{f}	**0.03^{de}**
	1	0	7.67^{ijkl}	12.67^{d}	4.50^{defg}	0.16^{bcdef}	0.06^{abcd}
	0	0.25	9.33^{efghi}	10.00^{def}	3.08^{ijk}	0.19^{abc}	0.07^{abcd}
	0	0.5	10.33^{def}	7.00^{ghij}	2.67^{kl}	0.14^{bcdef}	0.06^{abcd}
	0	0.75	11.33^{bcd}	5.67^{ijk}	2.50^{kl}	0.12^{cdef}	0.05^{abcd}
	0	1	7.67^{ijkl}	**3.67^{kl}**	**1.67^{l}**	0.19^{abc}	0.08^{abc}
	0.25	1	9.67^{defgh}	10.66^{de}	4.00^{fghi}	0.21^{ab}	0.09a
	1	**0.25**	**7.33^{jkl}**	**17.33^{bc}**	**5.33^{cd}**	**0.24^{a}**	**0.08^{ab}**
	LCD		1.68	2.99	1.01	0.08	0.04
	CV (%)		11.18	8.15	6.22	9.20	12.36

Values are means and means represented by the same lowercase letter within a column are not significantly different, whereas those with different letters are significantly different at $P<0.05$. No. = number, and wt = weight

4.4. Acclimatization

After being in the rooting medium for one month, those shoots that developed roots were transferred to different sterilized and unsterilized substrates (moist red soil, sand soil, compost in sole and mixed in 2:1:1 ratio, respectively) and allowed to grow in a greenhouse for three weeks. Results showed that plantlets grown on a mix of moist red soil: sand soil: and compost in 2:1:1 ratio hardened very well compared to other substrates used. Survival rates were (93.33%) and (86.66%) for Gudene and Belete varieties, respectively (Table: 4). However, plantlets which acclimatized in unsterile soil mix the survival rate were (53.33%) and (46.66%) for Gudene and Belete varieties, respectively. They were severely affected by cutworms which cut down the stem from the bottom. And some leaves were dried up and consequently detached from the shoots. This may be due to unrestricted loss of water from their leaves or low hydraulic conductivity of roots and root-stem connections (Pospišilova *et al.*, 1999; Kumar and Rao, 2012). Few of the dead plantlets were due to fungal contamination and rotting. (Table: 5).

Direct transfer of *in vitro* plantlets to *ex vitro* Environment may result in rapid wilt and death (Lesar *et al.*, 2012). Thus acclimatization is essential to enable the rooted plantlets to adapt to the natural environment in *ex vitro* conditions (Deb and Imchen, 2010). In the acclimatization stage of this experiment, a total of 240 well rooted plantlets (120 plants from each Irish potato variety) from each treatment of plant growth regulators were acclimatized to different sterilized and unsterilized culture substrate for three week by irrigating water daily to maintain the humidity of the plantlets. (Venkataiah *et al.*, 2006).

Loss of some plantlets might be due to the variation in environmental factors. Especially, the drop of relative humidity from near 100% in the culture vessels to much lower values in the greenhouse which might result in excessive water loss and death. The current result is in agreement with the report of Ali et al. (2008) who obtained (70%-80%) of greenhouse acclimatization potential for *in vitro* regenerated sugar cane at serialized culture substrate.

Finally, in the present investigation hardening of well rooted plantlets grown on plastic cell tray filled with sterilized Mixture of moist red soil, sand soil and compost soil, in 2:1:1 ratio

respectively under a reduced light intensity by covering with red polyethylene sheet and high humidity condition had a survival rate of as much as 93.33 % for Gudenie and 86.66% for Belete varieties. This result also revealed the highest survival rate of the variety Gudenie plantlets as compared to Belete Irish potato variety after 15 days in the open greenhouse environments. Further observation of survived individual plantlets in the greenhouse revealed no aberrant phenotypes. (Table: 4).

Table 4: Effect of different sterilized culture substrates on the survival rate of *in vitro* regenerated plantlets of Gudenie and Belete Irish potato varieties during acclimatization in greenhous

Variety	Type of culture Substrates	Total no. of explants transferred	No. of survived explants	No. of died explants	% of survived explants	% of died explants
Gudenie	Moist red Soil alone	15	10.83^{cd}	4.25^{bc}	72.20^{cd}	28.07^{bcd}
	Sand soil alone	15	9.00^{de}	6.07^{b}	60.00^{de}	40.00^{b}
	Compost soil alone	15	11.33^{bcd}	3.66^{bcd}	75.53^{bcd}	24.46^{cd}
	Mixture in 2:1:1 ratio	15	13.97^{a}	1.03^{e}	93.13^{a}	6.67^{e}
Belete	Moist red Soil alone	15	9.33^{d}	6.05^{b}	62.20^{cde}	37.80^{bc}
	Sand soil alone	15	7.83^{e}	7.17^{a}	52.20^{e}	47.80^{a}
	Compost soil alone	15	11.67^{bc}	3.33^{cd}	77.80^{bc}	22.20^{cde}
	Mixture in 2:1:1 ratio	15	13.01^{b}	1.99^{cde}	86.73^{b}	13.26^{de}
	Significance		**	**	*	*
	LSD		0.46	0.86	1.28	7.27
	CV (%)		5.21	7.28	3.17	3.37

Values are means and means represented by the same lowercase letter within a column are not significantly different, where as those with different letters are significantly different at $P<0.05$.

Table 5: Effect of different unsterilized culture substrates on the survival rate of *in vitro* regenerated plantlets of Gudenie and Belete Irish potato varieties during acclimatization in greenhouse

Varieties	Type of culture Substrates	Total no. of explants transferred	No. of survived explants	No. of died explants	% of survived explants	% of died Explants
Gudenie	Moist red Soil alone	15	5.33cd	9.67bc	35.33^{cd}	64.47^{bcd}
	Sand soil alone	15	2.99de	12.01b	20.00^{de}	80.00^{b}
	Compost soil alone	15	6.00bc	9.00cd	40.00^{bc}	60.00^{cde}
	Mixture in 2:1:1 ratio	15	8.15a	6.85e	**54.33^{a}**	45.67^{e}
Belete	Moist red Soil alone	15	5.67bcd	9.33bcd	37.80^{bcd}	62.20^{cd}
	Sand soil alone	15	2.87e	12.13a	19.13^{e}	80.87^{a}
	Compost soil alone	15	5.28^{bc}	5.28^{cd}	9.72bc	35.20cd
	Mixture in 2:1:1 ratio	15	7.00^{ab}	7.00^{b}	8.00de	**46.66^{b}**
	Significance		**	*	**	*
	LSD		1.06	0.59	2.37	1.43
	CV (%)		3.41	4.76	7.23	2.76

Values are means and means represented by the same lowercase letter within a column are not significantly different, where as those with different letters are significantly different at $P<0.05$.

\

5. SUMMARY

While tissue culture based rapid multiplication proved to be promising in different species of plants including Irish potato, optimizing efficient and reproducible *in vitro* rapid multiplication protocol for Ethiopian potato varieties from seed would increase the social, environmental and economic benefit derived from the species. Therefore, this work was undertaken with the following objectives: to develop effective explants-surface sterilization procedure; to asses effects of different types and concentrations of cytokinin and auxins on shoot proliferation and multiplication; to asses effects of different types and concentrations of auxins on root induction; and develop method to acclimatize *in vitro* seedling derived plantlets of *Solanum tuberosum L.*
The studies were conducted in Areka Plant Biotechnology Lab. The experimental design used was CRD with three replications except for sterilization experiment.

In the sterilization experiment, explants were excised using sterilized scalpel and washed with double distilled water. Then, it was dipped in 70% alcohol for one minute and immediately washed with distilled water. Then, it was sterilized in the laminar air flow cabinet with 0.1% Berekina local bleach and 1% NaOCl for 5 minutes. Surface sterilized segment was washed 4 to 5 times with sterilized distilled water .The establishment of culture shoot from sterile explants were carried out in full strength MS solid medium by different concentration (1,2,3 and 4)mg/l for BAP with(0.5,1.0,1.5,and 2.0)mg/l for IAA to induce shoots. Aseptically initiated propagules were transferred in to full strength MS medium supplemented by different BAP and KN concentrations (0.5, 1, 1.5, and 2 mg/l) for each of BAP and KN to shoots multiplication experiment.

Root induction experiment was carried out in full strength MS medium prepared by different IBA and NAA concentration (0.25, 0.5, 0.75, and 1.0 mg/l) for each of IBA and NAA to root induction experiment. Plantlets with well-developed shoots and roots were transplanted on a plastic cell tray containing mixture of both filled with sterilized and unsterilized culture substrates of moist red soil, sand soil and compost soil at alone and mixture in 2:1:1 ratio respectively, for acclimatization in greenhouse at low light intensity and high humidity condition.

Among the different disinfectant treatments investigated, a maximum induced shoot per/ explants percentage 91.67% in Gudenie variety was recorded. the highest number of shoot was multiplication per/explants were 10.67 in Gudene and The highest root number per/shoot were 23.34 in Gudenie variety.

With regard to shoot induction and multiplication, type and concentration of cytokinins had significantly affected the duration of shoot induction, rate of shoot proliferation, number and growth of shoot. Similar to the shoot induction and multiplication experiment, in the root induction too, IBA was superior to NAA. The highest number of root induction, (23.34), was recorded from two concentrations of the auxin (IBA: 1 mg/l) with (NAA: 0.25mg/l) in Gudenie variety.

Finally, hardening well rooted plantlets grown on plastic cell tray filled with sterilized mixture of moist red soil, sand soil and compost soil, in 2:1:1 ratio respectively, under a reduced light intensity by covering with red polyethylene sheet and high humidity condition a survival rate of as much as 93.33 % was obtained after 15 days in the open greenhouse environments.

6. CONCLUSIONS

This study provided optimal protocol for micro-propagation of Irish potato varieties through lateral bud culturing on MS basal medium supplemented with appropriate concentrations of different PGRs in sole or combination. This protocol can thus be utilized to micro-propagate the two Irish potato varieties to boost its production.

Lack of a balanced supply of good planting material was one of the bottle necks for the exploitation of the potential of Irish potato in Ethiopia. Mass propagation of Irish potato through lateral bud culture ensures quick availability of genetically uniform (true to type) diseases free planting materials within short period of time. In the present study, an effective protocol for subsequent in vitro plantlets multiplication from lateral bud explants was developed for two Irish potato varieties Gudene and Belete. Accordingly, irrespective of the type of carbon source 3% sucrose used in the MS solid medium, the combination of 2mg/l BAP with 1mg/l IAA was found to be the best combination for shoot induction of both varieties Gudene and Belete, while the combination of 0.5mg/l BAP with 2mg/l KN in Gudene and 2.0mg/l KN alone in Belete was found to be the best combination for shoot multiplication. KN was found to be the best for both varieties.

And the combination of 1mg/l IBA with 0.25mg/l NAA in both Gudene and Belete varieties were found to be the best combination for root induction. Regarding in vitro shoot induction, shoot multiplication and root induction the optimum protocol was obtained Gudene varieties. Hence, to make developing countries like Ethiopia beneficiary of micro propagation technology was best alternative to more improvement of the micropropagation protocol.

7. RECOMMENDATIONS

.

Based on the above findings, the following major recommendations were made and further investigation of Irish potato culture should focus on the following areas:-

- It is important to develop Lateral bud culture micropropagation protocol for other potato varieties.
- Explants other than lateral bud should be used for further development of the micropropagation protocol for better result.
- Further studies on the performance of the tested cytokinins in combination with other type of hormone (cytokinins and/or auxins) should be carried out for better result on shoot induction and multiplication.
- Research on further optimization of acclimatization should be done particularly for Gudenie variety to increase the survival rate.

- Use of the already developed micro propagation protocol for large scale production of the two Irish potato varieties and distributing them to farmers to increase seed potato production in Ethiopia should be given due attention.

8. REFERENCES

Abbasi, N.A., ahoor, M. Khan, H. and Qureshi, A. A. 2012. Effect of Encapsulated calcium carbide application at different growth stages on potato (*Solanum tuberosum L.*), growth yield and tuber quality. *Pakistan Journal of Botany*, 44(5): 1543-1550.

Abebe Chindi, Gebremedhin, W. Giorgis, Atsede Solomon, Lemma Tessama and Kassaye Negash. 2014. Rapid Multiplication Techniques (RMTs): A Tool for the Production of Quality Seed Potato (*Solanum tuberosum* L.) in Ethiopia. *Asian Journal of Crop Science, 6:176-185.*

Aggarwal, D., Barna, K.S. 2004. Tissue culture propagation of plants. *Plant Biochemical. Biotechnology*, 13: 77-79.

Ahmad, N., Khan, A. A., Khan, N. A., Binyamin, R., and Khan, M. A. 2011. Identification of resistance source in potato germplasm against PVX and PVY. *Pakistan Journal of Botany,* 43(6): 2745-2749.

Ahmad, Q., Hafeez, U.R., Ahmad, C.M.S. and Laghari, M. H. 2003. Effect of culture media and explant origin on in vitro propagation of Ceratonia siliqua L. via cuttings. *Biotech. Agron. Soc. Environ,* 12(3): 251 – 258.

Akin-Idowu PE, Ibitoye, D.O, Ademoyegun, O.T. 2009. Tissue culture as a plant production technique for horticultural crops. *Afr. J. Biotechnol*, 8(16): 3782-3788.

Ali, A, lqbalj,NazS,Siddiqui FA.2008. An efficient protocol for large scale production of sugare cane through micropropagation. *Pak.j.Bot*, 40 (1):139-149.

Anilkumar, M. and Nair, A.S. 2004. Multiple shoot induction in *Ipomoea* batatas L.cv. early California wonder. *Plant Cell Biotech. Mol. Biol*, 5: 95-100.

Anonymous. 2005. Area and production of crops, Addis Abeba, Ethiopia. *The Hindu India*, 29: 5-6.

Arya, S., Sharma, S. Kaur, R. and Arya, I.D. 1999. Micropropagation of *Dendrocalamus asp*er by shoot proliferation using seeds. *Plant Cell Report,* 18: 879-882.

Badoni, A. and Chauhan, J.S. 2009. Effects of growth regulators on meristem-tip development and *in vitro* multiplication of potato cultivar Kufri Himalini. *African Journal of Biotechnology*, 11 (17): 3993-3999.

Badoni, A. and. Chauhan J.S. 2010. Conventional vis-a-vis biotechnological methods of propagation in potato. *A review. Stem Cell*, 1: 1-6.

Berrie, A.M. 1984. Germination and growth regulators. In: Advanced plant physiology. Malcom, W.B. (ed.), *English Language Book Society. Longman.* Pp987.

Beukema, H.P. and van der Zaag D.E. 1990. Introduction to Potato Production. 2nd Edn., Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, The Netherlands, ISBN: 9789022009635, Pages: 208.

Beyl, C.A.2005. Getting Started with Tissue Culture: Media Preparation, Sterile Technique, and Laboratory Equipment.Pp 19-37. In: TrigianoR.N.,and Gray, D.J. (eds.) plant development and biotechnology. CRS Press, Boca Raton, Florida.

Bhat, M.A., Mujib, A., Junaid, A. and Mohamooduafar, M. 2010. *In vitro* regeneration of *Solanum nigrum* with enhanced solasodine production. Biologia Plantarum, 54(4): 757-760.

Brown, D.C.W. Thorpe, T.A .1995. Crop improvement through tissue culture. World J. Microbiol & Biotechnol, 11: 409-415.

Bruce, L.P, George, L.T.H. 1989. Phthorimaea operculella (Zell.), the potato tuber moth: new locality records for East Africa. *American Journal of Potato Research*, 66:583–586.

Camire, M.E., Kubow, S. & Donnelly, D.J. 2009. Potatoes and Human Health. *Critical Reviews in Food Science and Nutrition,* 49(10): 823-840.

Cassells, A.C, Doyle, B.M. 2005. Pathogen and biological contamination management: the road ahead. In: Loyola-Vargas VM, Vázquez-Flota F, editors. Plant Cell Culture Protocols, Humana Press. New York, USA: 35-50.

Central Potato Research Institute (CPRI), Shimla .1992. Tissue culture technique for potato health, conservation, micro propagation and improvement. CPRI, Shimla: 1-23.

Central Statistical Agency of Ethiopia (CSA). 2008. Agricultural sample survey: Report on FAO Stat. 2009. http://faostat.fao.org/site/567 Page ID = 567.

Central Statistical Authority (CSA) .2002. Ethiopia Agricultural Sample Enumeration, 2001/2002 (1994 E.C). Report on the preliminary result of area, production and yield of temporary crop (Meher season, private peasant holdings). Volume I, part II. Addis Ababa, Ethiopia.

Chawla, H. S. 2003. Plant Biotechnology: Laboratory Manual for Plant Biotechnology. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi.

Chiarappa, L. 1992. Worldwide losses due to seed-borne diseases. In: *Seed Pathology*. Proceedings of the CTA seminar held at Copenhagen, Denmark, in June 1988. Wageningen, p17-23.

Deb, C.R. and Imchen, T. 2010. An efficient *in vitro* hardening technique of tissue culture raised plants. *Biotechnology,* 9: 79-83.

Devi, Bengyella, L.W.S. and Sharma, G.J. 2012. *In vitro* seed germination and micropropagation of edible bamboo *Dendrocalamus giganteus* Munro using seeds. *Biotechnology, 2012*: 1-7.

Devi, W.S. and Sharma, G.J. 2009. *In vitro* propagation of *Arundinaria callosa* Munro- an edible bamboo from nodal explants of mature plants. *The Open Plant Science Journal* (3): 35-39.

Donnelly, D.J, Warren K.C. and Shirlyn, E.C. 2008. Potato microtuber production and performance: A review. *American Journal of Potato Research*, 80(2): 103-115.

El-Komy, M.H., Abou-Taleb, E.M, Aboshosha, S.M. and. El-Sherif, E.M. 2010. Differential expression of potato pathogenesis-related proteins upon infection with late blight pathogen: A case study expression of potato osmotin-like protein. *Int. J. Agric. Biol*, 12: 179-186.

FAO (Food and Agriculture Organization of the United Nations). 2008. The international year of potato, the global crop diversity trust and FAO's plant production and protection division, Rome, Italy, 1: 1-30.

FAO (Food and Agriculture Organization of the United Nations).2008. *International Year of the Potato (IYP): Potato World*. [Online] Available from: http://www.potato.rg.

FAOSTAT (Food and Agriculture Organization Statistics) Agriculture .2012. FAO statistical database. http://www.fao.org/corp/statistics/en/ read on, 29.09.2012.

FAOSTAT (Food and Agriculture Organization Statistics). [Online] Available from: http://faostat3.fao.org.

FAOSTAT (Food and Agriculture Organization Statistics). 2008. Country Profile on potato production and utilization: Ethiopia.

Franklin, K.A. 2009. Light and temperature signal crosstalk in plant development. *Current Opinion in Plant Biology*, 12: 63-68.

Garcia-Gonzales R, Quiroz K, Carrasco B, Caligari P. 10010. Plant tissue culture: Current status, opportunities and challenges. *Cienciae Investighatigation Agriculture,* 37(3): 5-30.

Gebremedhin Woldegiorgis.2001. National potato research program report. Ethiopian Agricultural Research Organization,Holetta Agricultural Research Center.

Geddes, A. M. W. 1990. The relative importance of crop pests in sub-Saharan Africa.NRI, Kent, UK: Natural Resources Institute Bulletin No. 36.

Gomes, J., peixoto, F., Sang, H., and Torres, P.J. 2006. Study of the temperature effect in three Chestnuts (*Castanea sativa* Mill.) cultivars behaviour. *Journal of Plant Physiology*, 163: 945-955.

Gomez-Alpizar, L., Carbone, I. & Ristaino, J.B. 2007. An Andean origin of Phytophthora infestans inferred from mitochondrial and nuclear gene, 17: 794-798.

Goodwin, P. B., Kim Y. C. and Adisarwanto T. 1980. Propagation of potato by shoot tip culture. Potato Res, 23:9-18.

Gopal, J., Kumar, V., Kumar, R. & Mathur, P. 2013. Comparison of different approaches to establish a core collection of andigena (*Solanum tuberosum* group andigena) potatoes. *Potato Research*, 56(1): 85 98.

Hartmann, H.T., Kester, E.D. and Davies, T.J.F. 1990. *Plant propagation: Principles and Practices*, *5th Edition*. USA, 459-601.

Hashem, A,Hussain,M.M.and Monnikhof,G. 1990. Seed potato Production at the private sector in Bangladesh .In: Seed potato in Bangladesh. Bangladesh Agricultural Development Corporation, Dhaka.pp, 100-105.

Haverkort, A.J., M.J. van Koesveld, H.T.A.M. Schepers, J.H.M. Wijnands, R. Wustman and X.Y. Zhang. 2012. Potato prospects for Ethiopia: On the road to. Praktijkonderzoek Plant and Omgeving, PPO Publication No. 528, the Netherlands and pp, 1-66.

HAWKES, J. G. 1978. Biosystematics of the potato. In The Potato Crop: *The Scientific Basis for Improvement* (P. M. Harris, Ed.). pp, 15-69. Chapman and Hall. London.

Hirpa, A., Meuwissen, M.P.M, A, Lommen, W.J.M. A.O. Lansink, A. and P.C. Struik. 2010. Analysis of seed potato systems, Am. J. Potato Res, 87: 537-552.

Hoque ME. 2010. *In vitro* tuberization in potato (*Solanum tuberosum* L.). *Plant Omics Journal*, 3(1): 7-11.

Hoque, M.I, Mila, N.B, Khan, M.S, Sarker, R.H, Islam, A.S .1996. Shoot regeneration and *in vitro* microtuber formation in potato (*Solanum tuberosum* L.). *Bangladesh* J. Bot, 25(1): 87-93.

Hossain, M.J. and Sultana, N. 1998. Effect of Benzyl Amino Purine (BAP) and Chloro Choline Chloride (CCC) on *in vitro* tuberization of potato. *Bangladesh Journal of Agricultural Research*, 23(4): 685-690.

Husain, M.K., Anis, M. 2009. Rapid *in vitro* multiplication of *Melia azedarach* L. (a multipurpose woody tree). *Acta Physiologiae Plantarum*, 31(4): 765-772.

Hussain, I., Muhammad, A., Chaudhary, Z., Asghar, R., Naqvi, S.M.S., Rashid, H .2005. Morphogenic potential of three potato (*solanum tuberosum*) cultivars from diverse explants, a prerequisite in genetic manipulation. Pak. J. Bot, 37(4): 889-898.

Ibaraki, Y. and Nozaki, Y. 2005. Estimation of light intensity distribution in a cultur vessel. *Plant Cell Tissue and Organ Culture*, 80: 111-113.

International Union for the Protection of New Varieties of Plants (UPOV). 1986. Guidelines for the conduct of tests for distinctness, homogeneity and stability. TG/23/5. Potato (Solanum *tuberosum L.), Geneva*, 27p.

Jana, R.K. 1982. Status of Irish potato cultivated in East Africa and its future. In R.L. Villarreal and Griggs T.D (eds). Irish potato, Proc. ml.Symp.AVRDC.Talwan, China, pp .63-76.

Kagona, J.D.K. 2008. The incidence of bacterial wilt *(Ralstonia solanacearum)* in informal potato planting materials used by farmers in Dedza and Ntcheu districts of Malawi. Norway: *Norwegian University of Life Sciences*.

Khadiga, G., Elaleem, A., Rasheid, S. M. and Mutasim, M. K. 2009. Effect of cultivar and growth regulator on *in vitro* micropropagation of potato (*Solanum tuberosum* L.). *American- Eurasian Journal of Sustainable Agriculture,* 3(3): 487-492.

Khadiga, G.A.E., Rasheid, S.M. and Khalafalla, M.M. 2009**.** Effect of plant growth regulators on callus induction and plant regeneration in tuber segment culture of potato (S*olanum tuberosum L*.) cultivar Diamant. *African. Journal of Biotechnology*, 8: 2529-2534.

Kishor, R.H. and S. Devi. 2009. Induction of multiple shoots in a monopodial orchid hybrid (*Aerides vandarum Reichb*.f X *Vanda stangeana Reichb*.f) using thidiazuron and analysis of their genetic stability. Plant Cell Tiss Organ Cult, (2009) 97: 121-129.

Knapp, S. 2008. Propitious esculent: The potato in world history. *Nature*, 455(7210): 170-171.

Knapp, S., Bohs, L., Nee, M. and Spooner, D.M. 2004. Solanaceae - a model for linking genomics with biodiversity. *Comparative and Functional Genomics*, 5(3): 285-291.

Kozai, Y., Kubota, C. and Jeong, B.R. 1997. Enviromental control for the large-scale production of plant through *in vitro* techniques. *Plat Cell Tissue and Organ Culture*, 51: 49-56.

Kuhn, N., Ormeno, N.J., Jaque, Z.G., and Perez, F.J. 2009. Photoperiod modifies the diurnal expression profile of VvPHYA and VvPHYB transcripts in the field grown grapevine leaves. *Journal of Plant Physiology*, 66: 1172-1180.

Kumar, K. and Rao, I.U. 2012. Morphophysiologicals problems in acclimatization of micropropagated plants in-*ex vitro* conditions- A Reviews. *Journal of Ornamental and Horticultural Plants* 2 (4): 271-283.

Lesar, H., Hlebec, B. Čeranič, N. Kastelec, D. and Luthar, Z. 2012. Acclimatization of terrestrial orchid *Bletilla striata* Rchb.f. (Orchidaceae) propagated under *in vitro* conditions. *Acta Agriculturae Slovenica* ,99 (1): 69-75.

Mack, M. 2009. Micro-propagation and secondary metabolites of *Sclerocarya birrea*. PhD uDissertation, Research Centre for Plant Growth and Development School of Biological Convention Sciences University of Kwazulu-Natal, Pietermaritzburg micropropagated plants to *ex vitro* conditions. *Biologia Plantarum,* 42 (4): 481-497.

Mih, M. and Atiri, G.I. 2006. Overview of irish potato viruses and viruses diseases. *Journal of Plant Disease*, 1: 80-83.

Miransaria, M. and Smith, D.L. 2014. Plant hormones and seed germination: Review. *Environmental and Experimental Botany*, 99: 110–121.

Mohamed, M.F., Mohamed, M.A.A. and Ahmed, A.M. 2007. Differential axillary-bud proliferation responsesof two sweet potato cultivars to benzyl adenine and thidiazuron. *Assiut University bulletin environment Research,* 10(2): 21-30.

Molla, M.M.H., Nasiruddin, K.M., Al-Amin, M., Khanam. D., Salam, M.A. 2011. Effect of Growth Regulators on Direct Regeneration of Potato. *International Conference on Environment and Industrial Innovation,* vol.12, IACSIT Press, Singapore.

Motallebi-Azar, A., Kazemiani, S., Kiumarsie, F., Mohaddes, N. 2011. Shoot Proliferation from node explants of potato (*Solanum tuberosum* cv. Agria). Effect of Different concentrations of NH4NO3, hydrolyzed casein and BAP. *Romanian Biotechnol, Letters,* 16: 6181-6186.

Muhammad, A.A. and Hakoomat, A. 2004. Effect of culture medium on direct organogenesis from different explants of various potato genotypes. *Biotechnology*, 3 (2):187-193.

Murashige, T. 1974. Plant propagation through tissue culture. *Annual Review of Plant Physiology*, 25: 135.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cell cultures. *Physiologia Plantarum,* 15: 473-497.

Mustafa, B.M. and Sarker, R.H. 2002. Regeneration and agrobacterium-mediated genetic transformation of two indigenous potato varieties of Bangladesh. *Plant Tissue Culture*, 12(1): 6977.

Muthoni, J., Mbiyu, M. and Kabira, J.N. 2011. Up-scaling production of certified potato seed tubers in Kenya: Potential of aeroponics technology. *Journal Horticultural Forestry*, 3: 238-243.

Naghmouchi, SKhouja, M. L., Rejeb, M.N. and Boussaid, M. 2008. Effect of growth regulators.and growth regulators on micro propagation of peach rootstock. GF 677. *Pak J. Bot,* **35**: 331-338.

Naik, P.S. 2005. Achieving self-sufficiency in quality seed production in roots and tuber crops with special reference to potato, in: K.L. Chadha, B.S. Ahloowalia, K.V. Prasad, S.K. Singh, (Eds.), and Crop improvement and production technology of horticultural crops. *New Delhi: The Horticultural Society of India*. Pp, 243-255.

Naik, P.S. and Karihaloo, J.L. 2007. Micropropagation for production of quality potato seed in asia-pacific. Asia-Pacific Consortium on Agricultural Biotechnology, NewDelhi, India, pp: 54. http://www.apcoab.org/uploads/files/1276940006potato pub.pdf.

Nyende, A.B., Schittenhelm, S., Mix-Wagner, G., Greef, J.M. 2005. Yield and Canopy growth characteristics of field grown synthetic seeds of potato. *European Journal of Agronomy*, 22, 175-184.

Patnaik, J. and Chand, P.K. 1996. Micropropagation of *Hemidesmus indicus* (L.) R. Br. through axillary bud culture. *Plant Cell Rep,* 15: 427-430.

Peter, G., Paul, D., Peter K., Mercy, W., Moses, N. and Thomas Z. 2007. Select the best trainers' manual. Positive selection to improve farm saved seed potatoes. CIP, Lima, Peru, 26pp. *Potato Association of American Hand book*. 2005. Sourced from:

Pospíšilová, J., Tichá, I., Kadleček, P., Haisel, D. and Plzáková, Š. 1999. Acclimatization of micropropagated plants to *ex vitro* conditions. *Biologia Plantarum,* 42 (4): 481-497.

Rabbani, A., Askari, B., Abbasi, N.A., Bhatti, M., Quraishi, A .2001. Effect of growth regulators on *in vitro* multiplication of potato. *Int. J. Agric. Biol*, 3(2): 181-182.

Roa, S., Barboza, C. and Zambrano, A. 2010. Yield stability of potato (Solanum tuberosum L.) variety for industrial processing in Tachira, Venezuela.*Revista De La Facultad De Agronomia De La Universidad Del Zulia,* 27(2): 173-192.

Roca, W.M., Bryan, J.E., Roca, M.R .1979. Tissue culture for the international transfer of potato genetic resources. *American Potato Journal*, 56: 1-10.

Roca, W.M., Espinoza, N.O., Roca, M.R., Bryan, J.E. 1978. A tissue culture methods for the rapid propagation of potatoes. *American potato Journal,* 55: 691-701.

Saini, R., Jaiwal, P.K. 2002. Age, position in mother seedling, orientation, and polarity of the epicotyl segments of blackgram (*Vigna mungo* L. Hepper) determines its morphogenic response. *Plant Science*, 163(1): 101-109.

Sanatombi, K. and Sharma, G.J. 2006. *In vitro* regeneration and mass multiplication of (*Ipomoea batatas* (L.) Lam.). *Artificial Environment*, **4**: 205-208.

Sarker, R. H. and Mustafa, B.M. 2002. Regeneration and Agro bacterium mediated genetic transformation of two indigenous potato varieties of Bangladesh, Plant Tissue Cult. 12(1): 69-77.

Shroti, R.K., Upadhyay, R., Niratkar, C. and Singh, M. 2012. Micropropagation of *Dandrocalamus asper* through inter nodal segment. *Bulletin of Environment, Pharmacology and Life Sciences,* 1 (3): 58-60.

Sood, A., Ahuja, P.S., Sharma, M., Sharma, O.P. and Godbole, S. 2002. *In vitro* protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro. *Plant Cell, Tissue and Organ Culture,* 71: 55-63.

Spooner, D.M., McLean, K., Ramsay, G., Waugh, R. and Bryan, G.J. 2005. A single domestication for potato based on multilocus amplified fragment, 37: 169-180.

Thiart, S. 2004. Manipulation of growth by using tissue culture techniques. Comb. Proc. *Int. Plant Prop. Soc,* 53: 61–67.

Thiruvengadam, M., Rekha, K.T. and Chung, I.M. 2011. Rapid *in vitro* micropropagation of *Bambusa oldhamii* Munro. *The Philippine Agricultural Scientist,* 94 (1): 1-8.

Triqui, Z., Guédira, A., Chlyah, A., Chlyah, H., Souvannavong, V., Haïcour, R. and Sihachakr, D. 2007. Effect of genotype, gelling agent, and auxin on the induction of somatic embryogenesis in potato, **1**: 116-126.

Tsoka, O., Demo, P., Nyende and K. Ngamau. 2012. Potato seed tuber production from *in vitro* and apical stem cutting under aeroponic system. *African Journal of Biotechnology,* 11: 12612-12618.

Tuan, T.T., T.u., H.L., D.D. and D.u., T.X. 2012. The increase in *in vitro* shoot multiplication rate of *Dendrocalamus asper* (Schult. f.) Back. ex Heyne. *TẠP CHÍ SINH HỌC,* 34(3se): 257-264.

Tugrul, S. and Samanci, B. 2001. Factors affecting tuber formation in potato (*Solanum tuberosum* L.). *Potato Abstr,* 26: 86

Velayutham, P. and Ranjitha Kumari, B.D. 2003. Direct shoot regeneration from leaf explants of chicory (*Cichorium intybus* L.). *Plant Cell Biotech Mol Bio,* 4(3&4): 125-130.

Velayutham, P., Jahir Hussain, G. and Baskaran, P. 2005. *In vitro* plantlet formation from nodal explants of *Plumbago zeylanica* L. - an important medicinal plant. J. Swamy Bot. Cl. 22: 117-120.

Venkataiah, P, Chirstophor T. and Subhash, K. 2006. *In Vitro* Shoot Multiplication and Plant Regeneration in Capsicum and Potato Species Using Thidiazuron. *Science Horticulture,* 107: 117-122.

Villafranca, M. J., Vermendi J., Sota V. and Mingo-Castel, A. M. 1998. Effect of physiological age of mother tuber and number of subcultures on *in vitro* tuberization of potato (*Solanum tuberosum* L.). *Plant Cell Reproduction,* 17: 787-790.

Vinterhalter, D., B. Vinterhalter and Calovic .1997. The relationship between sucrose and cytokinins in the regulation of growth and branching in potato cv. Désiree shoot cultures. *Acta Hortic,* 462: 319-323.

Waikhom, S.D. and Louis, B. 2014. An effective protocol for micropropagation of edible bamboo species (*Bambusa tulda* and *Melocanna baccifera*) through nodal culture. *Hindawi Publishing Corporatione, Scientific World Journal* Volume 2014, Article ID 345794: 1-8.

Wang, P.J. and C.Y. Hu. 1982. *In vitro* mass tuberization and virus-free-seed potato production in Taiwan. *Am. J. Potato Res,* 59: 33-37.

Wescott, R. J., Henshaw, G. G. and Roca, W. M. 1977. Tissue culture and storage of potato germplasm: Culture initiation and plant regeneration, *Plant Sci. Lett*, 9:309-315.

West cott RJ, Henshaw GG, Roca WM .1977. Tissue culture Storage of potato geramplasm Culture initiation and Plant regeneration. *Plant science Letter*, 9:309-315.

Woldegiorgis Gebremedhin.1994. Prospects of seed potato production in Ethiopia. *Proceedings of the 2nd National Horticultural Workshop of Ethiopia,* December 1-3. 1992.

Zaerr, J.B. and Mapes, M.O. 1982. *In* Bonga, J.M., Durzan, D.J. (Eds.). *Tissue Culture in Forestry*. Martinus Nijhoff/Dr. W. Junk Publisher,The Hague/Boston/London. Action of growth regulators, pp. 231 – 255.

9. APPENDIX

Appendix Table 1: Nutrient of stock solution Composition and concentration of full strength Murashige and Skoog Medium (1962)

Compounds	Concentration(mg/l)for stoke solution	Amount dispensed for 1 liter media(ml/l)
Macronutrients		
NH4NO3	16.5	50
CaCl2.2H2O	4.4	25
MgSO4.7H2O	3.7	25
KNO3	19.0	50
KH2PO4	1.7	25
Micronutrients		
H3BO3	0.62	5
FeSO4.7H2O	2.78	5
Fe-Na-EDTA	3.73	5
CoCl2.6H2O	0.0025	5
CuSO4.5H2O	0.0025	5
MnSO4.H2O	2.23	5
KI	0.083	5
Na2Mo.2H2OO	0.025	5
ZnSO4.7H2O	8.6	5
Common Organics additives		
Myo-insitol	10	10
Glycine	0.2	0.2
Glutamine	0.1	0.1
Proline	0.05	0.05
Nicotinic acid	0.05	0.05
Pyridoxine (B6)	0.05	0.05
Thiamine (B1)	0.01	0.01
Plant growth Regulators(mg/ml)		
BAP	25	1.0,2.0,3.0,4.0
IAA	25	0.5,1.0,1.5,2.0
BAP	25	0.5,1.0,1.5,2.0
Kn	25	0.5,1.0,1.5,2.0
IBA	25	0.25,0.5,0.75,1.0
NAA	25	0.25,0.5,0.75,1.0

Others: Sucrose (30gm/l), Agar (6gm/l) are used Source: Beyl (2005)

Appendix Table 2: Specific solvents of Plant Growth Regulators

PGR	Solvents
IAA,IBA,NAA	**NaOH(0.1N)**
BAP,KN	**HCL(0.1N)**

Appendix Table: 3. ANOVA for the effect of initiated explants of the two Irish potato varieties

Source of variation	DF	Mean square of initiated explants (%)
Varieties	1	20.833ns
Hormones	4	463.541***
Var*Hor	4	20.833*
Error	18	59.606
R^2		0.654
CV (%)		10.409
LSD		13.244

Note: ***=highly significant (P< 0.001) at P=0.05 significant level; R2= coefficient of determination; CV = coefficient of variation; DF= Degree of freedom; LSD=least Significant Difference; %=percentage

Appendix Table: 4. ANOVA for the effect of BAP and KN on *in vitro* shoot multiplication rate at number of nodes, number of days to shoot emerged, number of shoots proliferated, shoot length, shoot fresh and dry weight.

Source of variation	DF	Mean square of multiplied shoots					
		No. of days to shoot emergence(n)	No.of nodes per/exp. (n)	No.ofshoots per/exp .(n)	Shoot Length per/exp .(cm)	Shootfresh Wt.per/exp .(g)	Shootdry Wt.per/exp. (g)
Varieties	1	0.03**	0.71ns	1.34*	0.04ns	0.005**	0.02ns
Hormones	14	6.51***	25.68***	26.87***	16.27***	0.02***	0.07**
Var*Hor	14	0.59*	1.95***	1.99***	0.50*	0.02*	0.08*
Error	58	0.27	0.40	0.40	0.27	0.04	0.01
R^2		0.87	0.94	0.95	0.94	0.51	0.49
CV (%)		14.62	10.99	11.01	10.92	7.03	11.08
LSD		0.85	1.04	1.04	0.85	0.11	0.05

Note: ***, highly significant (P< 0.001) at P=0.05 significant level; R2= coefficient of determination; CV = coefficient of variation; DF= Degree of freedom; LSD=least Significant Difference; %=percentage

Appendix Table: 5. ANOVA for the effect of IBA and NAA on *in vitro* rooting of micro shoots

Source of variation	DF	Mean square of induced roots				
		Days to rooting Response(n)	Root no. Per shoot .(n)	Length of RootPer shoot.(cm)	Root fresh wt. Per shoot.(g)	Root dry wt. Per shoot.(g)
Varieties	1	1.88ns	157.34***	27.78***	0.02ns	0.04ns
Hormones	14	20.73***	162.52***	13.89***	0.08***	0.01*
Var*Hor	14	17.49***	3.73*	0.94**	0.05*	0.03**
1Error	58	1.05	3.36	0.38	0.02	0.07
R^2		0.89	0.93	0.92	0.61	0.40
CV (%)		11.18	8.15	6.22	9.20	12.35
LSD		1.68	2.99	1.06	0.07	0.04

Note: ***=highly significant (P< 0.001) at P=0.05 significant level; R^2= coefficient of determination; CV = coefficient of variation; DF= Degree of freedom; LSD=leastSignificant Difference

Appendix Figures indicates procedures in tissue culture laboratory work.


A


B

Figure: 1. cutting off mother explants from green house plantlet (A-B). Where, (A) Gudenie and (B) Belete Irish potatoes varieties.

.


A


B

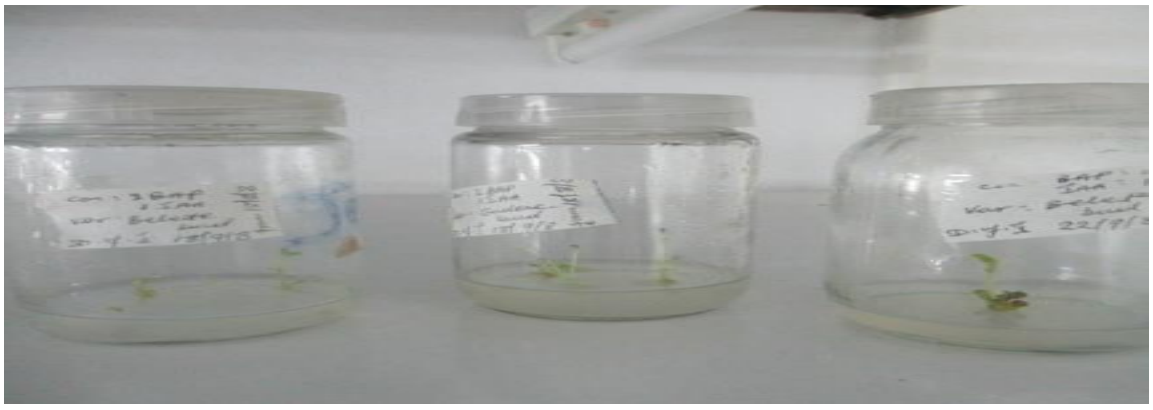
C

Figure: 2. *In vitro* shoot induction from the Lateral bud cultures of the two Irish potato varieties (A-C) .where, (A) Gudene varieties, (B) Belete varieties, (C) both Gudene and Belete varieties.

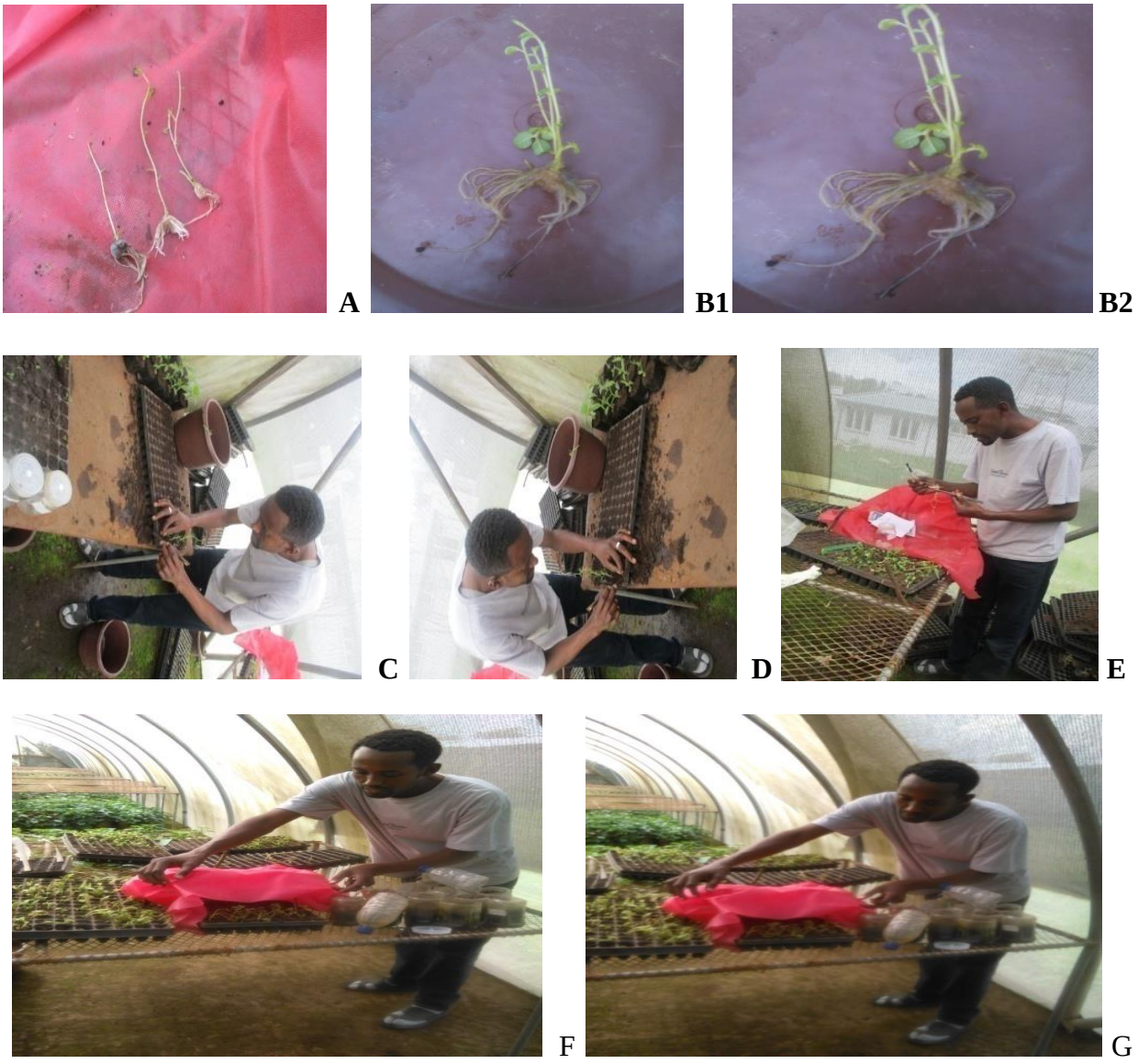

Figure: 3. Acclimatization of plantlets of Irish potato in the greenhouse (A-G). where, (A) washed Belete plantlet from agar medium;(B1 and B2) washed Gudenie plantlet from agar medium;(C and D) the explant planted on cell tray containing the culture Substrates,(E) when I was observed the planted shootletes were survived or not; (F - G) the explant planted on cell tray for three weeks, that acclamatized plants developed well green shoots.