Development of *in Vitro* Conservation Protocol for Banana Germplasm through a Slow Growth Technique

Obse Zewde Buli

A Thesis Submitted to the Department of Applied Biology,

School of Applied Natural Science,

Presented in Partial Fulfilment of the Requirement for the Degree of Master of Science in Applied Biology (Specialization in Biotechnology).

Office of Graduate Studies

Adama Science and Technology University

March, 2023

Adama, Ethiopia

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Obse Zewde Buli

Advisor:

Geleta Dugassa (Ph.D)

Co- Advisors:

Adugna Mosisa

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DECLARATION

I hereby declare that this MSc. Thesis entitled **"Development of in Vitro Conservation Protocol for Banana Germplasm through a Slow Growth Technique"** is my original work. Thus, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Name: **Signature** **Date:**

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We, the advisors of this MSc. thesis hereby certify that, we have read the revised version of the thesis entitled **"Development of In Vitro Conservation Protocol for Banana Germplasm through a Slow Growth Technique"** prepared under our guidance by **Obse Zewde** submitted in partial fulfillment of the requirements for the degree of Mater's of Science in Biotechnology. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

Major Advisor: Geleta Dugasa (PhD) Signature_____ Date_____

Co-advisor: Adugna Mosisa Signature _____Date _____

Approval of Advisors

I/we, the advisors of the MSc. thesis entitled **"Development of *In Vitro* Conservation Protocol for Banana Germplasm through a Slow Growth Technique"** and developed by **Obse Zewde,** hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor: Geleta Dugasa (PhD) Signature_____ Date_____

Co-advisor: Adugna Mosisa Signature _____ Date _____

Approval of Board Reviewers

We, the undersigned, members of the Board of Examiners of the thesis by **Obse Zewde** have read and evaluated the thesis entitled **"Development of *In Vitro* Conservation Protocol for Banana Germplasm through a Slow Growth Technique"** and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirements of the degree of Master of Science in Biotechnology.

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ABRIVIATION

BA	Benzyl Adenine
BAP	Benzylaminopurine
CK	Cytokinin
CRD	Completely Randomized Design
FAO	Food and Agricultural Organization of the United Nations
IAA	Indole Acetic Acid
IBA	Indole Butrayl Acid
INIBAP	International Network for the Improvement of Banana and Plantain
IUCN	International Union for Conservation of Nature
KI	Kinetin
MARC	Melkasa Agricultural Research Center
Mtr	Meta-Topolin riboside,
MemT	meta-Methoxy topolin, MemT
MemTTHP	Meta-Methoxytopolin9-tetrahydropyran-2-yl,) Methenoadenosine
N6	
NAA	Naphtal Acetic Acid
PGR	Plant Growth Regulator
TDZ	Thidiazuron

Abstract

The study mainly focused on to development of an *in vitro* conservation protocol for three randomly selected banana varieties through a slow growth technique. Banana (*Musaspecies*) is one of the most important fruit crops grown widely in Ethiopia and it plays a huge role in the rural communities by ensuring food security, generating income and creating job. They are the fourth most important food crops after rice, wheat, and Maize. Globally, the major banana producing countries are India, China, Brazil, Ecuador and Philippines. In Africa, Angola, Tanzania, Kenya, Brundi and Cameron are the major banana producers. Major producers of banana in Ethiopia are found in Southern and south western Ethiopia, with 59.64% (53,956.13 ha) of the total fruit area and 68% (478,251.04 ton). Field conservation of vegetative propagated crops such as banana is a key concern for germplasm curators Nowadays, slow growth storage (SGS) technique becomes an economical *in vitro* method to preserve several plant species by controlling the growth and development of plantlets. As a procedure surface sterilized shoot tip meristem of about one-centimeter square explants were inoculated on Murashige and Skoog (MS) medium supplemented with plant growth regulator (PGR) and different types of plant growth retardant (PGR) such as manitol, sorbitol and sucrose SGR at sorbitol and manitol 0.2,0.3, 0.5 and Manitol 0.3, 0.5, 0.7 concentration was found to be the best for long storage of banana varieties under in vitro condition.ANOVA revealed existence of significant difference among varieties, confirming the need to develop varieties specific protocols. The contaminated and survived per cent among the varieties are Poyo 13.3, Giant 8.90, Grande 20.0 per cent are contaminated and the survival rate are in Poyo 86.70, Giant 91.10, Grande 80.00 percent are survived in each variety and treatment. The generated plantlets had survived for 3, 6 and 9 months. Therefore, the present study successfully designed an *in vitro* preservation technique for banana to safeguard its biodiversity.

Key word: *ANOVA, Banana, In vitro conservation, Plant growth retardant, Slow growth storage.*

1. Introduction

Banana is an elongated, edible fruit botanically a berry produced by several kinds of large herbaceous flowering plants in the genus Musa Morton, (Julia F. 2013). In some countries, bananas used for cooking may be called "plantains", distinguishing them from dessert bananas. The fruit is variable in size, color, and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind, which may be green, yellow, red, purple, or brown when ripe Armstrong(Wayne,2013). The fruits grow upward in clusters near the top of the plant. Almost all modern edible seedless (parthenocarp) bananas come from two wild species Musa acuminata and Musa balbisiana. The scientific names of most cultivated bananas are Musa acuminata, Musa balbisiana, and Musa paradisiaca for the hybrid Musa acuminata M. balbisiana, depending on their genomic constitution. The old scientific name for this hybrid, Musa sapientum, is no longer used Merriam-Webster (2013).

Part of the ripe bananas is used to produce crunchy slices of dehydrated banana, or even banana flour. In some areas of Eastern Africa, ripe bananas are used to make a low alcohol beer. Other products are puree, juice, liquor and sweets. It also contributes significantly to the income (both in hard and soft currency) and employment generation efforts of many developing countries, in addition to being key staple foods (FAO, 2003).

There are more than 1000 varieties of bananas produced and consumed locally in the world, but the most commercialized is the Cavendish type of banana, which accounts for around 47 percent of global production (FAO, 2017). There are three categories of African banana that are East African banana (primarily dessert) bananas, the African plantain banana grown mainly in Central and West Africa, and the East African highland banana, used for cooking and beer preparation (Zinabu Ambisa.,2019). Globally, the major banana-producing countries are India (15% of total production), China, Brazil, Ecuador and Philippines (5–6% each) (FAOSTAT, 2020). In Africa, Angola, Tanzania, Kenya, Burundi and Cameroon are the major banana producers (FAOSTAT, 2020). Major producers of banana in Ethiopia are found in southern and south-western Ethiopia i.e., Arba Minch, Mizan Teferi, and Tepi (Seifu Gebre-Mariam, 1999). It covers about 59.64% (53,956.13

ha) of the total fruit area, about 68% (478,251.04 ton) of the total fruit produced, and about 38.3% (2,574.035) of the total fruit producing farmers (Zinabu Ambisa., 2019). Although Ethiopia is rich with a large number of banana germplasm, its production and productivity are largely affected by several biotic and abiotic stress Field conservation of vegetative propagated crops such as bananas is a key concern for germplasm curators in impoverished nations such as Ethiopia. Pests and illnesses, as well as other pressures (drought, cold, hailstorm, etc.) and wildfires, are some of the causes. Tissue culture, on the other hand, has been proved to be an effective method of storing a variety of vegetatively propagated commercial crops such as potatoes, palms, forest species, and fruit crops (Rao, 2004). Over the last three decades, immense effort has been done in the area of research for *in vitro* conservation of such plant genetic resources, including bananas, via tissue culture (Neitzsch, 1983 and Moges, 2003). Meristem derived explants such as shoot tips and buds were mostly suggested for their conservation under slow rate of growth (Bajji, 1986 and Shibli., 1994). Minimal growth conditions could be achieved through induction of osmotic stress with mannitol or sucrose, reduced temperature and/or low light intensity (George, 2013). Slow growth procedures have been developed for a wide range of species; they are routinely used for conservation of genetic resources of only a few species including *Musa* spp. This technique of germplasm conservation is as well acclaimed useful in the subsequent distribution of the materials at any time when needed (Wilkins, and Engels JMM. 2004).

Among Germplasm conservation system, *in vitro* Germplasm conservation through tissue culture system is considered to be best option in minimizing storage space, labor and costs. *In vitro* shoot cultures are used for the medium-term conservation of plant germplasm. *In vitro* conserved tissue follows multiplication by micro-propagation techniques. Micro propagation is the technique of clonal propagation of plants by tissue, cell and organ culture methods. It involves the aseptic culture of explants of tissues and organs in closed vessels using defined culture media in a controlled environment. The method is being widely used for mass propagation of elite and endangered plant species, medicinally important plants of commercial and social value, and for Germplasm conservation. Micro-propagation of *in vitro* conserved Germplasm also holds promise for regeneration of true to type planting materials. Hence, a plant tissue culture strategy-based micro propagation technique warrants mass cloning of elite plant that are uniform in their chemical and genetic constituents to that of the mother plant (Kiran Sharma, 2015). Commercial *in vitro*

multiplication of banana using shoot explants can increase the rate of seedling production and improve the seedling quality such as uniformity and being true to parental type. For *in vitro* germplasm conservation, organized cultures, especially the shoot tips are preferable because they have a capacity of maintaining strict genotypic and phenotypic stability under tissue culture conditions (Bennici, 2004). *In vitro* storage of plant genetic resources becomes a very useful alternative for genetic variability conservation, crop improvement programs, and production of certified seeds. The minimal growth plant method is a common midterm *in vitro* conservation system and involves the reduction of the plant metabolism and the increase of the subculture time without affecting the tissue or plant viability (Sarkar, 1999). To reduce the plant metabolism, the environmental conditions (temperature, photoperiod, light intensity, and so on) or culture media composition (organic and inorganic nutrients, osmotic regulators, or growth inhibitors) can be modified along the incubation period (Engels JMM. 2004), There are other alternatives to increase the *in vitro* conservation period such as the reduction of the oxygen flow and shoot defoliation (Bajaj, 2005). Bajaj (2005) 6also stated that adding osmotic regulators such as mannitol, sorbitol, or sucrose can result in a substantial reduction of *in vitro* plant growth (Bajaj, 2005). This study is therefore initiated to develop/optimize a proper protocol for conservation of dessert and cooking banana germplasm materials through the slow growth technique.

1.1 Statement of the Problem

Bananas play an important role in the food security of developing countries, especially in sub-Saharan Africa. Banana is the most produced fruit in Ethiopia for food and commercial purposes. Characterization and conservation of the available banana genetic resource is a potential option to improve its agronomic traits. The traditional method of preserving banana by maintaining clonal collections in the field are complained to need large space, tiresome, costly, and at constant risk of serious losses of germplasm because of biotic and abiotic stresses. Nowadays, *ex situ* conservation of plant genetic resources using the *in vitro* conservation method is widely used in several countries. *In vitro* conservation through slow growth storage (SGS) technique that involves controlling the growth and development becomes best alternative, economical, and less laborious. In Ethiopia, there is no/ limited research efforts to conserve banana germplasm under *in vitro* conditions.

Therefore, the present study targeted to develop an *in vitro* conservation protocol for banana Germplasm through a slow growth technique.

However, there are constraints in the production of banana. Among the banana production constraints, low propagation, low quality and quantity of yield. There is a need for biotechnological applications to improve the production of disease free, develop micro propagation protocols and quality plantlets. Therefore, to optimize development of a reliable and efficient regeneration and transformation protocol of farmer-preferred cultivars using in vitro conservation of banana germplasm through a slow growth technique for mass propagation of banana.

1.2 Significance of the Study

Bananas are one of the most studied plants in the field of plant biotechnology. The huge economic importance and the inherent problems associated with conventional propagation of bananas makes the species one of the highly prioritized research crops globally. Generally, the importance of this study is to develop *in vitro* conservation of banana Germplasm through a slow growth technique used in different labs for the rapid micro propagation and dissemination of elite banana varieties. It protocol provide an economical, simple and safe conservation of the available banana genetic resource. The work will have a significant impact in addressing food security issues by increasing production and productivity of the banana.

1.3 Objectives

1.3.1 General objective

Development of *In Vitro* Conservation Protocol for Banana Germplasm through a Slow Growth Technique.

1.3.2 Specific objectives

- ✓ To develop a proper protocol for conservation of different banana variety.
- ✓ To identify best slow growth retardant and concentration for *in vitro* conservation of banana.
- ✓ To determine the manitol, sorbitol, sucrose, for banana conservation.
- ✓ To evaluate the performance of banana varieties after short term storage under in vitro conservation.

1.4 Research question

1.4.1 Research question

- ➢ What is the optimum concentration of mannitol, sorbitol and sucrose for efficient *in vitro* conservation of banana through slow growth storage technique?
- ➢ Is there any significance difference in banana Germplasm performance after short term *in vitro* conservation?
- ➢ What are the optimum greenhouse conditions for efficient hardening of *in vitro* generated banana plantlets?

2. Literature review

2.1. Origin, domestication and distribution of banana

The genus *Musa* which is about 50 million years old has distributed globally i.e. most importantly in the Southeast Asian region where it is believed to be originated (OECD, 2009). *Musa* is widely distributed in the tropics, from 175˚ E to 150˚ W longitude and from 30˚ N to 23˚ S latitude (Nayar, 2010). All the wild bananas are warm-region plants and this genus has a limited tolerance for low temperature but a few species have resistance for a cool temperature and no species have resistance for drought (Nayar, 2010).

2.2 Morphology of the banana plant

Drawing of a Banana mat showing the 'true' stem (shown in blue) inside the pseudo stem. The clump formed by the fruit-bearing stem, the suckers and the rhizome is called a mat. In commercial plantations the number of suckers is kept down by pruning.

The banana is a tree-like perennial herb. It is an herb because it does not have woody tissues and the fruit-bearing stem dies down after the growing season. It is a perennial because suckers, shoots arising from lateral buds on the rhizome, take over and develop into fruit-bearing stems.

What looks like a trunk is not a woody stem but a pseudostem, a compact assemblage of overlapping and spirally arranged leaf sheaths. The 'true' stem is made up of three parts: the underground rhizome, the aerial stem to which are attached the leaves, and the peduncle to which is attached the inflorescence (Mafla G, Panis B,). The stem starts on the rhizome's apical meristem, grows inside the pseudostem, and ends in the male bud.

Mat is the banana-specific horticultural term for the clump formed by the rhizome, the fruit-bearing stem (or stems as more than one stem can be fruiting at the same time) and the suckers. Some people say stool. The botanical term is genet. The above-ground shoots are called ramets. Barring mutations in the lateral buds, the shoots on a genet are genetically identical to each other (i.e. clones).(Debouck D, Dumet D, Escobar R ,2011,) Wild species of bananas also form genets but, unlike cultivated bananas, they also reproduce sexually since their flowers are fertile. Pollination is required for the ovules to develop into seeds, which in turn stimulates pulp development in the fruit. Banana cultivars, which have flowers that are mostly sterile, produce fruits parthenocarpically, in the absence of pollination. Root system The root system is the means by which the plant takes up water and nutrients from the soil. The roots are produced by the underground structure called a rhizome . The primary roots originate from the surface of the central

cylinder (see below), whereas secondary and tertiary roots originate from the primary roots. Rhizome the rhizome is commonly referred to as a corm, and occasionally as a bulb, but the botanically correct term is rhizome (Robinson, J.C., 2010) Rhizomes are characterized by horizontal underground growth; production of roots from multiple nodes; and production of clonal shoots (Panis B, and Panta A, 2011). Corms, on the other hand, are vertical enlarged compact stems with a tunic of thin leaves and roots arising from a single node6; features that do not describe well the banana's underground structure. In the vegetative phase, the terminal growing point of the rhizome, the apical meristem, has the form of a flattened dome. At the transition from the vegetative to the floral stage, the meristem area becomes convex and rises above the surrounding leaf bases. Flower bracts appear in place of leaves. Swellings, which differentiate into female flowers and then male flowers, appear at the base of the flower bracts .Pseudo stem the stem is visible in the center of the pseudo stem. The pseudo stem is the part that looks like a trunk. This 'false stem' is formed by the tightly packed overlapping leaf sheaths. The pseudo stem continues to grow in height as the leaves emerge one after the other and reaches its maximum height when the stem, which has been developing inside the pseudo stem, emerges at the top of the plant. Even though the pseudostem is very fleshy and consists mostly of water, it is quite sturdy and can support a bunch that weighs 50 kg or more (Dumet D, 2011) . Stem Banana plants stripped of their leaves to reveal the stem. The 'true' stem provides support to the leaves and flowers, some of which will develop into fruits. The leaves and flowers are attached to a node, and the sections between nodes are internodes. The stem is subdivided in three parts: the underground rhizome (see above), the aerial stem, and the peduncle (Robinson, J.C, 2010). The aerial stem begins to develop after the formation of flowers on the rhizome's apical meristem. As it develops, it carries the inflorescence and the leaf bases upwards inside the pseudostem. When the aerial stem emerges at the top of the plant, it is called the peduncle. The aerial stem is often called the floral stem. But this is wrong because the flowers are attached to the peduncle. Only the leaves are attached to the aerial stem (Escobar R, 2011). Leaf The leaf is the main photosynthetic organ. Each leaf emerges from the center of the pseudostem as a rolled cylinder (see cigar leaf below). The distal end of the elongating leaf sheath contracts into a petiole, that is more or less open depending on the cultivar. The petiole becomes the midrib, which divides the blade into two lamina halves. The upper surface of the leaf is called adaxial while the lower one is called abaxial. The first rudimentary leaves produced by a growing sucker are called scale leaves. Mature leaves that consist of sheath, petiole, midrib and blade are called foliage leaves. Lamina veins run parallel to each other in a long S shape from midrib to margin. Veins do not branch, which results in leaves tearing easily (Endress, P.K. 2010) . Cigar leaf The cigar leaf is a recently emerged leaf still rolled as a cylinder. The lapse of time in which a leaf unfolds varies. Under favourable climatic conditions, it takes about seven days, but it can take up to 15 to 20 days under poor conditions. The new leaf is tightly coiled, whitish, and particularly fragile. The extension at the tip of the leaf is called the precursory appendage.

After emergence, it withers and falls off (Clonal colony , 2017). .Sucker a sucker is a lateral shoot that develops from the rhizome and usually emerges close to the parent plant. Other names for sucker are keiki (in Hawaii) and pup. A sucker that has just emerged through the soil surface is called a peeper. A full grown sucker bearing foliage leaves is called a maiden sucker. Morphologically, there are two types of sucker: sword suckers (right on the photo), characterized by narrow leaves and a large rhizome, and water suckers (left on the photo), which have broad leaves and a small rhizome. Water suckers have a weak connection to the parent plant and as such will not develop into a strong plant. The number of suckers produced varies with the type of cultivar. The sucker selected to replace the parent plant after fruiting is called the follower or ratoon (Robinson, J.C. and Galan Saúco, V. 2010).

2.3. Taxonomy and genetics of banana

The genus Musa was created by Carl Linnaeus in 1753 Blench, Roger (2016). The name may be derived from Antonius Musa, physician to the Emperor Augustus, or Linnaeus may have adapted the Arabic word for banana, mauz. According to Roger Blench, the ultimate origin of musa is in the Trans–New Guinea languages, whence they were borrowed into the Austronesian languages and across Asia, via the Dravidian languages of India, into Arabic as a Wanderwort Blench, Roger (2016).

Musa is the type genus in the family Musaceae. The APG III system assigns Musaceae to the order Zingiberales, part of the commelinid clade of the monocotyledonous flowering plants. Some 70 species of Musa were recognized by the World Checklist of Selected Plant Families as of January 2013; several produce edible fruit, while others are cultivated as ornamentals Bailey, Liberty Hyde (1916).

The classification of cultivated bananas has long been a problematic issue for taxonomists. Linnaeus originally placed bananas into two species based only on their uses as food: Musa sapientum for dessert bananas and Musa paradisiaca for plantains. More species names were added, but this approach proved to be inadequate for the number of cultivars in the primary center of diversity of the genus, Southeast Asia. Many of these cultivars were given names that were later discovered to be synonyms (Valmayor et al. 2000).

In a series of papers published from 1947 onwards, Ernest Cheesman showed that Linnaeus's Musa sapientum and Musa paradisiaca were cultivars and descendants of two wild seed-producing

species, Musa acuminata and Musa balbisiana, both first described by Luigi Aloysius Colla. Cheesman recommended the abolition of Linnaeus's species in favor of reclassifying bananas according to three morphologically distinct groups of cultivars – those primarily exhibiting the botanical characteristics of Musa balbisiana, those primarily exhibiting the botanical characteristics of Musa acuminata, and those with characteristics of both. Researchers Norman Simmonds and Ken Shepherd proposed a genome-based nomenclature system in 1955 (Valmayor et al. 2000). This system eliminated almost all the difficulties and inconsistencies of the earlier classification of bananas based on assigning scientific names to cultivated varieties. Despite this, the original names are still recognized by some authorities, leading to confusion, Porcher and Michel H.(2002).

The accepted scientific names for most groups of cultivated bananas are Musa acuminata Colla and Musa balbisiana Colla for the ancestral species, and Musa × paradisiaca L. for the hybrid M. acuminata × M. balbisiana Porcher,Michel H.(2011).

2.4. Economic importance of banana

bananas exported worldwide in the period 1985-2002 grew at an unprecedented average annual rate of 5.3 percent; twice that of the previous 24 years (2.4 percent between 1960 and 1984). This expansion was accompanied by technological changes and changes in the world trade scenario including: the opening of socialist economies to world markets in the early 1990s; bilateral and multilateral efforts to liberalize trade (General Agreement on Tariffs and Trade-GATT and the World Trade Organization-WTO); rising environmental awareness (Montreal Protocol in 1987 and the Earth Summit in Rio de Janeiro in 1992); the creation of the Single European Market in 1993; an unprecedented period of economic growth fostered by multimedia technologies and “the new economy” in the developed world; the implementation of structural adjustment policies in banana producing countries; and a significant concentration of trade at retail level. The publication starts with an overview of the evolution of production and trade in the last 15 years, and follows with an in-depth description and analysis of the events and causes underlying such developments. It reviews banana production and exports in the major exporting regions of the world; the evolution of imports and of import policies of major markets; technology changes at production and transportation levels; environmental and social concerns, policies and instruments; and the roles of transnational companies in the world banana economy FAO, 2018.

2.5. Banana cultivation in Ethiopia

Banana, especially the dessert banana is the major fruit crop in Ethiopia leading both in area and production. Dessert banana is the major fruit crop that is most widely grown and

consumed in Ethiopia. It is cultivated in several parts of the country where the growing conditions are favorable. Especially in the south and southwestern as well as south eastern parts of the country, it is of great socioeconomic importance contributing significantly to the overall wellbeing of the rural communities including food security, income generation and job creation. About 104,421.81 hectares of land is under fruit crops production in Ethiopia. Bananas contributed about 56.79% of the fruit crop area followed by avocadoes that contributed 17.26% of the area. More than 7,774,306.92 quintals of fruits were produced in the country. Bananas, Mangoes Avocados, Papayas, and Oranges took up 63.49%, 13.50%, 10.47%, 6.99% and 3.93% of the fruit production, respectively Tekle et al,(2014). Local cultivars of banana were under cultivation in Ethiopia for long period of times. These local varieties are low yielder and less demand on market. As a result a number of high yielding banana varieties were introduced and adapted in the country. According to the ministry of Agriculture research and development (MoARD) (2006), in Ethiopia there are seven dessert and five cooking type banana varieties released by the research system. Besides, different local varieties that are produced in almost all part of the country by small-scale farmers as garden crop mainly for home consumption and in some cases for sale in local markets.

2.6 Types of banana grown

Cultivated bananas are derived from two species of the genus Musa, namely from Musa acuminate and Musa balbisiana. Musa acuminate originates from Malaysia, while Musa balbisiana originates from India. African banana is grouped into three categories, including East African (mainly dessert) bananas, the African plantain banana grown mainly in Central and West Africa, and the East African Highland banana, used for cooking and beer preparation (Ambisa et al., 2019).In Ethiopia, even though both dessert and cooking types/ varieties of banana are released by the research system, the types of varieties that are under production are dessert type that has been under production since the early 1970s. In major banana producing areas, farmers produce formerly recommended varieties such as Dwarf Cavendish, Giant Cavendish, and Poyo. Most of them produce Dwarf and Giant. They produce these varieties for market. Dwarf has short plant height, which is easy to manage, while Giant and Poyo have good fruit size and quality for market. Some farmers also grow Ducasse Hybrid variety, East African Highland Cooking banana, which is used as windbreak and tolerant to stresses such as drought. Ducasse hybrid is starchy type and is not preferred as dessert, but other African countries use it for brewing (Zinabu et al. 2019)

2.7. Nutritional Composition of Banana

Bananas are a nutritious fruit in terms of their carbohydrate and sugar contents. A ripe fruit contains as much as 22% of carbohydrate, mainly as sugar, and is high in dietary fiber, potassium, manganese, and vitamins B6 and C. Almost all the modern edible parthenocarpic bananas come from the two wild Species-M. acuminata and M. balbisiana. Other than fresh fruits, it can be consumed as processed in various forms like chips, powder, flakes, etc. Banana pseudo stem is chopped and used as cattle feed. A medium sized banana has about 105 calories.

2.8. Banana Propagation

2.8.1. Conventionna propagation

Include how banana propagates through conventional method, and also the problems associated with this method in terms of getting disease free planting material in large amount.

2, 8.2. *In vitro* propagation of Banana

Multiplication of plants using plant tissue culture technique is used as an alternative to conventional method to circumvent the drawbacks associated with the latter. Tissue culture is the propagation of a plant part or single cell or group cell in a test tube/ jar under controlled and aseptic conditions. It has laid the foundation of production of uniform, high-quality, disease-free planting material and true-to type plants at a mass scale. The tissue culture technique in which plant cells, tissues, or organs are used to generate multiple identical true to type plants under *in vitro* condition is called micropropagation or *in vitro* mass propagation. The technique has several advantages including rapid initial propagation of new varieties, regeneration of large number of plantlets from a small tissue, elimination of pathogens and storage of plant germ-plasm under aseptic condition. Micro propagation is user-friendly technique which does not require much expertise and is suitable for adoption by small and marginal farmers and success of micro propagation depends on method, variety and price of initiation media. *In vitro* multiplication of banana plantlets is an excellent alternate and a number of countries in the world like Israel (Israeli *et al*., 2005), France (Amutha 2013), Australia (Drew and Smith 2009), Cuba and many African countries (Amutha 2013), are using this technique. The micro propagation of banana has been achieved using shoot tip (Cronauer and Krikorian, 2004) and from male floral apices (France (Amutha 2013). There are also reports of somatic embryogenesis and regeneration

in liquid medium (Novak *et al*. 2001). One of the most important factor affecting the efficiency of micro propagation system is the rate of multiplication. It has been observed that banana multiplication rate is genotypic dependent as well as variable behavior has been observed among cultures initiated from same banana genotypes cultured *in vitro* IFrance (Amutha, 2013).

Nowadays, micropropagation is considered to be the only realistic method of achieving rapid, large-scale production of disease-free planting materials of newly developed varieties in order to speed up the breeding and commercialization process in crop plants. However, micropropagation of plants can be influenced by a several factors including genotype (explant type, source and size) media composition, culture environments, and availability of micropropagation protocols.

2.8.3 Media composition

The composition of the culture medium is considered to be one of the most important factors governing the growth and morphogenesis of plant tissues in culture (Bhojwani and Razdan, 2006). According to Bhojwani and Razdan (2006), there are a number of media formulations for plat tissue culture work which include those described by Murashige and Skoog (2009), White (2003), Nitsch and Nitsch (2009), Schenk and Hilderbrandt (2009), Gamborg et al. (2003), and Lloyd and McCown (2001). Murashige and Skoog's MS medium, Schenk and Hildebrand's SH medium, and Gamborg's B-5 medium are high in macronutrients, while the other media formulations contain considerably less of the macronutrients.Qadir and N. Khan. (2017) is the most widely used medium in plant tissue culture. Jalaja et al. (2008) stated that with some minor modifications with addition of vitamins, hormones and sugars, MS basal medium is used by different laboratories to suit their needs. Inline with this, many workers used MS basal medium for sugarcane micropropagation (Bekesha et al., 2002, Singh, 2003; Ali et al., 2004; Sharma, 2005; Ali et al., 2008; Khan et al., 2006; Behera and Sahoo ,2009; Khan et al., 2009).

Plant tissue and cell culture media are generally made up of some or all of the following components: inorganic nutrients (macronutrients, and micronutrients), vitamins, amino acids or other nitrogen supplements, sugar (s), other undefined organic supplements, solidifying agents or support systems, and growth regulators (George and Klerk, 2008).

2.8.4. Culture environment

The lighting, temperature, and humidity conditions given inside the growth room all play a role in the success of an in vitro procedure. The intensity, quality, and duration of light are the most important elements impacting in vitro culture growth Qadir and N. Khan (2017). discovered a 16-hour cycle. The relative humidity in the culture room has been set to 55%. Banana shoot tip cultures are cultured at 28°C in a light cycle of 12-16 hours with a photosynthetic photon flux (PPF) of about 60E/m2/s at an ideal growth temperature of 28°C.

2.9. Micropropagation stages

2.9.1. Mother plant preparation

Loss of cultures due to contamination is one of the most serious problems in plant tissue culture. Explant contamination depends on the several plant and environmental related factors such as species, age, explant source and prevailing weather condition. A prior good care of stock plants may lessen the amount of contamination that is present on explants; plants grown in the field are typically more “dirty” than those grown in a greenhouse or growth chamber. Thus, to minimize the initial source of contamination, researchers grow donor plants in greenhouse. Mother plant preparation is the act of raising mother plants under greenhouse condition or more hygienic conditions to reduce the risk of contamination. Therefore, to minimize the contamination problem that can come from explants source, it is important to collect the explants from mother plants grown in protected areas under appropriate pretreatment with fungicides and pesticides.

2.9.2. Explants establishment

Living plant materials from the environment are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, so surface sterilization of starting materials (explants) in chemical solutions is important. Despite the best timing and selection efforts, it is almost impossible to eliminate contamination from in vitro grown plants and losses due to contamination in vitro average between 3 and 15% at every subculture in the laboratories. Fungi and bacteria are the most frequently reported contaminants in sugarcane micropropagation (Wagih *et al*., 2009). Several antibiotics have been found to be effective in reducing bacterial contamination in banana tissue culture. To improve the effectiveness of the sterilization procedure, disinfectants such as sodium hypochlorite, calcium hypochlorite, ethanol (or isopropyl alcohol), mercuric chloride,

hydrogen peroxide, silver nitrate, and bromine water are commonly used. A surfactant such as Tween-20 is frequently added to the sterilizing solution in general, and the sterilizing solutions containing the explants are continuously stirred during the sterilization period (Oyebanji *et al*., 2009). For instance, Anbazhagan *et al*. (2014) had surface sterilized banana explants with 70% ethanol for 60 seconds, 5 percent sodium hypochlorite solution for 10 minutes plus 0.1 percent $HgCl_2$ for 5 minutes.

2.9.3. Shoot Initiation

Studies revealed that initiation media containing varying concentrations of BAP and IAA produced a rigid meristematic ball-like shape for banana shoot tip culture. They stated that the cultured shoot tip changed its color from creamy white to brown. Four weeks later, the explants' exterior leaf primordia turned green, and a spherical hard coat mass emerged from which adventitious plantlets grew. Muhammad *et al*. (2004) reported that BAP is the most often employed cytokinin in banana tissue culture by Cronauer and Krikorian (1984) and Vuylsteke (1998).

2.9.4. In vitro multiplication

This is the most important stage in any propagation program since it determines the number of produced plants (Omar and Aouine, 2007). In this stage, the number of propagules brought from initiation medium is multiplied by repeated sub- and reculture until the desired (or planned) number of plants is attained. The primary goal of this stage is to achieve propagation without losing the genetic stability (IAEA, 2004). *In vitro* shoot multiplication is critically influenced by the type and concentration of plant growth regulator/s in culture media

In line with Aremu *et al*. (2012) studied the effects of five topolins (metaTopolin= mT; metaTopolinriboside =mTR; metaMethoxytopolin = MemT; metaMethoxytopolinriboside = MemTR and metaMethoxytopolin tetrahydropyranyl = MemTTHP) on shoot regeneration of micro propagated 'Williams' bananas and compared to benzyl adenine (BA). The study revealed that 30 µM mT resulted in the highest number of Shoots (7.3±1.0). Unlike other Cytokinins (CK) treatments requiring higher concentrations, optimum mean shoot number per explant was attained at the lowest concentration in MemT and MemTTHP (10µM) treatments. In terms of abnormality index, their study showed that mTR regenerated plantlets were of the best quality across all the CKs tested.

Similarly, Sipen and Davey (2012) studied the different concentrations of N6 benzylaminopurine (BAP) and Indole Acetic Acid (IAA) for their effect on shoot multiplication and plant regeneration of the Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. They reported maximum shoot on medium supplemented with BAP at 5 mg/l (Pisang Nangka), 6 mg/l (Pisang Mas and Pisang Berangan) and 7 mg/l (Pisang Awak) with 0.2 mg/l IAA. Likewise, Ahirwar *et al*. (2012) tested different concentration of BAP (0-10 mg/l), Kinetin (0-10 mg/l), NAA (0.3-0.5 Mg/l) and different combination of BAP (0-10 mg/l) and NAA (0.3- 0.5 mg/l). They observed the highest frequency of shoot regeneration (52.25), number of shoots regenerated per explants (3.25) and shoot length (4.69) at BAP concentration of 5 mg/l, Kinetin concentration of 5 mg/l and combination of 7.5 mg/l BAP + 0.3 mg/l NAA. The addition of 5 mg/l BAP showed better than Kinetin for shoot development from shoot tip or male inflorescence tip explants. Rahman *et al*. (2013) investigated the best plant growth regulators for shoot proliferation and multiplication for cultivar Agnishwar. Among different types and concentration of cytokinins viz. 6-benzylaminopurine (BAP), kinetin (KIN), N6 – (2-isopentyl) adenine (2iP) tested for multiplication of shoot; maximum multiplication (95%) was obtained in MS medium containing 4.0 mg/1 BAP. The highest average number of shoots for each explants (5.9) was found in MS medium fortified with 4.0 mg/l BAP, while maximum elongation of shoot (4.9 cm) was observed in MS medium containing 5.0 mg/l BAP.

Ramachandran and Amutha (2013) reported best shooting response for Cavendish Dwarf variety on basal MS medium supplemented with 4 mg/l BAP + 0.2 mg/l NAA. They also observed best multiplication on MS medium containing the combination of BAP 5 mg/l + NAA 0.3 mg/l . Ahmed *et al*. (2014) reported that MS medium supplemented with BAP 4.00 mg/l + IAA 2 mg/l was best for explant establishment and shoot multiplication in banana cv. Grand Naine. Shiv Shankar *et al*. (2014) did mass propagation of banana (*Musa spp*.) cv. Grand Naine through direct organogenesis by using Benzyl Adenine Purine and Kinetin. Benzyl Adenine Purine (BAP) in five different concentrations (control, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/l) were used for shoot proliferation and differentiation and shoot multiplication rate. The study revealed that medium supplemented with BAP 4.0 mg/l produced greater number of shoots (55) and longer shoot (3.0±0.012 cm) when compared with other treatments. Reddy *et al*. (2014) studied the effect of diverse concentration of 6-benzylamino purine (6-BAP) on shoots induction of Grandnaine plantlets (*Musa spp*).

Anbazhagan *et al*. (2014) cultured shoot tips of Musa spp. on MS medium supplemented with different concentrations of BAP, KIN and IAA both in individual and in combined form and the best results were obtained from MS medium supplemented with BAP + IAA at the concentration of 3.0 mg/l and 0.5mg/l respectively.

Furthermore, Jamir and Maiti (2014) studied the effect of various levels of cytokinin and auxin for in vitro regeneration of banana cultivars Grand Naine and Jahaji. They tested various concentration of BAP (0-6.5 mg/l). It was observed that 4.5 mg/l BAP was found to be the best concentration in induction of highest number of buds (an average of 7.05 and 7.2) with highest mean length of 0.65 cm and 0.7 cm of shoots. But, the shoot elongation was maximum at lower concentration of BAP (1.5mg/l). Shashikumar *et al*. (2015) tested the effect of BAP, TDZ and coconut water at various concentrations. They recorded high frequency of shoot initiation (93.33) at 5 mg/l BAP. The synergetic effect of BAP (4 to 6 mg/l), TDZ (0.1 to 1.2 mg/l) and coconut water (0.1 to 0.9 ml/l) induced multiple shoot buds and this was optimum at the concentration of 5 mg/l BAP, 0.5 mg/l TDZ and 0.5 ml/l coconut water with 15.90 ± 1.66 frequency of shoots per propagated. Suman and Kumar (2015) tested the micro propagation of banana cv. Malbhog on MS medium supplemented with different concentrations and combinations of IAA and BAP. This combination resulted in differentiation of adventitious shoots. They reported maximum differentiation of shoots (92.05 %) on MS medium with 0.57 μM IAA + 17.74 μM BAP. The number of shoots per culture was 16.75. The subculture of differentiated shoots on the same medium resulted in further differentiation (91.97 %) of more than 15 shoots per culture.

2.9.5 Acclimatization

In vitro plant material is not adapted to natural environmental conditions; acclimatization is required in the case of in vitro produced plantlets (Brainerd and Fuchigami, 1981). They are not qualified to withstand the low humidity, increased light levels, and more volatile temperature found outside (Wainwright, 1988). As a result, the three key parameters to be adjusted during acclimatization to a natural environment are light, temperature, and relative humidity. The physical, chemical, and biological aspects of the potting mixture play a role in the formation of in vitro grown plantlets. The problem of fungal infection is eliminated by thoroughly washing plantlets to remove residues of agar and nutrient media, dipping in 0.05% sarbendazim, and sterilizing the potting mixture (Anderson, 1980 ; Muniswamy *et al*. 1994). Two parts of a commercial growing media combination

(Sunshine Professional), 1 part perlite, and 3 parts vermiculite are used in the greenhouse potting mixture for growing out banana plantlets (medium to coarse grade). Before being transplanted to the field, plants are usually allowed to acclimate in the greenhouse for about 2 months and achieve a height of around 20 cm (8 inches) (Perez and Hooks (2008). Rai *et al*. (2012) hardened rooted plantlets of the banana variety Grand Naine (G9) in portrays containing various potting mixtures, including soil, sand, and cocopeat (1:1:1), soil, sand, and farmyard manure (1:1:1), and a mixture of cocopeat and sand (2:1), with the mixture of Cocopeat and sand (2:1) showing the highest plantlet survival (96%). Likewise, Elisama *et al*. (2013) investigated the influence of fertigation and the addition of IBA to nutritive solution on the growth of Musa cavendischii plantlets during the greenhouse acclimatization phase. The experimental unit consisted of one transplanted plant and the application of 10 ml of Steiner's nutritive solution at 10, 25, 50, 75, and 100 percent without and with 1 mg/l of auxin on a daily basis (IBA). They discovered higher plants with respect to plant fresh weight, dry weight, height, and leaf width after 11 weeks of acclimation, which corresponded to treatments ranging from 75 to 100% of Steiner's solution. They reported that the IBA treatment had no discernible effect on the M. cavendischii plants' growth. They reported no evidence of a link between fertigation and the use of IBAs.

2.10 Banana Germplasm conservation

Conservation of plant genetic resources is a priority for maintaining genetic variability–the basis of all plant breeding programmes. Banana is among many groups of species that are suffering from genetic erosion. (Shibli et al., 2006; Rai et al., 2009) pointed out that, little by little, cultivars and wild types of particular interest (e.g., Musa AA, BB, ABB, AAB, AAA, AAAB, etc.) are disappearing from the center of genetic diversity of this crop and from other areas as a result of natural disasters, nomadic agriculture and deforestation. Propagation in most Musa species is vegetative, however, because their fruits are parthenocarpy (Stover and Simmonds 1987). Due to the parthenocarpy nature of Banana, its corms are used for propagation, but their large size makes transport from place to place is very expensive. Hence, any user of Banana will benefit from *in vitro* germplasm conservation methods that improve the management of these propagules. Tissue culture is one such method and its use for *in vitro* collecting of explants in the field is a real possibility.

In addition to conventional propagation problems, both biotic and biotic factors affect the morph biochemical and physiological properties of plant (Jan et al., 2016; Jan et al., 2017). The crop of banana is at the risk of biotic stresses such as Banana streak virus, Banana bract mosaic virus, Cucumber mosaic virus and Banana bunchy top virus which are the four important and widely spread viral diseases. These diseases are disturbing the production rate of banana crop while the abiotic stresses such as the environmental factors also play role in minimizing banana's production specifically among the smaller scale farmers who have inadequate resources. Banana crop production is often put to an end usually because of natural disasters (Singh *et al*., 2011). Propagation of crops by using tissue culture technique (TC) is the simple and first solution of the problems of such rapidly evolving ailments (Hussain *et al*., 2013; Khan et al., 2014; Jan et al., 2015).

Amongst the methods of conservation available for banana, *in vitro* conservation is still advantageous over the other methods since it is technically easy, requires less labor and land, and facilitates easy exchange of germplasm (INIBAP, 2006). The Global Musa Germplasm Collection is housed at the International Transit Centre (ITC) in Belgium, in the form of the largest *in vitro* collection comprising 1,185 accessions (Panis, 2009).

The maintenance of plant stocks or material under aseptic and adequate environmental conditions can be conducted using the two main approaches. The first one of these approaches is based on conserving material without disturbing its growth, i.e., successive transfer in a fresh medium, while the second one is based on conservation under slow-growth condition (Withers, 1980; Engelmann 1991; Sarasan *et al*., 2006; Novikova *et al*., 2008). The shortcomings of a successive transfer are an increase in work expenses and the consumption of basic materials and nutrients (Cordeiro *et al*., 2014). It should also be taken into consideration that long-term subculture can be followed by a decrease and/or the loss of the morphogenetic potential of the culture as well as by an increase in the probability of genetic changes during long-term sub culturing Bessembinder *et al*., 2003; Hao and Deng, 2003). Furthermore, there is a risk of losing propagating material as a result of a human errors or microbial contamination in the process of subculture (Grout 1990); therefore, it is advisable to reduce frequent interventions during conservation. With due regard for all these factors, *in vitro* culture under slow-growth conditions is supposed to be the most effective method of plant germplasm conservation. The use of this approach is aimed at slowing down the growth of cultures and prolonging the interval between two successive transfers (Cordeiro et al. 2014), as well as raising the degree of safety during

the conservation of cultures as a result of a decrease in interferences in a culture system and the minimization of the risk of contamination during subculture (Grout 1990; Engelmann 2011). The success of the use of certain approach depends on numerous factors, such as the possibility of extending the time period between two successive transfers, how long the influence of a limiting factor lasts until the moment when that factor begins to negatively affect the culture, and how fast the regular developmental functions could be restored after reverting to standard culture conditions (Grout 1990). The essential condition for using slow-growth procedures is the study of vital capacities of various kinds of cultures and the stability/instability of the preserved material (Shibli *et al*., 2006; Rai *et al.*, 2009).

3. Materials and Methods

3.2 Location of the study area

This experiment was carried out at Melkessa Agricultural Research Center's Tissue Culture Laboratory, Ethiopian institute of agricultural research 108km far from Addis Ababa

3.2. Experimental design

Three treatments (mannitol, sorbitol and sucrose each with three concentration levels) with five replications and four explants per jar were used in a Laboratory experinment for all varieties (Poyo, Giant and Grande). (Genotype Poyo, Giant and Grande media type mannitol, sorbitol and sucrose used for 3,6 and 9 month for this experinment).

3.3 Experimental Procedure

3.3.1 **Preparation of stock plants and disinfection**

In this experiment three randomly selected banana varieties (Poyo, Giant and Grande) grown at Melkassa Agricultural Research center (MARC) were considered. The shoot-tip explants (≈ 4 cm x 2 cm) were cut from young suckers. The samples were washed with tap water and detergent for one hour in order to remove debris. Then, they were surface sterilized by dipping first in 70% ethanol for 30 seconds followed by three times washing sterile distilled water. Then after, the explants were immersed in 5% Clorox (commercial bleach) with 2 drops/100 ml of Tween-20 as a wetting agent followed by shaking on electrical shaker for 20 minutes. Then, the explants were rinsed three times with sterile distilled water under aseptic conditions. Also *in vitro* propagated banana plants (Grande Naine, Giant Cavendish and Poyo) samples were collected from the Plant Biotechnology Laboratory MARC. After 6 sub-cultures *in vitro,* grown shoots were used for the study as explants).

3.3.1 **Culture initiation**

Shoot tip meristem of about 1cm and leaf sheath about one centimeter square was extracted after trimming all the dead and chlorine affected tissues to be used as explants. These explants were then being inoculated in MS (Murashige and Skoog, 1962) medium with plant growth regulator (PGR)-free. The MS basal media was prepared by mixing the appropriate stock solutions of macro, micro nutrients, thiamin (1.0 mg/l), myo-inositol (100 mg/l), carbon source (30 g/l sucrose), and solidifying agent (8g/l agar-agar). The pH of the media was adjusted to 5.7 prior to autoclaving at 121^{o}C for 20 minutes. All cultures

were incubated in relatively controlled growth rooms with constant temperature of 27 ± 2^{o}C and a photoperiod of 16 h under a 2500 lux light intensity from cool white fluorescent lamps of 40W. The culture was maintained in multiplication media until five subcultures (it was cultured every one month). After five subculture the explants were transferred to the invitro conservation media for all genotype. 60 explants per genotype were used for these *invitro* conservation.

3.3.2 **Slow growth treatments**

MS medium supplemented with sorbitol, mannitol, (0.2, 0.3, 0.5%) sucrose (0.3, 0.5, 0.7%) singly was used for this experiment. Thirty milliliters of media solidified with 0.8% (w/v) agar was dispensed in baby food jar covered with plastic caps and autoclaved at 121 °C and 105 kPa for 20 minutes. During the experiment, baby food jar with plant material were placed in cultivation box and maintained under photoperiod 16/8 h (light/dark), temperature 25±2 °C and the intensity of light 2500 lux. Data were recorded at the 3rd, 6th and 9th months of transfer on to te SGR media. The treatment combinations were as indicated below. The experiment was triplicated.

Table 1: mannitol, sorbitol and sucrose *with three levels for each treatment.*

X	*Media*									
		sorbitol(mg/l)			*mannitol(gm/l)*			*sucrose (gm/l)*		
Genotype	*Time*	*0.20%*	*0.30%*	*0.50%*	*0.20%*	*0.30%*	*0.50%*	*0.30%*	*0.50%*	*0.70%*
Poyo	*3*	*T1*	*T2*	*T3*	*T4*	*T5*	*T6*	*T7*	*T8*	*T9*
	6	*T10*	*T11*	*T12*	*T13*	*T14*	*T15*	*T16*	*T17*	*T18*
	9	*T19*	*T20*	*T21*	*T22*	*T23*	*T24*	*T25*	*T26*	*T27*
Giant	*3*	*T28*	*T29*	*T30*	*T31*	*T32*	*T33*	*T34*	*T35*	*T36*
	6	*T37*	*T38*	*T39*	*T40*	*T41*	*T42*	*T43*	*T44*	*T45*
	9	*T46*	*T47*	*T48*	*T49*	*T50*	*T51*	*T52*	*T53*	*T54*
Grande	*3*	*T55*	*T56*	*T57*	*T58*	*T59*	*T60*	*T61*	*T62*	*T63*
	6	*T64*	*T65*	*T66*	*T67*	*T68*	*T69*	*T70*	*T71*	*T72*
	9	*T73*	*T74*	*T75*	*T76*	*T77*	*T78*	*T79*	*T80*	*T81*

3.3.3 Shoot Multiplication

After 3, 6 and 9 months of storage without subculture, clean stored shoots under slow growth conditions were transferred to the next multiplication medium. The medium at this stage of culture was MS basal medium supplemented with 30 g/l of sucrose, 100 mg/l of myo-inositol, 2 mg/l of thiamine, and solidified with 8 g/l of agar-agar. The medium was prepared with different concentration of BAP (2ml/g,3ml/g,4ml/g). The experiment was laid at CRD with three replications. Cultures were sub cultured on fresh medium with the same combination at 30-day intervals for 8 weeks.

Rooting

Individual shoots were clipped from regenerated multiple shoots and grown on ½ MS medium with various concentrations of for root induction. After 35 days, the rooting frequency data was recorded.

Acclimatization

Plantlets with well-developed shoots and roots were taken out of the culture vessels and thoroughly washed with tap water to remove all traces of the rooting medium. After that, the plantlets were temporarily soaked in a fungicidal solution (3 percent Kocide solution) before being placed on seedling trays filled with nursery growth substrate. They were kept under the humidity chamber made of poly tunnels after planting to supply the sensitive plants with enough relative humidity to avoid desiccation.

Data Collection and Analysis

Data was collected depending on the percentage of survival and contamination, and number and length of shoots on the culture initiation medium, number and length of shoots on the multiplication medium, number and length of roots on rooting media after 3, 6 and 9 month's storage.

The SAS statistical program was used to conduct suitable statistical analysis on the data acquired from each experiment. The significant means were separated using the proper approaches.

5 Result and Discussion

5.1 Percentage of contamination

In the current study an effort has been made to establish *in vitro* protocol for banana germplasm conservation (gene banks). Storage experiment was done for checking the performance of three genotype of Banana (Poyo, Giant and Grande) during short term *in vitro* maintenance, while in this *in vitro* germplasm maintenance experiments culture contamination performance after several month conservation was identified (Table 2).

To solve culture contamination problem surface sterilization of banana, shoot tip explants with NaoCl in the form of 5% NaoCl active commercial bleach (chlorox) was done in the present study. Shoot tip explants sterilization results of the tested banana varieties was varied in response to showing contamination percentage as presented in (Table 2).

Table 2. Percentage of culture contamination

Genotype	% contaminated	%Survived
Poyo	13.3	86.70
Giant	8.90	91.10
Grade	20.0	80.00

The data percentage of contamination was collected; total number of populations divided to number of contaminated explants times 100% for all varieties (Poyo, Giant, Grande). It was reported that about 40-60% of banana *In vitro* cultures lost in spite of using main aseptic reliable procedures (Msogoya et al., 2012). As the result indicated Grande were high contamination recorded genotype (20%). However, Poyo and Grande varieties were recording lower contamination 13.3% and 8.90% respectively (Table 2). According to the result Giant variety record high survival (91.1%) rate than poyo (86.7%) and Grande (80.0%) varieties. One of the main obstacles to the use of the in vitro micropropagation technique for the mass production of banana planting materials is microbial contamination (*Amany1 Eliway Ali, Mohamed1,2018*). Generally, the concentration of sorbitol, mannitol, and sucrose were not impact on the contamination rate of the cultivars. According to reports, the sub-culturing procedure is a significant source of contamination (5–15% for each sub-culture) since explants, growth material, working tools, and operators are not sufficiently sterilized (Omamor et al., 2007). The contamination rate was depended on the safety of the laboratory.

5.2 Effect of growth retardant reagents on banana *in vitro* cultures

In this experiment, the effect of different plant growth retardants (sucrose, sorbitol and mannitol) was tested on three banana genotypes for three different time length (3, 6 and 9 months) to find out the most suitable culture media formulation to induce slow-growth of banana micro-plants as germplasm *in vitro* conservation, and to reduce the interval time between the sub-cultures. The results showed that micro-plant survival percentage was highly and positively correlated with micro plants. As indicated in table 2 the treatment (mannitol, sorbitol and sucrose) effects were also significant for enhancing survival of conserved micro-shoot. This indicates there is need to develop genotype specific protocols for germplasm conservation. For conservation of plants in in vitro high survival percentage is mandatory. Adding different plant growth inhibiting reagents in the specific media directly affect the survival ratio of banana cultivars (Poyo, giant and grand) in the conservation period of three to nine (3-9) months. Genotype Grande showed maximum survival percentage (77%) followed by Poyo genotype (73%), while least in vitro survival percentage (58%) was recorded in Giant genotype. The data was gathered based on the survival percentage of cultivars that survived in 3, 6 and 9 months. As a result, indicated poyo variety on T2 (at 0.5% concentration of sucrose) had a high percentage of survival (100 percent the first three months, 90% six and nine months), for Giant variety T2 (at 0.5% concentration of sorbitol) had a high percentage of survival (95% for both three and six months;90% for nine month) and Grande variety T1 (at 0.2% concentration of sorbitol) had a high percentage of survival (100 % for all the first three, six and nine months). However, poyo variety had a poor percentage of survival on T1 (at 0.2% concentration of mannitol) 40% the first three, six and nine months, whereas Giant variety was 45,25 and 20% survival rate on T3 (at 0.5% concentration of sorbitol) the first three, six and nine months respectively. Grande variety (mannitol) had a poor survival rate 50% after three months, 15% after six and nine months at concentration 0.2% of mannitol (T1). As a previous result, the highest shoot multiplication rate was obtained on MS medium supplemented with a combination of BAP and IAA at different concentration for Giant and poyo, respectively, Dagnew; 2012. As a result of this study, the poyo variety on T2 (at 0.5 % sucrose concentration) had a high percentage of survival (100 % the first three months, 90 % the six and nine months. So when we try to see if this study has additional PGR like BAP and IAA, (Dagnew; 2012), so on this study, Banana plants conserve for nine months on nutrient media by using PGR like sucrose.

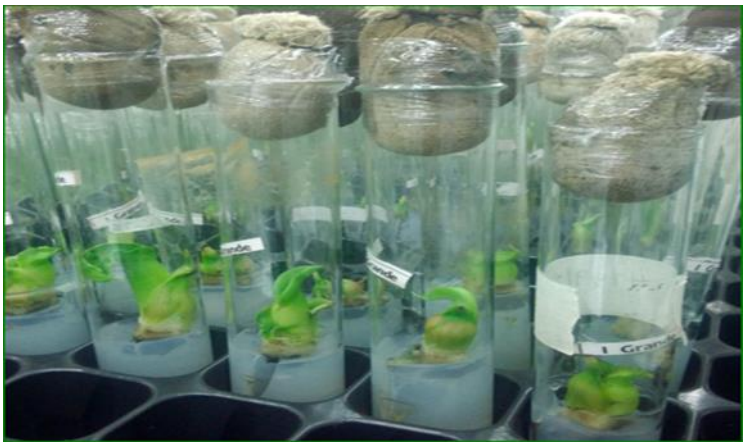


Figure 1: - Initiation stage

Table 3: survival percentage of culture initiation

	Variety		
	Poyo	Giant	Grande
% of survival for culture initiation	73	58	77

5.3 Effect of different concentrations of Sorbitol, Mannitol and Sucrose on shoot height, number of shoot and number of roots in MS medium

Genotype response to in vitro germ-plasm conservation culture media conditions was well studied. The shoot multiplication of the three genotype of Banana showed genotypic and treatment effect differences in shoot height, number of shoots, and root number throughout the tested culture media during in vitro storage. The analysis was performed with the mean of each parameter of different Banana genotype throughout the media conditions (Table 4). Shoot multiplication result showed that plants preservation reagents (Mannitol, Sorbitol and Sucrose), Banana variety and their interactions had significance effect on number of shoots, length of shoot and number of roots.

To clarify the preservation culture media effect on growth of Banana germplasm during in vitro storage, the genotype responses throughout culture condition were averaged and culture media supplemented with different type and concentration of plant preservative

reagents were compared (Table 4).As a result, for the three variables(Number of shoot, Length of shoot and root number), the genotype Giant Cavendish showed the highest value which was observed at 0.7% sucrose preserving reagent, followed by the Grande genotype at the same type and concentration of shoot preservation reagent, While Poyo genotype showed the lowest values in both factors(growth retardant concentration and preservation duration) except on root number parameter (Table 4). Number of shoots, shoot length and root number data analysis within the genotype showed genotypic differences and on some growth parameters no significance among genotypes within the treatments (Table 4). The genotypes did not show constant increasing and decreasing trends of results. But culture modification with different growth retardant reagent showed differences among genotype in at least one variable, and response varied with in tested Banana genotype (Table 4). Under growth retardant supplemented culture conditions, it was observed that the genotype reduced their growth during preservation while it maintains the multiplication ability of the plant. The duration of Banana preservation in vitro condition had no negative effect on shoot multiplication of the three Banana genotype because plant growth parameters showed best result at 9-month culture the remaining specified duration except on few treatments (Table 4 and figure 2C).

As shown in table 4, the number of shoots, length of shoot and root number increase when the concentration of Sorbitol and mannitol increased up to optimal concentration then afterward it showed redaction. While in the case of Sucrose except on few condition the all the parameters increased when the concentration of Sucrose increase (Table 4).

Figure 2. After three months; Figure 2B. After six months; Figure 2C. After nine months

Table 4: Means of parameters of all varieties (Poyo, Giant and Grande)

Variety				Sorbitol			Mannitol			Sucrose		
	Treatments		Month	T1	T2	T3	T1	T2	T3	T1	T2	T3
Poyo	Means of parameters	Height of shoot (cm)	3	2.15	2.05	1.75	1.20	2.30	1.33	2.38	3.13	3.48
			6	2.25	2.83	1.80	1.50	2.90	1.70	2.65	3.20	3.70
			9	2.85	3.15	2.05	1.65	3.14	2.15	2.95	3.53	4.13
		No of shoot	3	0.95	0.90	0.90	1.25	1.30	1.15	0.95	1.10	0.95
			6	0.90	0.95	0.95	1.30	1.55	1.15	1.00	0.90	0.90
			9	0.90	1.00	1.00	1.30	1.60	1.15	1.00	0.90	0.90
		No of root	3	2.55	1.90	1.65	2.03	2.40	2.00	2.60	2.55	2.80
			6	3.25	2.80	2.50	2.50	3.58	3.35	3.25	2.85	4.54
			9	3.33	2.85	2.60	2.65	3.90	3.50	3.30	3.05	4.87
Giant	Means of parameters	Height of shoot (cm)	3	1.15	2.08	1.03	1.58	1.43	1.13	2.53	2.53	2.48
			6	1.30	2.60	1.50	2.00	1.65	1.40	2.85	2.78	3.43
			9	1.30	2.65	1.65	2.24	1.87	1.57	3.05	2.91	3.96
		No of shoot	3	0.55	1.10	0.45	0.90	1.00	0.75	1.00	1.10	1.05
			6	0.65	1.20	0.35	0.85	0.90	0.65	1.00	1.15	1.10
			9	0.75	1.20	0.35	0.85	0.90	0.60	1.00	1.10	1.15
		No of root	3	1.15	1.65	0.75	1.10	1.00	0.75	2.55	2.90	3.10
			6	1.70	3.00	1.30	2.35	1.90	1.45	3.40	2.75	5.50
			9	2.06	3.15	1.52	2.68	2.07	2.00	4.12	3.00	6.14
Grande	Means of parameters	Height of shoot (cm)	3	1.15	2.08	1.03	1.58	1.43	1.13	2.53	2.53	2.48
			6	1.30	2.60	1.50	2.00	1.65	1.40	2.85	2.78	3.43
			9	1.36	2.98	1.89	2.32	1.75	1.68	3.00	2.95	3.86
		No of shoot	3	0.55	1.10	0.45	0.90	1.00	0.75	1.00	1.10	1.05
			6	0.65	1.20	0.35	0.85	0.90	0.65	1.00	1.15	1.10
			9	0.65	1.25	0.35	0.85	1.00	0.60	1.00	1.20	1.20
		No of root	3	1.15	1.65	0.75	1.10	1.00	0.75	2.55	2.90	3.10
			6	1.70	3.00	1.30	2.35	1.90	1.45	3.40	2.75	5.50
			9	2.00	3.54	1.57	2.78	2.14	1.61	3.65	2.84	5.87
CV %				20.2	18.1	17.3	19.5	18.0	20.1	20.6	16.0	15.3
LSD @ 5%				1.3	2.4	1.7	2.6	0.9	1.2	2.1	1.4	2.6

Note: T1= 0.2% Mannitol and sorbitol, 0.3% sucrose

T2=0.3% Mannitol and sorbitol, 0.5% sucrose

T3=0.5% Mannitol and sorbitol, 0.7% sucrose

The data grouped in this case based on the means of shoot height, shoot number, and root number of all cultivars at every three months (3, 6 and 9 months). The means of shoot height, number of shoots and number of root rate of all cultivars (Poyo, Giant and Grande) were found among the explants of the various criteria stated in above table.

5.3.1 Poyo Variety

As perthe experiment, maximum shoot height of poyo variety was recorded at 0.2% (T1) a nd 0.3% (T2) concentration of sorbitol; i.e., 2.15cm: 2.05cm: first three months, 2.25cm: 2.83cm the sixth and 0.85cm: 3.15cm nine months respectively. But at 0.5% concentration (T3) the lower shoot height was recorded. On the other hands, at 0.2% concentration of sorbitol (T1) 2.55,3.25 and 3.33 the maximum number of roots were recorded in the first three, six and nine months respectively. However, for all treatments, the number of shoots was relatively the same at all months. The highest height of shoot was recorded at 0.3% concentration of mannitol (T2) i.e.; 2.30cm 2.90cm and1.14cm on the third, sixth and ninth months respectively; and the lowest height of shoot was recorded at 0.2% concentration (T1) i.e.; 1.20cm 1.50cm and 1.65cm after three, six and nine months, respectively. The highest sucrose-induced shoot height was recorded at 0.7% concentration of sucrose (T3), while the lowest was at 0.3% (T1).

As the treatments showed a 73% survival Showed a 73% survival percentage, and as a result, indicates the poyo variety on T2 at 0.5% concentration of sucrose had a high percentage of survival: 100% the first three months and 90% at six and nine months. As a result, sucrose on three treatments and concentrations of 0.3%, 0.5%, and 0.7% showed a better survival rate than other treatments like sorbitol and Mannitol, and we got a 100 percent survival rate on treatment one with 0.3% sucrose. Mannitol already showed a 0% survival rate, which meant it wasn’t used for our banana conservation.

Statistically, this data was analyzed by SAS and used a one-way ANOVA to compare two means in this research. Result showed that the three level of each treatment mannitol sucrose and sorbitol on poyo variety of banana is given result like this height of plant by treatment of Manito 2.90 on T2, 1.70 on T1, and 1.50 on T1 statistically there is no significant pairwise difference among the means treatment level >0.05. The result showed

that the treatment like T3 on 2.40, T1 on 2.0, and T2 2.0 effect on number of shoots are statistically there is no significant pairwise difference among the means from the standard of 0.05 errors for comparison 2.18 is the critical value 3.78 for comparison 5.83. The result of number of roots by treatment are showed that by their level of treatment like there is no significant pairwise difference among the means, T2 is 3.57, T3 is 3.35, and T1 is 2.50. The result of height of plant by treatment sucrose, T3 3.48, T2 3.13, and T1 2.38 by the standard of 0.05 error comparison 0.67 critical P value comparison 1.80 it shows there is no significant difference on the result and Number of shoots by treatment: depending on a 0.5 standard, T2 is 3.57, T3 is 3.35, and T1 is 2.50, there is no significant difference between means.

5.3.2 Giant Variety

According to the study, at 0.3% concentration of sorbitol (T2) produced the maximum shoot
heightrecorded for Giant variety, whereas at concentration 0.2% and 0.5% recorded the lo west heights of shoot. Additionally, at concentration of 0.3% (T2) produced maximum nu mber of shoots, whereas at concentration 0.2% and 0.5% produced the lowest number of sh oots. At 0.3% concentration of sorbitol (T2) produced the greatest number of roots, whereas at 0.5%
(T3) produced the lowest number of roots. The best shoot height, shoot number and numbe r of roots were obtained at 0.2% (T1), 0.3% (T2) and 0.2% (T1) concentration of mannitol respectively, and the best shoot height and number of roots were obtained at 0.7% concentration of sucrose (T3). However, the number of shoots for each treatment was around the same.

As the treatments showed at 58% survival rate. Giant variety on T2 at 0.5% concentration of sorbitol had a high percentage of survival: 95% for the first three and six months and 90% for the ninth month. As a result, the data indicate that sorbitol on the Giant variety had been successfully conserved at different concentrations (0.2%, 0.3%, and 0.5) and treatments like sucrose and mannitol, while it showed a good survival percentage on the treatment sorbitol and 0. %. Sucrose had a medium percentage of survival and on Mannitol there is a poor percentage of survival. Mannitol T2 at 0.2 concentration has a survival rate of 45.5%, 25.0% and 20% survival rate on Sorbitol the first three six and nine month for the r giant variety, sorbitol at T2 0.5% concentration is preferred to conservation technique as I try to see.

The result showed that the three level of each treatment Mannitol sucrose and sorbitol on Giant variety of banana is given result like this height of plant by treatment of Sorbitol the height of plant by treatment T3 1.50, T2 2.60 and T1 1.30 the result is an alpha of 0.05 with a standard error of 0.73 and a critical value of 3.78 for comparison at 1.95; the results show there is no pairwise significant difference on mean. Number of shoots by treatment T2 (1.10), T1 (0.55), and T3 (0.45), alpha value 0.05, standard error for comparison 0.23, and critical value 3.78 for comparison 0.63, there is no significant difference between T2 and T3 from one another. Number of roots by treatment mannitol T2 1.65, T1 1.15, and T3 0.75. The result showed alpha 0.05 standard error for comparison is 0.83. Critical value: 3.78 for comparison 2.23 there are no significant pairwise differences among the means this mean no effect was observed. The results showed on treatment mannitol are T1 1.57, T2 1.42, and T3 1.12; alpha value 0.05 standard error for comparison. 0.48 And the critical value for comparison are 3.78. There are no significant pairwise differences among the means according to the analysis. Number of shoots by treatment T3 0.65, T2 0.90, and T1 0.85, with an alpha of 0.05 and a standard error of 0.05 for comparison. 0.22 For the critical value, 3.78 for the comparison, and 0.59 for the result show there is no significant pairwise difference among the means. According to the result showed on number of roots by treatment T2 1.10, T11.00, and 0.75. The result showed in ANOVA the alpha 0.05 standard error for comparison 0.57 and critical value 3.78 for comparison 1.55, and then the result is that there is no significant pairwise difference among the means. The result of treatment sucrose height of plant by treatment T3 2.15, T1 1.55, and T2 0.05 are the alpha error standards for comparison. 0.51 critical Q value: 3.78 for comparison at 1.38 there is no significant pairwise difference among the means. Number of shots by treatment T1 1.25, T3 0.80, and T2 0.75 alpha 0.05 error comparison 0.32 critical values 3.78 for comparison 0.87, there are no significant pairwise differences among the means. Number of roots by treatment T1 1.90, T2 1.80, and T3 1.55; alpha 0.05; standard error comparison 0.79 critical value: 3.78 for comparison 2.13 there are no significant pairwise differences among the means.

5.3.3 Grande Variety

Finally, the highest shoot height, shoot number and root number for Grande variety were obtained using 0.3% concentration of sorbitol (T2), and the highest shoot height and number of roots were recorded at 0.2% concentration mannitol (T1). The poor height of plant, number of shootandnumber of roots was recorded at 0.2% and 0.5% concentration of sorbitol. Additio nally, the lowest highest shoot height and number of roots were recorded at 0.3% and 0.5% concentration of mannitol. However, all treatments had nearly the same number of shoots.

As the treatments showed a maximum survival percentage of 77% on sucrose. Grande had a good survival percentage on each treatment at 0.3%, 0.5%, and 0.7%, which showed a better survival rate than other treatments like sorbitol and Mannitol. Mannitol had a poor survival rate of 50 percent after three months, and 15 percent after six and nine months at a concentration of 0.2% of Mannitol (T1).

The result showed that the three level of each treatment mannitol sucrose and sorbitol on Grande variety of banana is given result like this height of plant by treatment of Manitol T2 is 0.55, T0 is 47, and T1 statistically there is no significant pairwise difference among the means treatment level >0.05. Number of shots by treatment T1 0.80, T2 0.55, and T3 0.55, with an alpha of 0.05 and a standard error of 0.05 for comparison 0.42 critical value; 3.78 critical value for comparison 1.14 There are no significant pairwise differences among the means. Number of roots by treatment T3 0.25, T1 0.15, and T2 0.15, with an alpha of 0.05 and a standard error of 0.05 for comparison 0.19 critical value; 3.78 critical values for comparison 0.52 there are no significant pairwise differences among the means. The result of sucrose type of treatment on height of plant by treatment T3 3.10, T1 2.57, and T2 alpha 0.05 standard error for comparison 0.41 critical value; 3.78 critical value for comparison 1.09 There are no significant pairwise differences among the means. Number of shots by treatment T1 1.20, T2 1.20, and T3 1.15 the result showed alpha 0.05 critical value 3.78 critical values for comparison. 0.85, there are no significant pairwise differences among the means. Number of roots by treatment T3 3.05, T1 2.35, and T2 1.25; alpha 0.05 standard error for comparison. 0.68 critical value; 3.78 critical values for comparison 1.82 there are no significant pairwise differences among the means. The result of treatment sorbitol on the height of plant by treatment T3 2.15, T1 1.55, and T2 0.05 are the alpha

error standards for comparison. 0.51 critical values: 3.78 for comparison At 1.38 there is no significant pairwise difference among the means. Number of shots by treatment T1 1.25, T3 0.80, and T2 0.75 alpha 0.05 error comparison 0.32 critical value: 3.78 for comparison 0.87, there are no significant pairwise differences among the means. Number of roots by treatment T1 1.90, T2 1.80, and T3 1.55 alpha 0.05; standard error comparison 0.79 critical value: 3.78 for comparison 2.13 there are no significant pairwise differences among the means.

According to previous research sucrose has been widely used in plant tissue culture as the major carbohydrate source to supply the energy to cell membrane. The length of root was not influenced with the increase in sucrose concentration in invitro culture medium; however, length and lower rate of shoot multiplication were observed without sucrose. Sucrose is considered as an important component in *invitro* culture medium where it serves as a source of carbon and energy Zahra, M et al. 2017. The multiple shoot thus obtained from shoot experiments either on hormone- free or cytokinin supplemented media developed roots parses. Roots origination from shoots was thick, long and fibrous and easy to handle. All plants derived from tissue culture survived and grew extremely vigorously and reached a 3 to 4m height after 4 months cultivation in the field, G. Reyes et al. 2017.

5.4 Acclimatization

After remove the agar, thoroughly wash the roots with water. Then plantlets were temporarily soaked in a fungicidal solution 0.05% sarbendazim and sterilizing the potting mixture. Acclimatized in vitro rooted plantlets by using different treatments so, well developed and healthy in vitro raised plantlets (about 6cm with 3-4 leaves) were transferred into small polybags filled with different soil media mix, and kept inside small chambers covered with transparent plastic in the greenhouse for primary hardening for a week. Plantlets were disinfected with randomly (2.0 g/l) for 10 minutes to prevent fungal infections before transplanting. Individual plantlets were then transferred into small polyethylene bags. Plantlets were then transferred to big polybags filled with soil media for acclimatization. All the plants were acclimatized (78% survival), and the highest

vegetative plant growth in terms of plant height, pseudo stem girth and number of photosynthetic leafs per plant was recorded on media containing sugarcane filter cake and sand at 3:1 ratio (v/v) as indicated in Under Melkessa condition the acclimatization of plantlets took from 10 to 14 weeks depending on the type of banana cultivar. The hardened plants were transferred to the field, successfully established, and produced large bunches with high quality fruits.

Figure 3: Plants were planted in soil media for acclimatization and survived.

6 Conclusion and Recommendation

6.1 Conclusion

Banana is one of the most important fruit crops grown widely in Ethiopia and the fourth most important food security crops after rice, wheat, and Maize globally. Globally, the major banana producing country are India, China, Brazil, Ecuador and Philippines. In Africa, Angola, Tanzania, Kenya, Brundi and Cameron are the major banana producers. Major producers of banana in Ethiopia are found in Southern and south western Ethiopia, with 59.64% (53,956.13 ha) of the total fruit area and 68% (478,251.04 ton). However, its production and productivity are largely affected by biotic and abiotic stress. Its traditional field conservation has several limitations including demand of large space, laborious, high transportation cost and subjected to biotic and abiotic factors. Nowadays, slow growth storage (SGS) technique becomes an economical *in vitro* method to preserve several plant species including banana by controlling the growth and development of plantlets. The

present study targeted to optimize the best banana Germplasm conservation method for long time under *in vitro* condition. The study confirmed significant difference among varieties in their survival rate in different SGR at different storage duration. It was observed that MS medium supplemented with manitol, sorbitol and sucrose SGR at sorbitol and manitol 0.2, 0.3, 0.5 and Manitol 0.3, 0.5, 0.7 concentration was found to be the best for long storage of banana varieties under in vitro condition.

Thus, the present study had successfully optimized an appropriate protocol for *in* vitro conservation of banana for long time to safeguard banana genetic resource.

The study investigated which one of banana variety by which treatment it may conserve and how many are contaminated. The work will have a significant impact in addressing production and productivity of the banana. Banana had better survival rates than plantains regardless of the method used. The contaminated and survived per cent among the genotype are Poyo 13.3, Giant 8.90, Grande 20.0 per cent are contaminated and the survival rate are in Poyo 86.70, Giant 91.10, Grande 80.00 percent are survived in each variety and treatment. Grande variety (mannitol) poor survival rate 50 percent after three months, 15 percent after six months and nine months at concentration of 0.2% of mannitol (T10. The shoot multiplication of the three genotypes of banana showed genotypic and treatment effect difference in shoot height, number of shoots, and root number. The Poyo variety on T2 had a high percentage of survival (100% the first three months, 90% the six and nine months). We try to see the objective of this study we are seceded by our work we get what we won't to see like percentage of contamination and develop a proper protocol for the conservation of different banana genotypes. All of the means that analysed are no significant pairwise differences.

6.2 Recommendation

The finding in this research are used to be in one to anther ways the method that used to develop the protocol for this finding is by using mannitol sorbitol and sucrose in different concentration with MS media in five replication in three treatments. The media were prepared from stock solutions of macronutrient, thiamine (1.0mg/l), myo-inositol (100mg/l), carbon source (30g/l), and solidifying agent (8g/l0 in agar agar. This medium is used slightly because of the different concentrations, and the best one is sucrose. As most of the treatment protocols I was working on especially, which is Mannitol shows much contamination protocol due to laboratory hygiene and humidity at the process of shoot,

root induction. I recommend for those who try to modify this research, they have to take care of the laboratory safety. In addition, light and temperature amount should be taken under consideration since it will harm the treatment protocols. For the above reasons the Mannitol treatment protocol did not show any results on Grande variety so it was dismissed.

References

Andrew J. Lack; David E. Evans (2005). Plant Biology. Garland Science. pp. 199–. ISBN 978-0-415-35643-5.

Armstrong, Wayne P. "Identification Of Major Fruit Types". Wayne's Word: An On-Line Textbook of Natural History. Archived from the original on November 20, 2011. Retrieved August 17, 2013.

Bajaj, Y. 2005. Cryopreservation of plant cell, tissue and organ culture for the conservation of germplasm and biodiversity, p. 3–28. In: Bajaj, Y. (ed.). Biotechnology in agriculture and forestry 32. Springer-Verlag Press, New York.

Blench,Roger (2016). "Things your classics master never told you: a borrowing from Trans New Guinea languages into Latin". Academia.edu. Academia, Inc.

Blog post Would the true peduncle please stand up? published 3 March 2016 in Under the peel, the blog of the ProMusa community.

"Banana facts & figures". How is banana production cost structured?. fao.org. 2013. Archived from the original on July 18, 2019. Retrieved July 24, 2019.

Bennici A, Anzidei M. and Vendramin G.G. (2004). Genetic stability and uniformity of Foeniculum vulgare Mill. Regenerated plants through organogenisis and somatic embryogenesis Plant Sci. 166, 221-227.

Brian R. Chapman; Eric G. Bolen (31 August 2015). Ecology of North America. John Wiley & Sons. pp. 98–. ISBN 978-1-118-97154-3.

Chia, C.L.1981. Bananas. Commodity Fact Sheet BA-3 (A) Fruit. www.extento.hawaii.edu/kbase/crop/crops/i_banana.htm

Carolin, Roger C.; Tindale, Mary D. (1994). Flora of the Sydney region (4th ed.). Chatswood, NSW: Reed. p. 23. ISBN 0-73-010400-1.

Clonal colony or genet, retrieved 20 July 2017

Corm, Rhizome, retrieved 9 September 2020

Cronauer, S.S. and A.D. Krikorian. 1984. Multiplication of Musa from excised stem tips. Annals of Botany, 53:321-328.

Dagnew, Surafel Shibiru, and Abel Debebe..,Micropropagation of Banana varieties (Musa spp.) Using Shoot -Tip culture.2012. In Melkassa Agricultural Research center. J.Sci 22:14-25

De Langhe, E.A.L. 1984. The role of in vitro techniques in germplasm conservation. Pp. 131-137 in Crop Genetic Resources: Conservation and Evaluation (J.H.W. Holden and J.T. Williams, eds.). George Allen and Unwin, London.

Differences between roots and rhizomes, retrieved 16 March 2016

Endress, P.K. 2010. Disentangling confusions in inflorescence morphology: Patterns and diversity of reproductive shoot ramification in angiosperms. Journal of Systematics and Evolution, 48(4):225-39.

FAO (Food and Agricultural Organization of the United Nations). 2003. The world Banana Economy 1985-2002. Food and Agriculture Organization. Rome Italy.

George L., Tokoporo, A. A., Elhassan and Ali, M. A 2013. Effect of nutrient medium concentration and temperature on short-term *in vitro* conservation of shoot-tip explants of banana. JONARES,1:37-40.

Guillermo Reyes, Jose Garcia, Femando Pina.., 2017. *Invitro* proliferation and cryonconsercation of Banana and plantain elite clones. Journal of Horticultural Research 2017, vol.25 (20:37-47.

Higgins, C.A., Yokoyama, K.M., Wanitprapha, K., Nakamoto, S.T. and Chia, C.L. 1990. Banana Economic Fact Sheet: No. 11, CTAHR, University of Hawaii. www.extento.hawaii.edu/kbase/crop/crops/i_banana.htm

Hanba, Y.T.; Kogami, H.; Terashima, L. The effects of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. Plant Cell and Enviroment, v.25, p.1021-1030, 2002.

Hiebert-Giesbrecht, Mickel Randolph; Novelo-Rodríguez, Candelaria Yuseth; Dzib, Gabriel Rolando; Calvo-Irabién, Luz María; Arx, Georg von; Peña-Rodríguez, Luis Manuel (2017-11-09).

Herb-chronology as a tool for determining the age of perennial forbs in tropical climates". Botany. 96: 73–78. doi:10.1139/cjb-2017-0167.

Hussain, A., I.A. Qarshi, H. Nazir, I. Ullah, M. Rashid and Z.K. Shinwari. 2013. In vitro Callogenesis and Organogenesis in Taxus wallichiana Zucc. The Himalayan Yew. Pak. J. Bot., 45(5): 1755-1759.

Iain J. Gordon; Herbert H.T. Prins (14 September 2007). The Ecology of Browsing and Grazing. Springer Science & Business Media. pp. 220–. ISBN 978-3-540-72422-3.

"Is A Banana A Fruit Or A Herb? | Lexico". Lexico Dictionaries | English. Archived from the original on June 6, 2020.

INIBAP (2006) Global conservation strategy for Musa (Banana and Plantain). International Network for Improvement of Banana and Plantain, Montpellier, 27 pp.

INIBAP (International Network for the Improvement of Banana and Plantain). 1992. Annual Report. Montpellier. France. p. 48.

IPGRI-INIBAP and CIRAD. 1996. Descriptors for banana (Musa spp.). IPGRI, Rome, Italy. 55p.

JV Escalant, C Teisson- Plant cell Reports, 1989-Sringer. Somatic embryogenesis and plant from immature zygotic embryos of the species Musa acuminita and Musa Balbisiniana. C.by 137.

Lee, D.W.; Oberbauer, S.F.; Johnson, P.; Krishnapilay, B.; Mansor, M.; Mohamad, H.; Yap, S.K. Effects Of Irradiance And Spectral Quality On Leaf Structure And Function In Seedlings Of Two Southeast Asian *Hopea* (Dipterocarpaceae) Species. American Journal Of Botany, V.87, P.447-455, 2000.

Jaramillo, R. 1983. Conservación del germoplasma y mejoramiento genético del banano y del plátano. Inf. Mensual UPEB 7:8-10.

Jan, S.A., N. Bibi, Z.K. Shinwari, M.A. Rabbani, S. Ullah, A. Qadir and N. Khan. 2017. Impact of salt, drought, heat and frost stresses on morpho-biochemical and physiological properties of Brassica species: An updated review. J. Rural Dev. Agri., 2(1): 1-10.

Jan, S.A., Z.K. Shinwari and M.A. Rabbani. 2016. Agromorphological and physiological responses of Brassica rapaecotypes to salt stress. Pak. J. Bot., 48(4): 1379-1384.

Jan, S.A., S.H. Shah, S. Ali and G.M. Ali. 2015. The effect of plant growth regulators on callus induction and somatic embryogenesis of hybrid tomato. Pak. J. Bot., 47(5): 1671-1677.

Kailash Chandra Bebarta (2011). Dictionary of Forestry and Wildlife Sciences. Concept Publishing Company. pp. 224–. ISBN 978-81-8069-719-7.

Khan, M.A., B.H. Abbasi and Z.K. Shinwari. 2014. Thidiazuron enhanced regeneration and Silymarin content in Silybum marianum L. Pak. J. Bot., 46(1): 185-190.

Kirchoff. B.K. 1992. Ovary structure and anatomy in the Heliconiaceae and Musaceae (Zingiberales). Canadian Journal of Botany, 70(12): 2490-2508.

Lentfer, C., ed. (June 2002 – June 2003). Tracing antiquity of banana cultivation in Papua New Guinea (Report). The Australia & Pacific Science Foundation. PBF 02-3. Archived from the original on August 29, 2007.

Meatia Zahra; Aveshek Datta; and patchareey Boonkorkaew. 2017. The effect of Different media, Sucrose Concentrations and Natural Additives on Plantlet Growth of Phalaenopsis Hybrid ‘Pink’ pp 11. Food/ feed science and Technology.

Moges, A.D., Karam, N.S and Shibli. R.A, 2003. Slow growth in vitro preservation of Africa violet(Saintpulia ionatha Wendl.) shoot tips. Advanced HortScience, 17:1-8.

Morton, Julia F. (2013). "Banana". Fruits of warm climates. Echo Point Books & Media. pp. 29–46. ISBN 978-1-62654-976-0. OCLC 861735500. Archived from the original on April 15, 2009 .

Morton, Julia F. (2013). "Banana". Fruits of warm climates. Echo Point Books & Media. pp. 29–46. ISBN 978-1-62654-976-0. OCLC 861735500. Archived from the original on April 15, 2009

Neitzsch, W. 1983. Germplasm preservation. In Hand book of plant cell culture. New York, Macmillan, pp: 82-805.Rao, N.K. (2004): Plant genetic resources: Advancing conservation and use through biotechnology. African Journal of Biotechnology, 3: 136–145.

Patrick L. Osborne (31 August 2000). Tropical Ecosystems and Ecological Concepts. Cambridge University Press. pp. 50–. ISBN 978-0-521-64523-2.
Panis B (2009) Cryopreservation of Musa germplasm, 2nd edn. In: Engelmann F, Benson E (eds) Technical guidelines No. 9. Biodiversity International, Montpellier, 48 pp.

Picq, Claudine & INIBAP, eds. (2000). Bananas (PDF) (English ed.). Montpellier: International Network for the Improvement of Banana and Plantains/International Plant Genetic Resources Institute. ISBN 978-2-910810-37-5. Archived from the original (PDF) on April 11, 2013. Retrieved January 31, 2013.
Ramachandran, K. Amutha 2013. Invitro Micropropagation of banana (Musa Spp.) By variant concentration of growth regulators.

Robinson, J.C. and Galán Saúco, V. 2010. Bananas and plantains. Crop production science in horticulture. CABI, Wallingford (GBR). 297p.

Roca, W.M., D.I. Arias, and R. Cha´vez. 1991. Me´todos de conservacio´n in vitro del germoplasma, p. 697–712. In: Roca,W.M. and L.A. Mroginski (eds.). Cultivo de tejidos en la agricultural: Fundamentos y aplicaciones. Cali: Centro Internacional de Agricultural Tropical.

Sarasan, Rcripps, MM Ramsay, C Atherton, 2006. Conserviation in vitro of threatened plants-progress in the past decade. Review.

S Shankar, U shanker- 2014. Arsenic contamination of groundwater: a review of sources, prevalence, health risks, and strategies for mitigation. The scientific world Journal, 2014-hindawi.com.

Shibli, R.A. and Al-Juboory, K.H., 2000. Cryopreservation of Nabali olive (*olea europeae* L.) somatic embryos by encapsulation-dehydration and encapsulation-vitrification. Cryoletters, 21:357-366.

Skutch, A.F. 1932. Anatomy of the Axis of the Banana. Botanical Gazette 93(3):233-258.

Stover, R. and N. Simmonds. 1987. Bananas. 3rd ed. Longman Scientific Technical, New York.

Sarkar, D., S.K. Kaushik, and P.S. Naik. 1999. Minimal growth conservation of potato microplants: Silver thiosulfate reduces ethyleneinduced growth abnormalities during prolonged storage in vitro. Plant Cell Rep. 18:897–903.

Saurabh Bhatia, Kiran Sharma, in Modern Applications of Plant Biotechnology in Pharmaceutical Sciences, 2015.

Stover & Simmonds 1987, p. 212.

Singh, H.P., S. Uma, R. Selvarajan and J.L. Karihaloo. 2011. Micropropagation for Production of Quality Banana Planting Material in Asia- Pacific. Asia-Pacific Consortium on Agricultural Biotechnology (APCoAB), New Delhi, Vol. 92.

Wilson G. Pond (16 November 2004). Encyclopedia of Animal Science (Print). CRC Press. pp. 425–. ISBN 978-0-8247-5496-9.

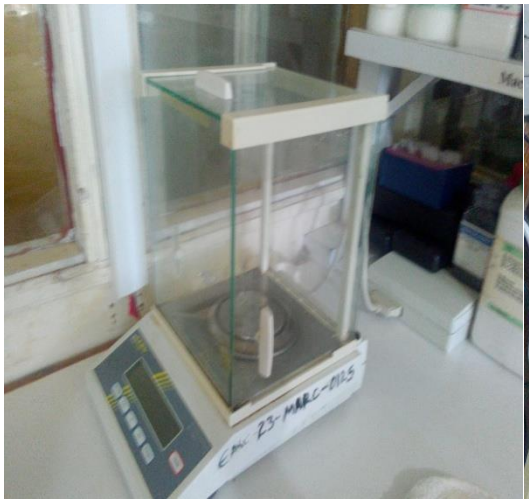
Wilkins, C.P. and J.H. Dodds, 1983. Tissue culture conservation of woody species. In: Dodds, J.H. ed. Tissue Culture of Trees. Croom Helm, London, pp: 113-138.

Appendices

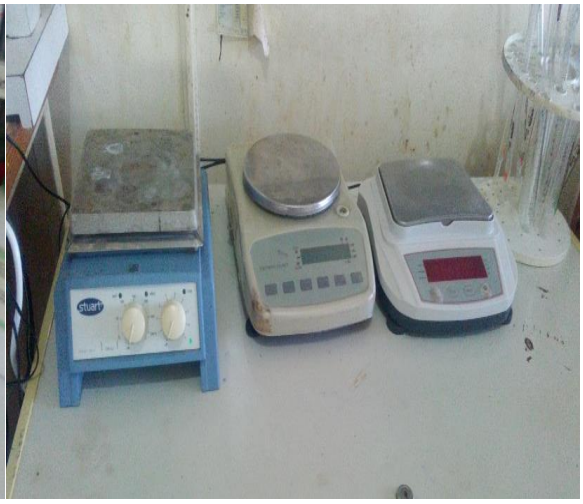
Stock solution

Autoclave

Sensitive balance



Balance



PH meter

poyo

Table 5: **ANOVA** table for poyo sorbitol: plant height, number of root and number of shoots

Height of plant

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.23068	0.11534	0.16	0.8524
Error	12	8.54956	0.71246		
Total	14	8.78024			

Number of shoots

Source	DF	SS	MS	F	P
Trt	2	0.00833	0.00417	0.09	0.9173
Error	12	0.57500	0.04792		
Total	14	0.58333			

Number of roots

Source	DF	SS	MS	F	P
Trt	2	2.1583	1.07917	0.77	0.4847
Error	12	16.8250	1.40208		
Total	14	18.9833			

Table 6: ANOVA table of Mannitol poyo: height of plant, number of root and number of shoot

Height of plant

Source	DF	SS	MS	F	P
Trt	2	3.6381	1.81905	0.97	0.4068
Error	12	22.4958	1.87465		
Total	14	26.1339			

Number of shoots

Source	DF	SS	MS	F	P
Trt	2	0.0583	0.02917	0.01	0.9897
Error	12	33.8750	2.82292		
Total	14	33.9333			

Number of roots

Source	DF	SS	MS	F	P
Trt	2	0.501	0.2505	0.02	0.9792
Error	12	142.959	11.9132		
Total	14	143.459			

Table 7: ANOVA table of poyo Sucrose: height of plant, number of root and number of shoots

Height of plant

Source	DF	SS	MS	F	P
Trt	**2**	3.1583	1.57917	1.39	0.2865
Error	**12**	13.6366	1.13638		
Total	**14**	16.7949			

Number of shoots

Source	DF	SS	MS	F	P
Trt	2	0.07500	0.03750	2.57	0.1176
Error	12	0.17500	0.01458		
Total	14	0.25000			

Number of roots

Source	DF	SS	MS	F	P
Trt	2	0.1750	0.08750	0.05	0.9513
Error	12	20.9250	1.74375		

Total	14	21.1000			

Giant variety

Table 8: ANOVA table for Sorbitol Giant: height of plant, number of root and number of shoots

Height of plant

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	3.2923	1.64613	1.98	0.1801
Error	12	9.9540	0.82950		
Total	14	13.2463			

Number of shoots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	1.22500	0.61250	4.39	0.0371
Error	12	1.67500	0.13958		
Total	14	2.90000			

Number of roots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	2.0333	1.01667	0.58	0.5729
Error	12	20.9000	1.74167		
Total	14	22.9333			

Table 9: ANOVA table for mannitol Giant: height of plant, number of root and number of shoots

Height of plant

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.52500	0.26250	0.65	0.5396
Error	12	4.84756	0.40396		
Total	14	5.37256			

Number of shoots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.15833	0.07917	0.79	0.4754
Error	12	1.20000	0.10000		
Total	14	1.35833			

Number of roots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.3250	0.16250	0.19	0.8266
Error	12	10.0750	0.83958		
Total	14	10.4000			

Table 10: ANOVA table for **S**ucrose Giant: height of plant, number of root and number of shoots

Height of plant

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.0087	0.00434	0.00	0.9963

Error	12	14.0993	1.17494		
Total	14	14.1080			

Number of shoots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.02500	0.01250	0.15	0.8623
Error	12	1.00000	0.08333		
Total	14	1.02500			

Number of roots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	3.6083	1.80417	1.22	0.3285
Error	12	17.7000	1.47500		
Total	14	21.3083			

Grande variety

Table 11: ANOVA table for Sorbitol Grande: for height of plant, number of root and number of shoots

Height of plant

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	1.37125	0.68563	1.03	0.3881
Error	12	8.02508	0.66876		
Total	14	9.39633			

Number of shoots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.17500	0.08750	1.14	0.3536
Error	12	0.92500	0.07708		
Total	14	1.10000			

Number of roots

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.3250	0.16250	0.10	0.9041
Error	12	19.1750	1.59792		
Total	14	19.5000			

Table 12: ANOVA table for mannitol Grande: for height of plant, number of root and number of shoots

Height of plant

Source	**DF**	**SS**	**MS**	**F**	**P**
Trt	2	0.10192	0.05096	0.18	0.8355
Error	12	3.35108	0.27926		
Total	14	3.45300			

Number of shoots

Source	DF	SS	MS	F	P
Trt	2	0.20833	0.10417	0.23	0.8008
Error	12	5.52500	0.46042		
Total	14	5.73333			

Number of roots

Source	DF	SS	MS	F	P
Trt	2	0.03333	0.01667	0.17	0.8425
Error	12	1.15000	0.09583		
Total	14	1.18333			

Table 13: ANOVA table for sucrose Grande: for height of plant, number of root and number of shoots

Height of plant

Source	DF	SS	MS	F	P
Trt	2	1.82500	0.91250	3.81	0.0524
Error	12	2.87484	0.23957		
Total	14	4.69984			

Number of shoot

Source	DF	SS	MS	F	P
Trt	2	0.00833	0.00417	0.02	0.9838
Error	12	3.05000	0.25417		
Total	14	3.05833			

Number of roots

Source	DF	SS	MS	F	P
Trt	2	8.2333	4.11667	3.53	0.0623
Error	12	14.0000	1.16667		
Total	14	22.2333			