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Review on *In Vitro* Conservation Protocol for Banana Germplasm through a Slow Growth Technique (*Musa sapientum L.*)

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Abstract

Banana (*Musa species*), is one of the most significant fruit crops grown widely in Ethiopia. It is extremely essential for ensuring food security in rural areas as well as for providing revenue and jobs. After rice, wheat, and maize, these are the next most significant food crops. India, China, Brazil, and Ecuador Field conservation of vegetatively propagated crops such as bananas are a key concern for germplasm curators. Nowadays, the slow growth storage (SGS) technique has become 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 mannitol, sorbitol, and sucrose.

Introduction

A 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 (parthenocarpy) 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 SapientumL, is no longer used by Merriam-Webster (2018) [1,2].

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 a key staple food (FAO, 2014) [3]. 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 bananas 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 et al.;2019) [4].

Globally, the major banana-producing countries are India (15% of total production), China, Brazil, Ecuador, and the Philippines (5-6% each) (FAOSTAT, 2020). In Africa, Angola, Tanzania, Kenya, Burundi, and Cameroon are the major banana producers (FAOSTAT, 2020). Major producers of bananas in Ethiopia are found in southern and south-western Ethiopia i.e., Arba Minch, Mizan Teferi, and Tepi (Seifu Gebra-Mariam, 2009). It covers about 59.64% (53,956.13 ha) of the total fruit area, about 68% (478,251.04 tons) of the total fruit produced, and about 38.3% (2,574.035) of the total fruit-producing farmers (Zinabu Ambisa et al., 2019) [5].

Pests and diseases as well as other pressures (drought, cold, hailstorms, etc.) and wildfires, are some of the causes. Tissue culture, on the other hand, has been proven to be an effective method of storing a variety of vegetative propagated commercial crops such as potatoes, palms, forest species, and fruit crops (RAO, 2014) [6]. Over the last three decades, immense effort has been made in the area of research for in vitro conservation of such plant genetic resources, including bananas, via tissue culture (Neitzsch, 2016 Moges, 2019). Minimal growth conditions could be achieved through the induction of osmotic stress with mannitol or sucrose, reduced temperature, and/or low light intensity (George, 2017). Slow-growth procedures have been developed for a wide range of species; they are routinely used for the conservation of genetic resources of only a few species including Musa spp. This technique of germplasm conservation is as well acclaims med useful in the subsequent distribution of the materials at any time when needed (Winks, et al.; 2004) [7,8].

Micro-propagation of in vitro conserved Germplasm also holds promise for the regeneration of true-to-type planting materials. Hence, a plant tissue culture strategy-based micropropagation technique warrants the mass cloning of elite plants that are uniform in their chemical and genetic constituents to that of the mother plant (Kiran Sharma, 2019). Commercial in vitro multiplication of bananas 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 the capacity to maintain strict genotypic and phenotypic stability under tissue culture conditions (Bennici, 2014). In vitro, storage of plant genetic resources

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becomes a very useful alternative for genetic variability conservation, crop improvement programs, and production of certified seeds [9].

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 et al., 2009). 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 during the incubation period. Therefore, this review is initiated to develop/optimize a proper protocol for the conservation of dessert and cooking banana germplasm materials through the slow growth technique [10].

Literature Review

Origin, domestication, and distribution of banana

The genus Musa which is about 50 million years old has been distributed globally i.e., most importantly in the Southeast Asian region where it is believed to have 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, 2017). All the wild bananas are warm-region plants and this genus has a limited tolerance for low temperatures but a few species have resistance to cool temperatures and no species have resistance to drought (Nayar, 2017) [11].

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 (Zahra, M et al. 2017). 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 [12]. It is perennial because suckers, shoots arising from lateral buds on the rhizome, take over and develop into fruit-bearing stems (Amany1 Eliway Ali, Mohamed1, 2018). What looks like a trunk is not a woody stem but a pseudo stem, 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 which is attached to the leaves, and the peduncle which is attached to the inflorescence (Mafla G, Panis B,). The stem starts on the rhizome's apical meristem, grows inside the pseudo stem, and ends in the male bud (Zahra, M et al. 2017) [13].

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 (Debouck D, Dumet D, Escobar R, 2011) [14]. 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 [15]. The primary roots originate from the surface of the central cylinder whereas secondary and tertiary roots originate from the primary roots (Amutha, 2017). 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; the production of roots from multiple nodes; and the production of clonal shoots (Panis B and Panta A, 2011). Corms, on the other hand, are vertically 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 [16].

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. Pseudostem the stem is visible in the center of the pseudostem (Muhammad Munir Iqbal, 2013). The pseudostem is the part that looks like a trunk. This 'false stem' is formed by the tightly packed overlapping leaf sheaths (G. Reyes et al.; 2017). The pseudostem continues to grow in height as the leaves emerge one after the other and reach their maximum height when the stem, which has been developing inside the pseudostem, emerges at the top of the plant [17].

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 are 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 into 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 [18].

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 pseudo stem 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 Ahirwar et al., (2012). The petiole becomes the midrib, which divides the blade into two lamina halves [19]. 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 a 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. 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 favorable climatic conditions, it takes about seven days, but it can take up to 15 to 20 days under poor conditions [20].

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 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 suckers: 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. et al.; 2010) [21,22].

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, a 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, 2000) [23].

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 SapientumL 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 (Ahmed, S et al.; 2014). Many of these cultivars were given names that were later discovered to be synonyms (Valmayor et al. 2000) [24]. In a series of papers published from 1947 onwards, Ernest Cheesman showed that Linnaeus's Musa SapientumL and Musa paradisiaca were cultivars and descendants of two wild seed-producing species, Musa acuminate 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 exhibiting the botanical characteristics of Musa acuminata, and those with characteristics of both Al-Amin et al. (2009) [25].

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 × paradisiacal L. for the hybrid M. acuminata × M. Balbisiana Porcher (Michel H, 2011) [26].

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); rising environmental awareness (GAT and Trade-GATT and WTO); rising environmental awareness (Montreal Protocol in 1987 and ES 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 the retail level (Gray R. and Daniels, D. 2015) [27]. 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 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 [28].

Banana cultivation in Ethiopia

Banana, especially the dessert banana is the major fruit crop in Ethiopia leading both in area and production. (Amutha, 2017). 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 southeastern parts of the country, it is of great socioeconomic importance contributing significantly to the overall well-being of the rural communities including food security, income generation, and job creation (Hussein, N. (2012). About 104,421.81 hectares of land are under fruit crop production in Ethiopia. Bananas contributed about 56.79% of the fruit crop area followed by avocadoes which 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 F et al, 2014) [29].

Local cultivars of bananas were under cultivation in Ethiopia for a long period of time. These local varieties are low-yielders and have market demand. As a result, several high-yielding banana varieties were introduced and adopted in the country. According to the Ministry of Agriculture Research and Development (MoARD, 2016), in Ethiopia, there are seven desserts and five cooking-type banana varieties released by the research system. Besides, different local varieties are produced in almost all parts of the country by small-scale farmers as garden crops mainly for home consumption and in some cases for sale in local markets (Hussein, N. 2012) [30].

Types of bananas grown

Cultivated bananas are derived from two species of the genus Musa, namely Musa acuminate and Musa Balbisiana. Musa acuminate originates from Malaysia, while Musa Balbisiana originates from India (Jamir, S. and Maiti, C. S. 2014)). 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 (Amutha, 2017) [31].

In major banana-producing areas, farmers produce formerly recommended varieties such as Dwarf Cavendish, Giant Cavendish, and Poyo. Most of them produce Dwarf and Giants. They produce these

varieties for the market (Amutha, 2017). 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 the Ducasse Hybrid variety, East African Highland Cooking banana, which is used as a windbreak and tolerant to stresses such as drought. Ducasse hybrid is a starchy type and is not preferred as a dessert, but other African countries use it for brewing (Zinabu et al.: 2019) [32].

Nutritional composition of banana

Bananas are a nutritious fruit in terms of their carbohydrate and sugar content. Ripe fruit contains as much as 22% of carbohydrates, mainly sugar, and is high in dietary fiber, potassium, manganese, and vitamins B6 and C (Amutha, 2017). Almost all the modern edible parthenocarpy bananas come from the two wild Species. Acuminata and M. Balbisiana. Other than fresh fruits, they can be consumed as processed in various forms like chips, powder, flakes, etc. Banana pseudo stem is disease-free planting material in large amounts (Zinabu et al.: 2019) [33].

Micropropagation of banana

Multiplication of plants using the plant tissue culture technique is used as an alternative to the conventional method to circumvent the drawbacks associated with the latter (Amutha, 2017). Tissue culture is the propagation of a plant part single cell or group cell in a test tube/jar under controlled and aseptic conditions. It has laid the foundation for the production of uniform, high-quality, disease-free planting material and true-to-type plants at a mass scale (Amutha, 2017) [34]. The tissue culture technique in which plant cells, tissues, or organs are used to generate multiple identical true-to-type plants under in vitro conditions is called micropropagation or in vitro mass propagation. The technique has several advantages including rapid initial propagation of new varieties, regeneration of a large number of plantlets from a small tissue, elimination of pathogens, and storage of plant germ-plasm under aseptic conditions. Micropropagation is a user-friendly technique that does not require much expertise and is suitable for adoption by small and marginal farmers success of micropropagation depends on the method, variety, and price of initiation media (Zinabu et al.: 2019) [35,36].

In vitro multiplication of banana plantlets is an excellent alternative and a number of countries in the world like Israel (Israeli et al., 2005), France (Amutha, 2017), Australia (Drew and Smith 2019), Cuba, and many African countries (Amutha 2013), are using this technique. The micropropagation of bananas has been achieved using shoot tips (Cronauer and Krikorian, 2004) and from male floral apices (France (Amutha 2013). There are also reports of somatic embryogenesis and regeneration in a liquid medium (Novak et al. 2018). One of the most important factors affecting the efficiency of the micropropagation system is the rate of multiplication. It has been observed that the banana multiplication rate is genotypic dependent as well and variable behavior has been observed among cultures initiated from the same banana genotypes cultured in vitro in France (Amutha, 2020) [37].

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, the micropropagation of plants can be influenced by several factors including genotype (explant type, source, and size) media composition, culture environments, and availability of micropropagation protocols Elisama et al. (2017) [38].

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 plant tissue culture work. 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 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 the addition of vitamins, hormones, and sugars, MS basal medium is used by different laboratories to suit their needs [39].

In line with this, many workers used MS basal medium for sugarcane micropropagation (Bekesha et al., 2002). 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, 20017) [40].

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 Elisama et al. (2013). 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 [41].

Micropropagation Stages

Mother plant preparation

Loss of cultures due to contamination is one of the most serious problems in plant tissue culture Elisama et al. (2013). Explant contamination depends on several plant and environmental-related factors such as species, age, explant source, and prevailing weather conditions (Bekesha et al., 2002). Prior good care of stock plants may lessen the amount of contamination that is present on explants; plants grown in the field are typically "dirtier" than those grown in a greenhouse or growth chamber. Thus, to minimize the initial source of contamination, researchers grow donor plants in the greenhouse (George and Klerk, 20017). Mother plant preparation is the act of raising mother plants under greenhouse conditions or more hygienic conditions to reduce the risk of contamination. Therefore, to minimize the contamination problem that can come from explant sources, it is important to collect the explants from mother plants grown in protected areas under appropriate pretreatment with fungicides and pesticides (Bekesha et al., 2012) [42,43].

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 (Anbazhagan et al.; 2019). 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 (RAO, 2019). Fungi and bacteria are the most frequently reported contaminants in sugarcane micropropagation (Wagih et al., 2019). 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., 2015) [44]. For instance, (Anbazhagan et al.; 2019) had surface sterilized banana explants with 70% ethanol for 60 seconds, 5 percent sodium hypochlorite solution for 10 minutes, and 0.1 percent HgCl2 for 5 minutes.

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 Muhammad et al. (2014). 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. (2014) reported that BAP is the most often employed cytokinin in banana tissue culture by Cronauer and Krikorian (2010) and Vuylsteke (2010) [45].

In vitro multiplication

This is the most important stage in any propagation program since it determines the number of produced plants (Omar and Aouine, 2017). In this stage, the number of propagules brought from the 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 genetic stability (IAEA, 2016). In vitro, shoot multiplication is critically influenced by the type and concentration of plant growth regulator/s in culture media [46].

In line with Aremu et al. (2012) studied the effects of five topologies (metaTopolin=mT; metaTopolinriboside=mTR; metaMethoxytopolin=MemT; metaMethoxytopolinriboside=MemT R and metaMethoxytopolin tetrahydropyranyl=MemTTHP) on shoot regeneration of micro propagated 'Williams' bananas and compared to benzyl adenine (BA) Elisama et al. (2013). The study revealed that 30 µM mT resulted in the highest number of Shoots (7.3 ± 1.0). Unlike other cytokines (CK) treatments requiring higher concentrations, the optimum mean shoot number per explant was attained at the lowest concentration in MemT and MemTTHP (10 µM) treatments. In terms of abnormality index, their study showed that mTR regenerated plantlets were of the best quality across all the CKs tested (Brainerd and Fuchigami, 2011) [47].

Similarly, Sipen and Davey (2012) studied the different concentrations of N6 benzyl aminopurine (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 concentrations of BAP (0-10 mg/l), Kinetin (0-10 mg/l), NAA (0.3-0.5 Mg/l), and different combinations of BAP (0-10 mg/l) and NAA (0.3-0.5 mg/l) Brainerd and Fuchigami, (2011) [48]. They observed the highest frequency of shoot regeneration (52.25), number of shoots regenerated per explants (3.25), and shoot length (4.69) at a 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 (Wagih et al., 2019). 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 the cultivar Agnishwar. Among different types and concentrations of cytokinins viz. 6-benzyl amino purine (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 [49]. The highest average number of shoots for each explant (5.9) was found in the MS medium fortified with 4.0 mg/l BAP, while the maximum elongation of the shoot (4.9 cm) was observed in the MS medium containing 5.0 mg/l BAP.

Ramachandran and Amutha (2013) reported the best shooting response for the Cavendish Dwarf variety on basal MS medium supplemented with 4 mg/l BAP + 0.2 mg/l NAA. They also observed the 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 [50].

The study revealed that medium supplemented with BAP 4.0 mg/l produced a greater number of shoots (55) and longer shoots (3.0 ± 0.012 cm) when compared with other treatments. Reddy et al. (2014) studied the effect of diverse concentrations of 6-benzylamine purine (6-BAP) on shoot induction of GrandeNaine 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 in the concentration of 3.0 mg/l and 0.5 mg/l respectively [51].

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 concentrations of BAP (0-6.5 mg/l). It was observed that 4.5 mg/l BAP was found to be the best concentration in the induction of the highest number of buds (an average of 7.05 and 7.2) with the highest mean length of 0.65 cm and 0.7 cm of shoots. However, the shoot elongation was maximum at a lower concentration of BAP (1.5 mg/l). Shashikumar et al. (2015) tested the effect of BAP, TDZ, and coconut water at various concentrations. They recorded a 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 micropropagation of banana cv. Malbhog on MS medium supplemented with different concentrations and combinations of IAA and BAP Elisama et al. (2017). This combination resulted in the differentiation of adventitious shoots (Brainerd and Fuchigami, 2011). They reported maximum differentiation of shoots (92.05 %) on MS medium with 0.57 mL. 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 Elisama et al. (2013) [52,53].

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, 2011). They are not qualified to withstand the low humidity, increased light levels, and more volatile temperatures found outside (Wainwright, 2016). 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% carbendazim, and sterilizing the potting mixture (Anderson, 2020; Muniswamy et al. 2021) [54,55].

Furthermore, 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) Shibli et al., (2006). 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 the nutritive solution on the growth of Musa Cavendish plantlets during the greenhouse acclimatization phase [56].

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) Elisama et al. (2013). 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 (Shibli et al., 2017). They reported that the IBA treatment had no discernible effect on the M. Cavendish plants' growth. They reported no evidence of a link between fertigation and the use of IBAs [57].

Banana germplasm conservation

Conservation of plant genetic resources is a priority for maintaining genetic variability-the basis of all plant breeding programs. Banana is among many groups of species that are suffering from genetic erosion. (Shibli et al., 2016; Rai et al., 2019) 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 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, 2007). Due to the parthenocarpy nature of bananas, their corms are used for propagation, but their large size makes transport from place to place very expensive. Hence, any user of Banana will benefit from in vitro germplasm conservation methods that improve the management of these propagules (Wagih et al., 2019). Tissue culture is one such method and its use for in vitro collecting of explants in the field is a real possibility [58].

In addition to conventional propagation problems, both biotic and abiotic factors affect the morph biochemical and physiological properties of plants (Jan et al., 2016; Jan et al., 2017). The crop of bananas is at 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 crops while the abiotic stresses such as environmental factors also play a role in minimizing banana 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., 2015). Propagation of crops by using the tissue culture technique (TC) is the simple and first solution to the problems of such rapidly evolving ailments (Hussain et al., 2013; Khan et al., 2014; Jan et al., 2015). Among the methods of conservation available for bananas, 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) [59].

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 conditions (Withers, 2012; Engelmann 2010; 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 subculturing (Bessembinder et al., 2013; Hao and Deng, 2013) [60].

Additionally, there is a risk of losing propagating material as a result of human error or microbial contamination in the process of subculture (Grout, 2013); therefore, it is advisable to reduce frequent interventions during 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. 2017), 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, 2013; Engelmann 2020). The success of the use of certain approaches depends on numerous factors, such as the possibility of extending the 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 2010). The essential condition for using slow-growth procedures is the study of the vital capacities of various kinds of cultures and the stability/instability of the preserved material (Shibli et al., 2006; Rai et al., 2009) [61].

Conclusion

Consumable bananas (Musa spp.) are a vital source of revenue for rural areas in tropical and subtropical countries as well as the main

staple food for rural and urban consumers. In Ethiopia, the banana is the most widely used naturally occurring product for both food and commerce. In any event, there are requirements for banana production. Low productivity, low quality, and variable quality are some of the banana generation limits. The question was directed at reviewing a fundamental, extensive, and effectively monotonous norm for banana (Musa SapientumL.) micropropagation. Germplasm conservation system, in vitro Germplasm conservation through tissue culture system is considered to be the best option in minimizing storage space, labor, and costs. Due to the parthenocarpy nature of bananas, their corms are used for propagation, but their large size makes transport from place to place 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. So, the methods of conservation available for bananas, in vitro, conservation is still advantageous over the other methods since it is technically easy, requires less labor and land, and facilitate easy exchange of germplasm.

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

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