

Review on Tissue Culture of Banana (*Musa sapientum* L.)

Aduugna Mosissa Kajela*

Ethiopian Institute of Agricultural Research Center, Melkassa Agricultural Research Center P.O.B, 436, Ethiopia

Abstract

Edible bananas (*Musa* spp.) are the major staple food for rural and urban consumers in tropical and subtropical countries and an important source of rural income. Banana is the most produced fruit in Ethiopia for food and commercial purposes. However, there are constraints in the production of bananas. Among the banana production constraints, are low production low quality, and quality of yield. The study aimed to review a simple, comprehensive, and efficiently repetitive protocol for micropropagation of bananas (*Musa sapientum* L.).

Introduction

Bananas (*Musa sapientum* L.) are the world's most important food crop, with an annual production of over 102 million tons. It is the most well-known and widely distributed fruit in the world, and it can be consumed raw or cooked, as well as processed into starch, chips, beer, vinegar, or dried items [1]. Bananas are high in vitamins C and A, as well as calories (128 kcal/100 g). Banana fruits have a high potassium (K) content and a low sodium (Na) ratio, providing a protective effect of K against excessive Na intake in the diet (FAO, 2012).

Edible bananas (*Musa* spp.) are a key source of rural income and a staple food for both rural and urban consumers in tropical and subtropical nations. The *Musa* genus (family Musaceae) has its origins in Asia (Simmonds, 1962). Bananas are cultivated from two diploid species of the genus *Musa*. Parent genomes of *M. acuminata* (Malaysia) and *M. balbiana* (India) (Stover & Simmonds, 1987; Simmonds, 1962; George et al., 2000) [2]. Carbohydrates, proteins, vitamins, and minerals are all abundant in bananas. Many pests and illnesses (particularly viral diseases such as banana mosaic virus) limit banana output, resulting in major environmental repercussions due to pesticide use. The lack of disease-free, true-to-type planting material, low fertility due to triploidy, slow propagation, and a long-time span between generations are also important restrictions in the banana production system. Because of the high degree of sterility and polyploidy of edible cultivars, traditional breeding is challenging (Stover & Simmonds, 1987) [3].

Because practically all cultivated banana cultivars are triploid, seedless, or seed sterile, bananas belong to a category of crops that are generally propagated through the vegetative components of the plant. Corms, large and small suckers, and sword suckers are among the components utilized in traditional propagation (Cronauer & Krikorian, 1984; Arias, 1992; Haq and Dahot, 2007). For banana crop enhancement that is based on reliable plant regeneration protocols, mass propagation of selected genotypes, somaclonal variation approaches, genetic engineering, and other biotechnological applications can be used. Tissue culture is also important for germplasm distribution, conservation, safe internal planting material exchange, and quick multiplication of newly selected hybrid cultivars. Micropropagation has been used to regenerate *Musa* spp. by several researchers (Cronauer & Krikorian, 1986; Jarret, 1986; Diniz et al., 1999; Nauyen & Kozai, 2001; Krishnamoorthy et al., 2001; Kagera et al., 2004; Muhammad et al., 2004; Roels et al., 2005; Madhulatha et al., 2004). However, propagation percentage and technique repeatability are issues that require a thorough, repeatable, and applicable approach for a wide range of genotypes to promote disease-free banana crop production on a commercial scale [4,5].

Bacterial contamination is a major issue in banana in vitro

micropropagation. Although surface sterilizing initially succeeds, microbial contamination at the explant's base emerges 7 to 15 days after inoculation. Endogenous bacteria destroy a large proportion of explants in the culture (Habiba et al., 2002). The current work proposes a quick banana multiplication technique from the shoot meristem by employing a medium with an optimal auxin/cytokinin concentration. Many publications on in vitro propagation are available, with sophisticated techniques but lower shoot proliferation percentages, resulting in fewer regenerated plants per culture [6].

Ethiopian farmers had a long history of growing and harvesting this fruit. However, they were unable to get plant components that were devoid of pollutants. As a result, perfecting in vitro protocols for selected cultivars is critical to supplying farmers with desired planting materials. Bananas are propagated asexually by detaching the daughter suckers from the mother plant or through tissue culture. Unlike sucker-derived planting material, tissue culture provides several advantages [7]. Producing homogeneous planting materials, clean, disease-free materials, and a large number of plants in a limited space are among them. It allows for large-scale propagation and the use of clean planting material. They are less expensive to transport than traditional suckers, and the combination of virus indexing enables safe transit, germplasm exchange, and conservation [8].

Furthermore, bananas grown utilizing tissue culture technology are said to be more vigorous, yield more, and produce higher-quality fruits than those grown using traditional methods (De Langhe, 1985). Despite these benefits, tissue culture technology is either unknown or underutilized in Africa (Murungikahangi. E, 2010). As a result, protocol optimization work is unavoidable in order to fully harness the benefits of this technology. In vitro regeneration of crop plants in an artificial medium requires growth regulators for this plant. Auxins aid in the rooting of proliferated shoots, while cytokines aid in shoot proliferation. However, the number of cytokines and auxins required

***Corresponding author:** Aduugna Mosissa Kajela, Ethiopia Institute of Agricultural Research, Melkassa Agricultural Research Center POB; 436, Ethiopia, E mail: adugnamosia@gmail.com

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sterilizing procedure should be chosen so that it kills the bacteria while having no negative impact on the plant tissues. Endogenous bacteria that escape initial disinfection or microorganisms introduced during tissue culture operations might induce contamination in tissue culture. Several antibiotics have been found to be effective in reducing bacterial contamination in banana tissue culture [23].

Oliveira et al., (2000) tested two techniques of banana explant disinfection as well as the use of three different indicator culture mediums for banana micropropagation. The cultivar Pioneira's shoot tips were disinfected by submerging them in 80 percent alcohol for 2 minutes and then submerging them in sodium hypochlorite (2 percent active chlorine) with 4 drops of Tween-20 for 10 minutes while shaking. The contamination was purely bacterial in origin. Lima and Moraes (2006) used NaOCl, rifampicin antibiotic, and their combinations to test bacterial contamination management strategies in banana explants cv. Caipira. Immersion in 1 percent (v/v) NaOCl for 10 minutes, followed by immersion in 300 mg/l rifampicin for 20 minutes, was the best treatment for explants. Immersion in 1 percent NaOCl for 10 minutes, followed by immersion in 300 mg/l rifampicin for 24 hours in the dark, was the best treatment after contamination [24].

Sodium hypochlorite (which dates back to the mid-eighteenth century), calcium hypochlorite, ethanol (or isopropyl alcohol), mercuric chloride, hydrogen peroxide, silver nitrate, and bromine water are all commonly used disinfectants. To improve the effectiveness of the sterilization procedure, a surfactant like Tween-20 is frequently added to the sterilizing solution (and in some laboratories, a mild vacuum is applied during the procedure). During the sterilization period, the sterilizing liquids containing the explants are generally constantly agitated (Oyebanji, et al., 2009). The effects of several disinfectants on explants were investigated by Jing-Yan et al., (2011) [25]. The sucker buds of the Longxuan banana used as explants were sterilized with mercuric chloride (0.1%) and sodium hypochlorite (0.2%) solutions. On the disinfection of explants, sodium hypochlorite performed better than mercuric chloride; the sterilization rate was 90.47 percent, and the explants developed well without intoxication [26].

Goswami and Handique (2013) sterilized explants by first treating them with Savlon for 15 minutes, then sterilizing them for 45 minutes with Tween-20 and a mixture of 2 percent NaOCl+1 gm/l Captan or DithaneM-45 and rifampicin (0.1 percent). After that, explants are quickly dipped in 70% ethanol for 15 seconds. Explants treated with NaOCl (0.5-1%) for 15 minutes followed by 0.1 percent HgCl₂ for 7 minutes performed better than those treated with the other therapies in a laminar airflow cabinet. Bohra et al., (2014) investigated the efficacy and phytotoxicity of antibiotics in the establishment of aseptic cultures in the difficult-to-establish native Ney Poovan banana (Musa AB). In order to obtain aseptic cultures in the native banana variety Elakki Bale, they standardize antibiotic supplementation. Chloramphenicol was shown to produce 100% aseptic cultures, followed by rifampicin + chloramphenicol, rifampicin, and chloramphenicol + streptomycin combinations. Rifampicin was discovered to have the least amount of phytotoxicity. In successive cycles, the plantlets grew properly, and 96.3 percent of the plantlets survived the transfer to ex vitro settings [27,28].

The explants were surface sterilized by rinsing in tap water for 30 minutes, then gently rinsing with 70% ethanol for 60 seconds and a 5 percent sodium hypochlorite solution for 10 minutes, according to Anbazhagan et al., 2014. In addition, sterilizing operations were carried out in a laminar airflow chamber for 5 minutes using 0.1 percent HgCl₂. The lighting, temperature, and humidity conditions given inside the growth room all play a role in the success of an in vitro

procedure [29]. The intensity, quality, and duration of light are the most important elements impacting in vitro culture growth (Murashige, 1974, 1977). Murashige (1977) discovered that a 16-hour photoperiod is sufficient for a wide variety of plant species. The relative humidity in the culture room has been set to 55%. Banana shoot tip cultures are grown at a temperature of 28°C in a light cycle of 12-16 hours, with a photosynthetic photon flux (PPF) of roughly 60 E/m²/s [30].

Effects of various types and concentrations of growth hormone on the beginning of new shoots

In initiation media containing varying concentrations of BAP and IAA, in vitro culture of banana shoot tips produced a rigid meristematic ball-like shape. In a few days after inoculation, the cultured shoot tip changed color from creamy white to brown (Muhammad Munir Iqbal, 2013). As described by Muhammad et al., (2004) employing BAP, the exterior leaf surface of explants turned green and a spherical hard coat mass appeared from which adventitious plantlets were generated four weeks later. BAP was also reported as the most often employed cytokinin in banana tissue culture by Cronauer and Krikorian (1984) and Vuylsteke (1998). Furthermore, following 30 to 35 days of inoculation, Al-Amin et al., (2009) observed a color shift of culture meristems to brown in 4 to 5 days and the growth of a green hard ball-like structure. Various types and concentrations of growth hormone have different effects on shoot multiplication [31].

Cronauer and Krikorian (1984) claimed that sliced banana and plantain shoot tips might be used to develop additional shoots. The most often used PGRs for banana micropropagation have been reported to be auxins and cytokinins (Vuylsteke, 1998) [32]. In numerous *Musa* spp., adenine-based cytokinins, particularly BAP, are the most often used cytokinins to influence shoot multiplication rate (Cronauer and Krikorian, 1984; Vuylsteke, 1998). Under in vitro circumstances, differences in the rate of multiplication of different *Musa* genotypes have also been documented (Vuylsteke, 1998; Muhammadi et al., 2004). Multiple shoots have been reported to be formed from sliced banana and plantain shoot tips (Cronauer and Krikorian, 1984). The most often used PGRs for banana micropropagation have been reported to be auxins and cytokinins (Vuylsteke, 1998). In numerous *Musa* spp., adenine-based cytokinins, particularly BAP, are the most often used cytokinins to influence shoot multiplication rate (Cronauer and Krikorian, 1984; Vuylsteke, 1998) [34].

Under in vitro circumstances, differences in the rate of multiplication of different *Musa* genotypes have also been documented (Vuylsteke, 1998; Muhammadi et al., 2004). Yellow friable calluses of banana (*Musa* spp.) 'Gros Michel' cultivated on Murashige and Skoog (MS) solid medium supplemented with 2, 4-dichlorophenoxy acetic acid (2, 4-D) and coconut water were observed by Srangsam and Kanchanapoom (2003). (CW) [35]. The calluses were small, spherical, and compressed. Friable calluses were placed in half-MS liquid media with 1.5 mg/l 2, 4-D, 1.5 mg/l 2, 4-D in combination with 5% CW, or half-MS liquid media without 2, 4-D and CW. These media did not result in any shootings. Embryogenic calluses were produced, and the spherical, compact calluses were then subcultured to half-MS solid medium in the presence of thidiazuron (TDZ). On MS germination media with 2.0 mg/l-naphthalene acetic acid (NAA) and 1.0 mg/l 6-benzyl adenine, these embryogenic calluses produced shoots (BA) [35].

In 2004, Muhammad et al. cultured the shoot ends of banana cv. Basrai grew plants on Murashige & Skoog basal medium supplemented with 5.0 mg/l BAP, averaging 124 plants per shoot tip after five subcultures. Al-Amin et al., (2009) investigated the effects of different

BAP and NAA concentrations on banana plant regeneration and shoot multiplication. BARI is a Japanese word that means “banana” in English. At a concentration of 7.5 mg/l BAP + 0.5 mg/l NAA, the highest shoot proliferation, longest shoot production, maximum number of leaves, and longest leaves were observed. Jing-Yan et al. (2011) used several hormonal combinations (0-6.0 mg/l 6-BA and 0.1-0.2 mg/l NAA) in MS medium to generate adventitious buds and facilitate multiplication in banana cv. Longxuan. MS + 3.0 mg/l 6-BA and MS + 4.0 mg/l 6-BA + 0.2 mg/l NAA were shown to be the optimal induction and multiplication medium for adventitious buds, respectively [36].

Aremu et al., (2012) investigated the effects of five topolins (metaTopolin=mT; metaTopolinriboside=mTR; metaMethoxytopolin=MemT; metaMethoxytopolinriboside=MemTR; metaMethoxytopolin 9 tetrahydropyranyl=MemTTHP) on shoot regeneration in micro propagated ‘Williams’ bananas, comparing them to benzyl adenine (BA) [37]. At 30 M mT, the maximum number of shoots (7.31.0) was obtained. Unlike other CK therapies that require greater concentrations, MemT and MemTTHP (10 M) treatments achieved the optimal mean shoot number per explant at the lowest dose. The quality of mTR regenerated plantlets was the best of all the CKs evaluated in terms of abnormality index. Mondal et al. (2012) investigated the effects of coconut water and ascorbic acid on banana variety Dwarf Cavendish shoot regeneration. They infected the shoot tips with BAP (Benzyl Amino Purine) (5.0 mg/l) supplemented with coconut water at various concentrations (0, 50, 100, 150, and 200 ml/l) and ascorbic acid in various concentrations (0, 25, 50, 75, and 100 mg/l) [38].

When the concentrations of coconut water and ascorbic acid were increased to 100 mg/l and 50 mg/l, respectively, there was a significant rise in the frequency of explant in shoot regeneration, the number of shoots regenerated per explant, and shoot length. The effect of varying quantities of N6 benzylaminopurine (BAP) and Indole Acetic Acid (IAA) on shoot multiplication and plant regeneration in the Malaysian banana cultivars Pisang Mas, PisangNangka, PisangBerangan, and PisangAwak was investigated by Sipeen and Davey (2012). On medium supplemented with BAP at 5 mg/l (PisangNangka), 6 mg/l (Pisang Mas and PisangBerangan), and 7 mg/l (PisangAwak) with 0.2 mg/l IAA, the maximum shoot was produced [39]. Ahirwar et al. (2012) also used various concentrations of BAP (0-10 mg/l), Kinetin (0-10 mg/l), NAA (0.3-0.5 mg/l), and combinations of BAP (0-10 mg/l) and NAA (0.3-0.5 mg/l). BAP concentrations of 5 mg/l, Kinetin concentrations of 5 mg/l, and a combination of 7.5 mg/l BAP + 0.3 mg/l NAA were shown to have the highest frequency of shoot regeneration (52.25 percent), number of shoots regenerated per explant (3.25), and shoot length (4.69 cm).

For shoot development from shoot tip or male inflorescence tip explants, 5 mg/l BAP was found to be superior to Kinetin. Rahman et al. (2013) looked at the optimal plant growth regulators for cultivar Agnishwar shoot proliferation and multiplication. When different types and concentrations of cytokinins, such as 6-benzylaminopurine (BAP), kinetin (KIN), and N6-(2-isopentyl) adenine (2iP), were evaluated for shoot multiplication, the MS medium containing 4.0 mg/l BAP yielded the highest multiplication (95%). The MS medium containing 4.0 mg/l BAP had the largest average number of shoots for each explant (5.9), while the MS medium containing 5.0 mg/l BAP had the maximum elongation of shoots (4.9 cm). Ramachandran and Amutha (2013) conducted a study on the Cavendish Dwarf banana type. The most suitable combination for shooting was Murashige and Skoog’s basal media supplemented with 4 mg/l BAP and 0.2 mg/l NAA [40].

Furthermore, Ahmed et al. (2014) discovered that MS medium

supplemented with 4.00 mg/l BAP and 2 mg/l IAA was optimum for explant establishment and shoot multiplication of banana cv. Grand Naine. Shiv Shankar et al. (2014) used PGRs Benzyl Adenine Purine and Kinetin to mass propagate the banana (*Musa* spp.) cv. Grand Naine through direct organogenesis. Shoot proliferation and differentiation, as well as shoot multiplication rate, were studied using Benzyl Adenine Purine (BAP) in five different concentrations (control, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/l). When compared to other treatments, medium supplemented with 4.0 mg/l BAP generated a higher number of shoots (55) and longer shoots (3.00.012 cm). Reddy et al. (2014) investigated the effect of different concentrations of 6-benzylamine purine (6-BAP) on Grandnaine plantlet shoot induction (*Musa* spp). Anbazhagan et al., (2014) cultured *Musa* spp. shoot tips on Murashige and Skoog (MS) medium supplemented with various concentrations of BAP, KIN, and IAA, both individually and in combination, and found that MS medium supplemented with BAP + IAA at concentrations of 3.0 mg/l and 0.5 mg/l produced the best results [41]. The effect of different amounts of cytokinin and auxin on in vitro regeneration of the banana cultivars Grand Naine and Jahaji was examined by Jamir and Maiti (2014). They examined BAP concentrations ranging from 0 to 6.5 mg/l. The concentration of 4.5 mg/l BAP was found to be the most effective in inducing the most buds (an average of 7.05 and 7.2) with the longest mean length of 0.65 cm and 0.7 cm of shoots. However, at a lower concentration of BAP (1.5 mg/l), shoot elongation was greatest [42].

Shashikumar et al., (2015) used BAP, TDZ, and coconut water at various doses in their research and found a high frequency of shoot initiation (93.33 percent) at 5 mg/l BAP. Multiple shoot buds were induced by 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), and this was optimum at 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) used Murashige and Skoog (MS) medium supplemented with varied amounts and combinations of Indole Acetic Acid (IAA) and Benzyl Amino Purine to micro-propagate banana cv. Malbhog (BAP). The adventitious branches were differentiated as a result of this combination. On MS medium with 0.57 m IAA + 17.74 m BAP, the highest differentiation of shoots (92.05 percent) was observed. A total of 16.75 shoots were produced per culture. Subculture of differentiated shoots on the same medium resulted in more than 15 shoots per culture being distinguished (91.97 percent) [43].

The effects of various growth hormone concentrations on rooting

For root initiation in banana cv. Al-Amin et al. (2009) employed half-strength MS medium supplemented with varied amounts of IBA (0, 0.5, 1.0, and 1.50 mg/l) and IAA (0, 0.5, and 1.0 mg/l). BARI-1. On 0.5 mg/l IAA + 0.5 mg/l IBA, the most roots (6.50) and the longest root length (5.88 cm) were achieved. In Malaysian banana cultivars Pisang Mas, PisangNangka, PisangBerangan, and PisangAwak, Sipeen and Davey (2012) discovered that half-strength MS medium enriched with 1 mg/l NAA was suitable for root regeneration from scalps. They discovered a maximum of 7 roots in cv. PisangNangka and Pisang Berangan, with a mean root length of 4.5 cm in both cultivars. Rai and colleagues (2012) infected banana cv. For root induction, Grand Naine (G-9) was grown on rooting media containing IBA or NAA (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/l) and Charcoal (2 g/l). IBA (2 mg/l) and Charcoal (2 g/l) produced the most roots (8.5) and the most root hairs. Rahman et al. (2013) looked for the optimum plant growth regulator for root induction in banana cv. Agnishwar. Among the various forms and concentrations of auxin, such as Indole-3-Butyric Acid (IBA)

and-naphthalene acetic acid (NAA), IBA at 1.0 mg/l was determined to be the most effective for rooting shoots (96 percent rooting) [44]. Ramachandran and Amutha (2013) grew banana cv. Cavendish Dwarf cultivated plants on media with activated charcoal and hormone (NAA-1.5 mg/l). On MS (half strength) medium fortified with 1.00 mg/l IBA and 200 mg/l activated charcoal, Ahmed et al. (2014) obtained rooting. Aremu et al. (2014) used IAA, IBA, NAA, smoke-water (SW), and karrikinolide in their rooting studies (KAR1). In SW and KAR1, the quantity and length of roots were significantly higher than in the control. When compared to BA, topinin showed a greater mean shoot number per explant (MemT and MemTTHP) at lower cytokinin concentrations, as well as ease of rooting throughout the shoot proliferation phase (MemTTHP). Shiv Shankar et al. (2014) studied the growth of roots in *Musa* spp. cv. Grand Naine regenerated shoots by supplementing the shoots with five different doses of kinetin (0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) in the MS medium. Root development was not observed in the hormone-free media. In the medium containing 1.0 mg/l kinetin, there was an increase in the number of roots (4.0), the length of roots (6.0 cm), and the length of shoots (6.5 cm). Anbazhagan et al. (2014) discovered that half-strength MS media supplemented with IBA at 1.0 mg/l resulted in the best root production (96%) and root number/explant (11.80) of in vitro-grown *Musa* spp. shoots [45,46].

The effect of IBA and NAA on the roots of the banana cultivars Grand Naine and Jahaji was investigated by Jamir and Maiti (2014). NAA and IBA were employed separately at concentrations of 0, 0.1, 0.2, and 1 mg/l for rooting. On medium with 1 mg/l NAA, 100% rooting and the largest number of functional roots (6.33 and 5.2) with moderate root length (2-4 cm) were achieved [47]. In banana cv. Karibale Monthan, Shashikumar, et al. (2015) discovered that MS medium supplemented with 1.0 mg/l IBA generated a 5.33 1.21 number of roots with a mean root length of 7.50 1.87 cm. The effect of various doses of growth regulators IAA (0.0, 0.5, 1.0, and 1.5 mg/l) and IBA (0.0, 0.5, 1.0, and 1.5 mg/l) and their interaction on roots in banana (*Musa paradisica*) cv. Grand Naine was investigated by Paulo et al. (2015) [48]. The short durations were 8.9 days for 0.5 mg/l IAA and 9.4 days for 1.5 mg/l IBA, whereas a combination of both 0.5 mg/l IAA and 1.5 mg/l IBA gave nearly a week of root induction (7.33) day. Treatment with 0.5 mg/l IAA produced the most roots, 6.2 and 7.8 at 15 and 30 DAI, respectively; 1.5 mg/l IBA produced 5.1 and 7.1 roots at 15 and 30 DAI, respectively; and a combination of 0.5 mg/l IAA + 1.5 mg/l IBA produced 7.0 and 8.0 roots at 15 and 30 DAI, respectively. At 15 and 30 DAI, it was discovered that 0.5 mg/l IAA produced the longest root size of 5.7 and 6.7 cm, respectively. In 15 and 30 DAI, the interaction of 0.5 mg/l IAA + 0.5 mg/l IBA yielded 6.33 and 7.33 cm lengths, respectively. Suman and Kumar (2015) investigated the impact of IBA on banana cv. Malbhog roots. On MS medium enriched with 4.92 M IBA, the in vitro generated shoots demonstrated 100 percent rooting, according to the researchers [49].

Acclimatization

Because in vitro-created 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 ill-equipped to withstand the low humidity, increased light levels, and more volatile temperatures found outside (Wainwright, 1988) [50]. 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 percent carbendazim, and sterilizing the potting mixture (Anderson, 1980 and Muniswamy et al., 1994). 2 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) [51,52].

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) [53]. Perez and Hooks (2008; Perez and Hooks, 2008; Perez and Hooks, 2008). Rai et al., (2012) hardened rooted plantlets of the banana variety Grand Naine (G9) in pottrays 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%). Elisama et al., (2013) investigated the influence of fertigation and the addition of Indole Butyric Acid (IBA) to nutritive solution on the growth of *Musa cavendishii* plantlets during the greenhouse acclimatization phase [54,55]. 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. The IBA treatment had no discernible influence on the *M. cavendishii* plants' growth. There was no evidence of a link between fertigation and the use of IBAs [56,57].

Ahmed et al. (2014) used various treatments to harden and acclimate in vitro rooted plantlets. Plants transplanted at 4 weeks following root initiation had the highest rate of survival (100%) during transplanting. These plants were hardened individually or in groups in glass beakers and polythene bags. By individually covering the plantlets with glass beakers and keeping them in the culture room, the highest survival throughout hardening (100%) was observed. The potting combination including soil, sand, and FYM (2: 1: 1 v/v/v) provided the most height and plantlet survival of all the potting mixtures tested. The findings also revealed that during primary hardening, soil: sand, and plantlets in coconut coir pith outperformed all other potting combinations (FYM, soil, sand, and vermiculite) [58-62].

Conclusion

The *Musa sapientum* L. plant, which produces about 102 million tonnes of bananas annually, is the most significant food crop in the world. Protocol optimization work is required to fully reap the rewards of this technique because bananas grown using tissue culture technology are also believed to be more vigorous, yield more, and produce higher-quality fruits than those grown using traditional methods. Growth regulators are necessary for this plant when growing agricultural plants in a laboratory setting on a synthetic medium. While cytokines promote shoot proliferation, auxins help proliferated shoots take root. However, depending on the banana species and culture conditions, different amounts of cytokines and auxins are needed.

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