



Study of Macropropagation Techniques and Economic Evaluation of Banana (*Musa* spp.) in Amhara, Ethiopia

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Abstract

Banana (*Musa* spp.) is propagated naturally by its sucker and corm. However, they are very slow and prone to the prevalence of insects and pests. It can be propagated commercially using tissue culture. However, this is skill demanding and unaffordable for small-scale farmers. To address this gap, macropropagation techniques have been introduced as an alternative propagation technology, called the Plant Issues of Shoot fragments technique, which means plants resulting from stem fragments. This method can be implemented with limited investment and skill on a small scale. Hence, the purpose of this study was to evaluate in situ and ex situ PIF techniques on two banana varieties (Poyo and Giant Cavendish). The data on the number of suckers for each decortication, days to emergence after decortication, and sucker vigor were collected and analyzed using an independent sample t-test. Furthermore, a cost comparison was conducted for the two techniques. The result showed that the in situ PIF technique could produce 16 suckers in three months with the cost of 0.43 Ethiopian Birr per sucker while the ex situ PIF technique could produce 7 suckers costing 8 ETB on one production cycle in three months period. The emergence date of the first decortication phase was shorter for in situ (12 days) than ex situ (44 days). The in situ technique could be a good alternative for banana seedling production under small-scale farming conditions. It is important to popularize this technique for the better production of banana suckers at a relatively low cost.

1. INTRODUCTION

Cooking bananas (*Musa acuminata*) and plantains (*Musa balbisiana*) are significant staple foods and sources of revenue for smallholders in the humid forest and mid-altitude agroecologies of sub-Saharan Africa regions (Emmanuel et al., 2013). Banana is an important fruit crop that produces high yields per unit area. It provides food security, nutrition, and income for many smallholder farmers in developing countries, such as Ethiopia. In Ethiopia, more than 4.7 million householders were engaged to support their livelihoods and bananas covered 95.96 thousand hectares of land in 2020–2021 (CSA, 2021). However, the productivity was not more than 9.4 tons (CSA, 2021). Banana production is constrained by many factors, such as the lack of

improved varieties, absence of clean planting material and prevalence of diseases and pests.

Banana belongs to the genus *Musa*, Musaceae family. It is native to Southeast Asia and grown in warmer regions. The cultivated edible bananas are hybrids of two wild species diploid species ($2n = 2x = 22$), resulting from inter or intra-specific hybridization of *M. acuminata* Colla (A genome) and *M. balbisiana* Colla (B genome) (D'Hont et al., 2012). The combinations of these genomes have resulted in various genotypes exhibiting different genomic composition (genome groups) and ploidy levels such as AA, AB, AAA, AAB, ABB, AABB, AAAB, or ABBB types. All commercially grown bananas are triploid and sterile, except for a few parthenocarpic diploid (AA and AB) bananas. The genome size of *Musa* ranges from 554 megabase (Mb) in *M. balbisiana*

to 523 Mb in *M. acuminata* (D'Hont et al., 2012). Currently, somaclones derived from a single triploid genotype (Cavendish) accounts for half of world banana production (D'Hont et al., 2012).

Banana can be propagated by three methods: Natural regeneration, macropropagation and tissue culture (Singh et al., 2011). Natural regeneration is a technique used by farmers to select planting material from existing plantations, establish new plantations or increase the size of existing ones. Because of hormone-mediated apical dominance in the main plant, this process is frequently slow and does not provide adequate planting material (Faturoti et al., 2002). A plant can develop 5–15 side suckers during its lifetime depending on the variety (Sajith et al., 2014). Through decortication this can be enhanced by a factor of 3–4 (i.e., removal of the apical meristem of emerging lateral buds) (Njeri et al., 2010). It is widely used all over the world but has a risk of spreading diseases and pests such as Banana wilt, Banana Xanthomonas, commonly known as BXW or BBW (banana bacterial wilt), Sigatoka leaf spot and other viral diseases (Saraswathi et al., 2024). Because natural suckers are not consistent, there is a high possibility that growers will not be certain of the quality of the variety (Sheela & Ramachandran, 2001). However, farmers can make decisions regarding the cultivar, age and size of the sucker at planting (Staver et al., 2010).

The macropropagation method known as plantlets Issues de Fragments of shoot (PIF) and plants issues from stem fragments (PISF) was developed by the International Institute of Tropical Agriculture (IITA), which is an ideal method for small-scale farmers to produce high-quality planting material at an economical cost (Tenkouano et al., 2006). Depending on the cultivar, this simple sucker multiplication technique has the potential to provide 25–150 shoots per sucker over the course 4–5 months (Faturoti et al., 2002; Baiyeri and Aba, 2005; Martha, 2013). To fill this gap in the supply of affordable, healthy banana planting materials, a cost-effective sucker production method was established in Africa. Numerous axillary buds with meristems at various stages of development have been observed in banana corms. Apical dominance is mechanically reduced during PIF macropropagation to promote lateral bud growth and hasten suckering rate (Faturoti et al., 2002). As a result, a single plant can produce more banana suckers that are economical and

high quality. This macropropagation method lies somewhere in the middle of traditional sucker production and tissue culture (Lorenzo, 2008). Macropropagation is inexpensive and simple, making it possible for farmers to practice. It is based on a simple, cost-effective technique that can be quickly put into practice with proper training and few resources.

In other countries, (Kenya, Cameroon and Nigeria) plants obtained from macropropagation are stronger in the field and have a consistent size than plantlets produced in tissue culture (Njeri, 2012; Martha, 2013). There are two common methods of macropropagation: in the field (in situ) or in a warm chamber (ex situ) (Singh et al., 2011). However, depending on the banana cultivar and bud manipulation, the sucker productivity of the two methods may differ (Tenkouano et al., 2006). The propagation efficiency of these methods is also influenced by temperature (Ntamwira et al., 2017).

Banana can be propagated by tissue culture (TC), but it is costly, skill demanding, and unaffordable for small-scale farmers. Tissue culture propagation techniques have been used in the country for the last few years. However, the development of mass propagation techniques through micropropagation (tissue culture) has not yet solved the demand for a large quantity of commercial clean planting materials on a large scale, as well as small-scale production. This is because of the high cost and sophisticated skills associated with technology (Sahijram et al., 2003; Kasyoka et al., 2010). Thus, the dissemination of the recommended commercial varieties to smallholder growers is restricted in Ethiopia. Therefore, an economical and simple approach to produce banana plantlets at the farmer's level is required to enhance banana production in small-scale agricultural systems (Kasyoka et al., 2010; Martha, 2013). Enset (*Ensete ventricosum*) commonly known as Ethiopian banana or False banana, has been used in Ethiopia to demonstrate simple plantlet growth techniques (Dougherty, 2002). This technique successfully produced a large number of plantlets for bananas and plantains.

To make the recommended banana varieties available to smallholders, it is crucial to evaluate and employ a relatively quick and clean material propagation method: Plantlets Issues of Shoot Fragments (PIF). Thus, the objective of this study was to evaluate this macropropagation method on two Bananas (*Musa acuminata*) varieties called Poyo and Giant Cavendish (GC).

2. MATERIALS AND METHODS

2.1. Description of the study area

This study was conducted at Ataye farmers training center (FTC) in Efratagidim district of North Shewa, Amhara region (Ethiopia) for two consecutive years (Fig. 1). The site is found at an altitude of 1514 m.a.s.l at 10° 17' 28" Northing and 39° 54' 27" Easting. The climate is semiarid lowland and it is characterized by mean annual rainfall 1010 mm. The temperature maximum and minimum were 27.7°C and 11.3°C, respectively (unpublished data, National Metrological Agency).

2.2. Plant material and Experiment procedure

In this experiment, two varieties of banana, Poyo

and Giant Cavendish (GC) were evaluated using in situ and ex situ Plantlets Issues of Shoot Fragments (PIF) macropropagation techniques. Both Poyo and GC are Cavendish sub-group AAA varieties grown on the study area and most of banana growing areas of Ethiopia.

In situ: In this propagation technique, the pseudo-stems of the mother corms or sword suckers, which were clean and free from any disease-causing pathogens were cut transversely 2 cm above the collar region. The apical meristems were then removed, leaving a cavity 2 cm in diameter and 4 cm in depth (decorticated). Suckers were chemically induced by pouring synthetic cytokinin (BAP, 40 ppm) into the decorticated cavity. The individual mats were covered with a mixture of equal parts of sandy loam soil and decomposed farmyard manure (FYM) to a thickness of 5 cm above ground level (Fig. 2). The chemical induction of lateral buds

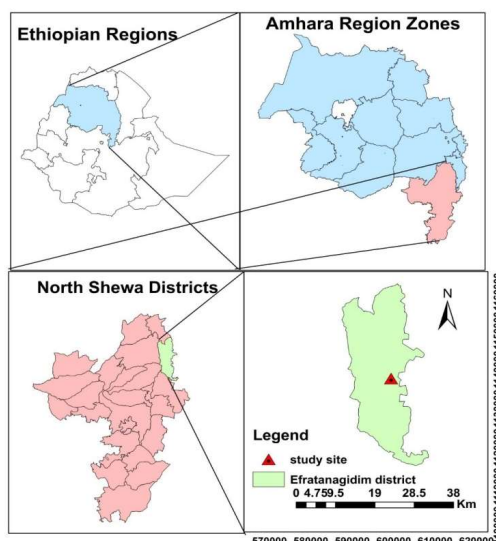


Fig. 1. Map showing the geographic localization of the study area



Fig. 2. In situ macro propagation procedure. a) Pseudostem of the mother corm, b) Decortication, c) Applying BAP after decortication, d) Primary suckers, e) Secondary Suckers, f) Third suckers

was performed on primary suckers and continued up to third suckers. The final suckers were separated from the mother corm and subsequently rooted in a sterile soil medium under intermittent mist.

Ex situ: In this technique, propagator chambers were constructed using wooden timber and filled with sawdust as a medium. The medium was then enriched with sterilized farmyard manure and placed in a propagator chamber. Corms of similar sized-scraped and the growing points were excised with a sharp knife (Fig. 3).

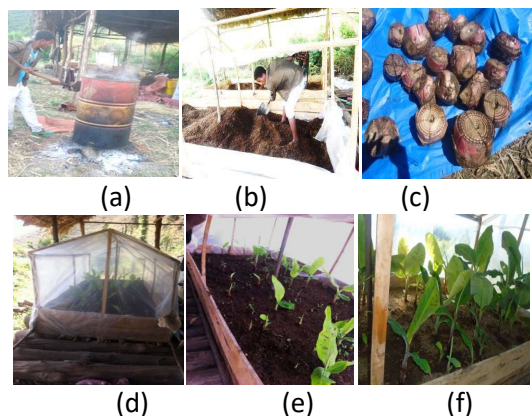


Fig. 3. Different stages of the ex situ macropropagation procedure of banana varieties.

- a) Farmyard manure sterilizing using a steam sterilizer, b) Medium enriched with sterilized farmyard manure in a growth chamber, c) Surface sterilized corm, d) Growth chamber, e) Primary suckers, f) Secondary suckers

The corms were surface sterilized by dipping in a bleaching agent (1% Hypochloride) and fungicide (Mancozeb 120 gm/60 L for 20 min) and allowed to dry for one day. Suckers and roots were chemically induced by pouring synthetic cytokinin (BAP 40 ppm) into the decorticated cavity and spraying synthetic auxin (IBA 40 ppm) on the base of the corm. Sterilized and induced corms were planted in the propagator chamber and covered with the prepared medium. After 35 days, the first decortication was performed when the side shoots emerged and attained a height of 15-20 cm with 3-4 leaves by heading back with a sharp knife followed by 3-4 transverse cuts. Secondary decortication was performed after 35 days of first decortication when the side shoots emerge and attained a height of 20-20 cm with 3-4 leaves by heading back with a sharp knife followed by 3-4 transverse cuts. Third-phase decortication was attempted after 25 days, with greater care. High humidity was achieved using an intermittent mist.

2.3. Data collection and data analysis

Data collected included the number of suckers per mat at each decortication for in situ propagation, and the number of suckers per corm for ex situ propagation techniques was counted at each decortication phase. For both techniques days of emergence from decortication and the cost of the various inputs used for establishing the macropropagation chamber were also recorded. Sucker quality (vigor) was recorded by visual observation. Data analysis was performed by using IBM SPSS version 24 independent sample T-test (IBM, 2019).

3. RESULTS AND DISCUSSION

3.1. Banana explant Emergence date

Result showed that the emergence date of the first decortication was shorter in situ (12 days) than in ex situ (44 days), as indicated in Fig. 4a and 4b.

This is because the mother corm (Fig. 3c) used for ex situ was planted as a new starting planting

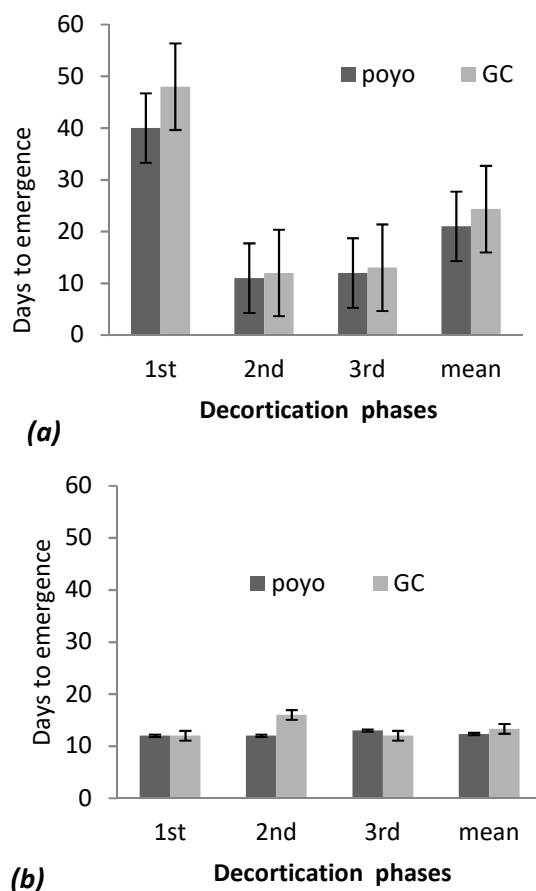


Fig. 4. Days to emergence of suckers in two banana varieties (Poyo and GC) evaluated using ex situ (a) and in situ (b) macropropagation techniques.

material while for the in situ technique, the pseudostem of the mother corm (Fig. 2a) was used, which was already established and continued immediate food provision in the field and suckers emerged earlier. This reduces the time required for the in situ technique. The second and third sucker emergency date of ex situ was become shorter (Fig 3a). This is because after the corm established in the chamber and start suckering the time will reduced because of the microenvironment in the chamber like temperature and relative humidity. In a similar study on ex situ macropropagation, days to produce primary suckers vary from 20-27 days depending on corm age (Saraswathi et al., 2024). In a study to identify the efficacy of in situ macropropagation techniques using additives such as bio-fertilizers and plant growth regulators, it was reported that the in situ technique with BAP+*T. viride* (bio-fertilizer) required 26 days for primary sucker emergence (Duarah et al., 2018). Plantlet production depends on variety and environmental factors. In addition, the planting material (type and age) plays a crucial role.

3.2. Banana sucker production

The results showed that in both years there was no significant difference ($P < 0.05$) between two varieties for both in situ and ex situ propagation techniques. However, there was a significant difference between chamber (ex situ) and field (in situ) propagation techniques (Table 1).

(Table 1). Field propagation (in situ) had the highest number of suckers. Under in situ condition, number of suckers showed an increasing trend for both GC and Poyo varieties (Fig. 5a). The number of suckers had an increasing tendency after the first decortication for GC under ex situ technique (Fig. 5b). Similarly, in 2018, number of suckers had an increasing tendency for both Poyo and GC varieties and for both in situ and ex situ techniques (Fig. 5c & d). This sucker production enhancement is related with the age and the size of the corm.

Result revealed that in both years, there was a significant difference between in situ and ex situ techniques. In total, under in situ 16 suckers, and under ex situ 7 suckers were produced after three phases of decortication with the application of 40ppm BAP and FYM which was highly related probably to environmental factors like temperature and humidity. Furthermore, the types of planting materials play a crucial role for the sucker production. In both years, there was no significant difference among the banana varieties which might be because of both varieties were from AAA types. Similar studies also found that there was no significant difference on sucker production between variety with in similar genome or groups (Ntamwira et al., 2017). The in situ technique gave the highest number of suckers, which was in agreement with the finding of Sajith et al., (2014) who reported that in situ technique with BAP (40ppm), plus *B.*

Table1. Banana sucker production difference between varieties, propagation techniques types and years studied in Amhara, Ethiopia.

		In-situ		Ex-situ		Poyo		GC	
		Poyo	GC	Poyo	GC	Ex-situ	In-situ	In-situ	Ex-situ
2017	Mean	5.87 ^{ns}	5.42 ^{ns}	2.8095 ^{ns}	2.5079 ^{ns}	2.7727	5.8667*	2.4848	5.4167*
	SD	1.89	1.84	0.8917	0.9867	0.8872	1.8868	0.969	1.8381
2018	Mean	4.417 ^{ns}	4.267 ^{ns}	1.567 ^{ns}	1.699 ^{ns}	1.567*	4.417*	4.267*	1.699*
	SD	1.279	1.149	0.461	0.572	0.461	1.279	1.149	0.572

Field propagation (in situ) had the highest number of suckers. In 2018, similarly to 2017, there was no significant difference in sucker production between banana varieties in field and Chamber propagation techniques. In the second year, there was a significant difference between propagation techniques at 5% probability level

subtilis (bio-fertilizer) gave the highest number of suckers (16.8) after three phases of decortication. In another study, the in situ technique with 0.04% BAP +30 g *T. viride* (bio-fertilizer) gave 28 suckers after three phases of decortication in 7-9 months (Duarah et al., 2018). Production of plantlets or suckers may

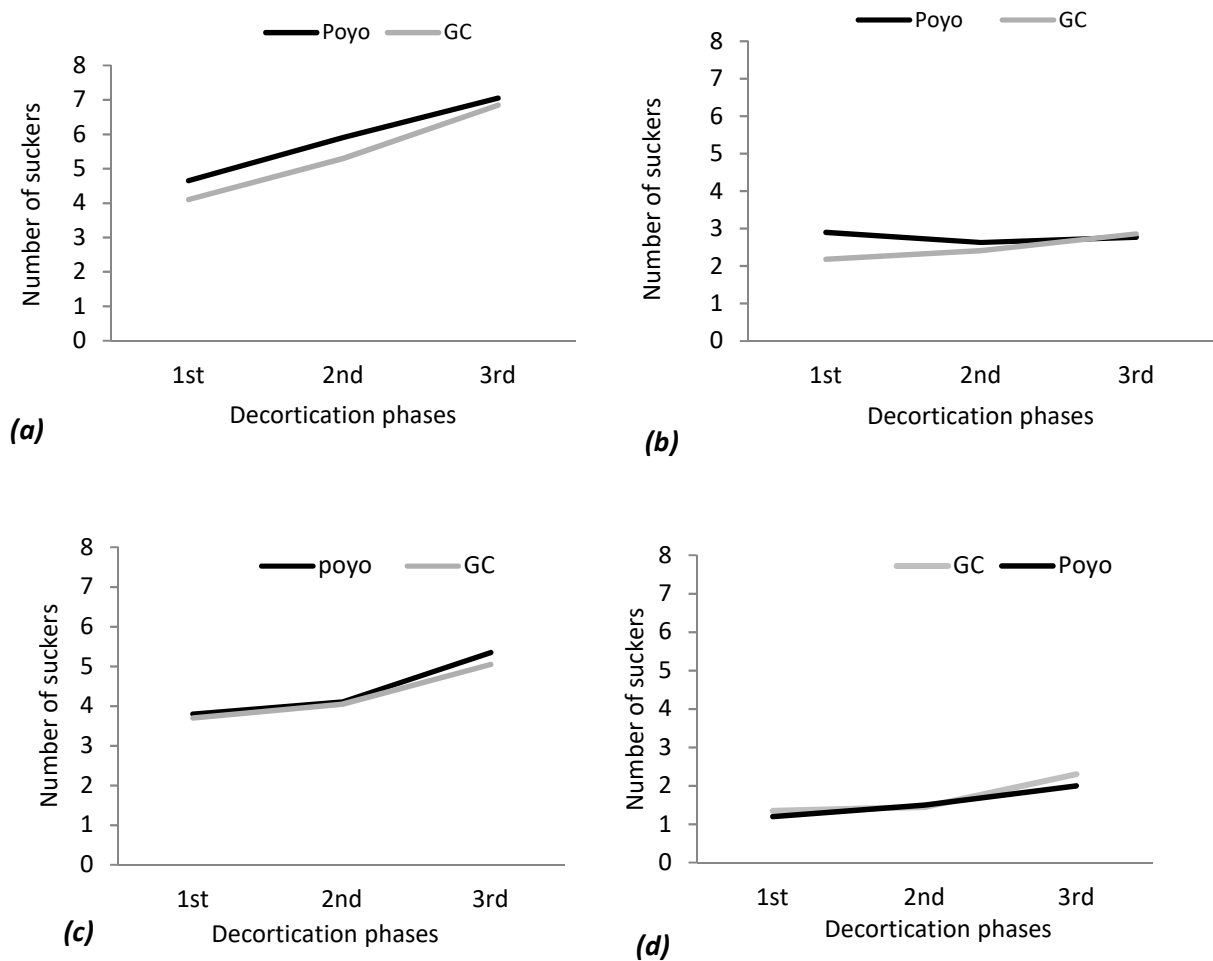


Fig. 5. Number of suckers by decortication phases of the two varieties: under macropropagation techniques of (a) In situ condition in 2017, (b) under ex situ in 2017, (c) under in situ condition in 2018 and (d) under ex situ in 2018.

vary with banana variety, agroecology, planting material and bud manipulation applied (Kasyoka et al., 2010; Saraswathi et al., 2024). Another study on effectiveness of macro propagation technique in production of healthy banana seedlings suggested that macro propagation technology is efficient in producing banana seedlings that are free from plant parasitic nematodes and the banana weevil which are cheap and easily adaptable when compared with micropropagation (Njeri, 2012).

3.3. Cost comparison among macro propagation techniques

In situ propagation offers a cheap alternative with a lot of potential for the production of quality planting material in banana. Depending on the variety, a banana plant can produce 5-15 side suckers during its life span. However, by using in situ macropropagation technique sucker number can be raised to 16 only in three up to four months. Further, production cost per

plant was less than 1.00 Ethiopian Birr (ETB), making it a cost-effective technique and affordable to small and marginal farmers without compromising on quality (Table 2). The other technique, ex situ cost 8 ETB per plant. The high initial cost and skills needed for establishing the ex situ macro propagation chamber have often hindered its adoption. In other similar studies, it was suggested that the low cost, use of local materials and comparable returns from the simple macropropagation units like modified in situ technique could be a good alternative for banana seedling production under small-scale farmer conditions (Ntamwira et al., 2017). Macropropagation was shown to be cost-effective and have high plantlet generation rate for small-scale farmers who have limited access to tissue culture plants (Sajith et al., 2014; Saraswathi et al., 2024). Most of banana production in Africa is from small scale farmers thus, macropropagation could be a cost effective alternative for those farmers.

Table 2. Comparison of the two techniques of banana macropropagation in Amhara, Ethiopia.

Inputs	Cost for 100 suckers (ETB)	Cost for one sucker in two treatments (ETB)	
		Ex situ	In situ
Polyethylene for chamber 20ETB/m	720	7.20	-
Wood box for chamber 260ETB/Timber	2340	23.40	-
Suckers 7ETB/ sucker	700	7	7
Sawdust 60ETB/ kg	1800	18	-
BAP (growth hormone) 0.05 ETB/mg	0.8	-	-
IBA (growth hormone) 0.06 ETB/mg	0.96	-	-
Fungicide 100 ETB/0.5kg	100		
Av. No. of plantlets produced		7	16
Cost for single plantlet (ETB)		8	0.43

4. CONCLUSION

In situ technique gives relatively healthy plants if source suckers are from healthy mother plants and contamination is minimized during the process. In situ PIF technique could produce 16 suckers in three months with the cost of 0.43 ETB per sucker while the ex situ PIF technique could produce 7 suckers costing 8 ETB on one production cycle in three months period. To ensure that risk of spreading disease is minimized, the corms used for propagation should not be acquired from areas where the disease has been reported. This technique can help to create the awareness of principles of plant health for the local farmers. Another advantage of In situ technique is that it can be done locally by low cost and with little training: at farmer level or it can be applied by different organization. The macropropagation of banana optimized in the present study is user-friendly, which requires minimum skill and expertise. Furthermore it is suitable for adoption by farmers at the farm level for the production of high-quality planting material in banana production.

The results additionally showed that including bio-fertilizers and growth hormones in the soil media improved the regeneration of primary, secondary, and tertiary buds as well as promoted the growth and development of plantlets. This reduced post-transplant shock and increased the percentage of plants that survived in the field. In situ propagation techniques offer a low-cost alternative with a lot of potential. Thus, under the study area condition, recommendation can be drawn to in situ macropropagation technique as the better multiplication option of both varieties of banana (Poyo and GC) which was cost effective and feasible to use. Efforts should be made in

popularizing this technology to unaddressed potential banana production areas to boost production. In addition, further studies are needed on the growth media and plant growth regulators effect on the production of banana suckers.

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AUTHOR CONTRIBUTION

Damtew A. conceived and designed the study and all authors (Tigist F., Damtew A., Getaneh G., Alemayehu and Natnael G.) were actively involved in the research investigation, documentation and publication process. The manuscript was drafted by Tigist F. All authors conceptualize the research work and reviewed the first draft of the manuscript. All authors read and approved the manuscript for publication.

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