Original Research Article

Efficacy of excised-bud (EB) and half-corm (HC) at four physiological growth stages on plantlet regeneration of *Musa* genotypes

.

ABSTRACT

Aims: This study was conducted to determine which of excised buds (EB) and half corms (HC) from 3 *Musa* genotypes at four growth stages would produce the most plantlets and also how scarification affects number of plantlets regenerated.

Study design: Treatments comprised three *Musa* genotypes at four growth stages and two macro-propagation methods – excised bud and half-corm in a randomized complete block design with 4 replications.

Place and Duration of Study: International Institute of Tropical Agriculture (IITA) High Rainfall Station, Onne (4°51'N, 7° 03'E, 10m above sea level), Rivers State, Nigeria for eighteen months.

Methodology: Propagules, excised buds and half corms from a tetraploid cooking banana hybrid BITA 3; tetraploid plantain hybrid PITA 14, and a cooking banana landrace Cardaba, at 6-month vegetative, pre-flowering, post-flowering and bunch harvest stages were planted to regenerate plantlets. At bunch harvest growth stage, additional excised buds and half corms were scarified to find out the effect on regeneration of plantlets.

Results: Excised buds and half corms did not differ significantly (P = .05) in number of plantlets produced in PITA 14 irrespective of growth stage but bunch harvest stage was best. In BITA 3, excised buds produced significantly more plantlets than half corms at the 6-month vegetative and bunch harvest stages. However, at the pre-flowering stage, half corms produced significantly more plantlets than excised buds. In Cardaba, half corms were significantly better at all growth stages especially bunch harvest stage. In all *Musa* genotypes, scarification increased significantly the number of plantlets.

Conclusion: Excised buds or half corms at any growth stage or at bunch harvest stage for PITA 14; and excised buds at 6-month vegetative or bunch harvest stage for BITA 3 with half corm at pre-flowering stage are best. For Cardaba, half corm at any growth stage or bunch harvest stage was best.

Keywords: [Musa genotype, Macro-propagation, scarification, excised bud, half corm}

1. INTRODUCTION

Musa is vegetatively propagated and planting materials can be produced either by micro-propagation or by macro-propagation. Natural replacement of suckers through regeneration of landraces, hybrids or clones, the method preferred by rural farmers [1,2] is very slow because apical dominance causes "shy suckering" which prevents buds from developing into suckers until the reproductive phase of the mother plant has commenced, causes slow regeneration [3]. Apical dominance is controlled by a growth hormone that is produced in the terminal bud and inhibits growth of the lateral shoots [4]. Besides being slow, natural suckering does not yield enough suckers of the desired varieties and pest and disease susceptibility can be quite high in the event of outbreaks [1,5,6] which can easily wipe-out whole plantations. The result is a serious shortage of clean planting materials and this shortage of planting materials is considered a serious constraint to the rapid expansion of *Musa* production [7]. While micro-propagation methods can provide large quantities and high

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quality planting materials [8], the tender plantlets require great care in the first 2 months of planting. Also the equipment, technical skills, cost and highly controlled environment required are beyond the reach of resource poor farmers [9]. Therefore, macro-propagation has remained an effective alternative method which requires less capital and skills to produce large numbers of better-quality *Musa* planting material by farmers. However, some problems associated with macro-propagation include use of large numbers of parent materials, large space required for multiplication, and lack of uniform size of plantlets. Macro-propagation techniques include traditional methods that use whole suckers or relatively large pieces of the parent plants to produce planting materials; these are usually bulky and difficult to transport. Common methods of macro-propagation include decapitation and false decapitation. Decapitation is the destruction of the terminal bud to increase the sprouting and development of suckers [10]. False decapitation also destroys the main apex in order to remove apical dominance, but it maintains the entire plant [11]. The rate of suckering using the above methods range from nine to fourteen suckers per annum [7,12]. Stripping of older sheaths to expose buds as well as mulching and earthing of the exposed buds have also been used to increase the number of suckers obtained from a mother plant [13,14,15]. Whole plants (peepers or sword) have also been used to produce planting materials [16,17]. The whole corm and corm-bits are used to produce few plantlets of uniform size [18]. The study [19] showed that the corm method could produce about five hundred suckers within eight months. More recent macro-propagation techniques involve methods that employ whole suckers or relatively large pieces of corm tissue to produce planting material in a propagator [20]. Other methods of macro-propagation utilize the whole corm, split-corm, split-bud and corm-bit techniques [21,22]. Depending on variety, one corm can yield an average of 10 seedlings, which can be increased by a factor of 3–4 by removal of the apical meristem of emerging lateral buds [23]. Hence, alternative methods based on bud excision are being investigated. The method requires that buds be removed from the mother corm, and incubated in the pre-nursery to generate shoots. Prior to transplanting, the shoots obtained could be further multiplied by making incisions-scarification, which could yield a higher number of uniform size plantlets. Different banana propagation techniques can give different number of shoots [24]; while number of shoots produced is also influenced by the banana's genotype [25,26]. To the best of our knowledge no studies have investigated the use of excised buds (EB) and half corms (HC) obtained at four physiological growth stages of different *Musa* species as propagules for production of planting material. It is important that such a study be conducted in order to provide critical information on the ideal physiological growth stage that can provide the maximum number of propagules for rapid multiplication of each *Musa* spp. This study was therefore conducted specifically to:

1. Asses and compare the rate of regeneration of excised buds (EB) and half corms (HC) obtained at four physiological growth stages as viable macro-propagation materials in 3 *Musa* genotypes
2. Find out how scarification of excised buds and half corms affect the rate of regeneration of plantlets of 3 *Musa* genotypes

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

This study was carried out at the International Institute of Tropical Agriculture (IITA) High Rainfall Station, Onne (4°51'N, 7° 03'E, 10m above sea level), in Rivers State, south-eastern Nigeria. The rainfall pattern is monomodal, distributed over a 10month period from February through December, with an annual average of 2400mm. Relative humidity remains high all year round with mean values of 78% in February, increasing to 89% in the months of July and September. The mean annual minimum and maximum temperatures are 25^0C and 27^0C, respectively, while solar radiation / sunshine lasts an average of 4hours daily [27]. The soil is derived from coastal sediments of the Niger Delta, freely drained and acidic (pH 4.3), and made up of mainly Kaolinite. Onne soils are also high in phosphorus 60mg kg^{-1}, manganese 0.2mmol kg^{-1}, but low in nitrogen [28,29].

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Preparation of Macro-propagation Materials

Three *Musa* genotypes comprising one tetraploid cooking banana hybrid BITA-3 (TMBx 5295-1) that is resistant to black Sigatoka disease; one tetraploid plantain hybrid PITA 14 (TMPx 7152-2) which is high yielding, short cycling, and resistant to black Sigatoka and to Banana streak virus diseases; and a cooking banana landrace (Cardaba) resistant to black Sigatoka disease were the source of the macro-propagation materials. Corms were harvested from 5 field-grown plants of each of these genotype source materials at each of the four physiological stages of growth as follows:

(i) At 6-month vegetative growth stage,
(ii) At onset of flowering growth stage,
(iii) At end of flowering growth stage, and
(iv) At bunch harvest growth stage

The harvested corms were immediately washed under a running tap. Roots were trimmed off and plant debris removed to expose all buds on the corm, after which each corm was split into two equal halves. One part was used as half-corm while buds were excised from the other half. Buds of about 150kg each were excised from the corms with a locally fabricated mechanical extractor to ensure uniformity in size of buds. These two macro-propagation methods, excised-bud (EB) and half-corm (HC) were used in multiplication of plantlets in order to determine which technique produced the highest number of healthy plantlets.

Treatment Applications and Experimental Design

Treatments were the three *Musa* genotypes and four physiological stages described earlier and two macro-propagation methods – excised bud (EB) and half-corm (HC) giving a 3 X 4 X 2 factorial combination in a randomized complete block design with 4 replications. The excised buds were initially surface sterilized with 20% solution of Sodium hypochlorite, and allowed to stand for 5minutes in a solution of 6g copper-oxychloride in one litre of water to prevent decay, after which they were allowed to air-dry for 4hours. The treated materials were planted at a spacing of 20cm by 20cm in a germination chamber consisting of a concrete basin filled with a mixture of sawdust and poultry manure at a ratio of 3:1 and watering was done as required. At the bunch harvest stage of physiological growth, an additional set of excised buds and half corms were scarified. Scarification was by making 2 incisions on the excised buds and on the growing point of the half corms in order to find out how scarification would affect the number of regenerated plantlets compared to non scarified ones.

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Data Collection and Statistical Analyses

Sprouting was considered to have occurred when the buds grew about 5cm above the soil level. The final number of regenerated plantlets was recorded. The data were subjected to square-root transformation, prior to analysis of variance (ANOVA) to test treatment effects. All data were analysed using the general linear model procedure of Statistical Analyses Software [30] and any effects found to be significant have been tested at a significance level of 5% while means were compared using the Least Significant Difference (LSD) at $P = .05$.

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3. RESULTS

Plantlet Regeneration at 6-month Vegetative Growth Stage

At the 6-month vegetative stage of growth, excised buds (EB) from the cooking banana hybrid (BITA 3) produced significantly ($P = .05$) more (333% more) plantlets than its half corm (HC) counterpart (Fig 1). However, there was no significant difference ($P = .05$) in the number of plantlets produced by the excised buds and the half corms in the plantain hybrid (PITA 14). In the cooking banana (Cardaba) the half corms produced significantly ($P = .05$) more (600% more) plantlets. In fact, excised buds did not produce any plantlets in Cardaba.

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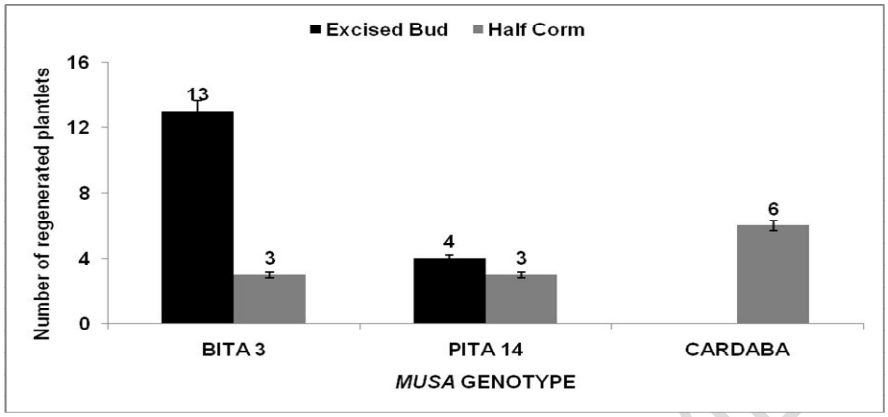

Fig. 1. Number of regenerated plantlets from excised buds and half corms obtained at the 6-month vegetative stage of growth in 3 *Musa* genotypes

Plantlet Regeneration at Pre-Flowering Growth Stage

In Figure 2, at the pre-flowering growth stage, half corms (HC) produced significantly (P = .05) more (120% more) plantlets than the excised buds (EB) in the cooking banana hybrid (BITA 3) . There was no significant difference (P = .05) in the number of plantlets produced by the half corms and the excised buds in the plantain hybrid (PITA 14). Again at this stage of growth, half corms produced significantly (P = .05) more (900% more) plantlets than excised buds which did not produce any plantlets in the cooking banana landrace Cardaba

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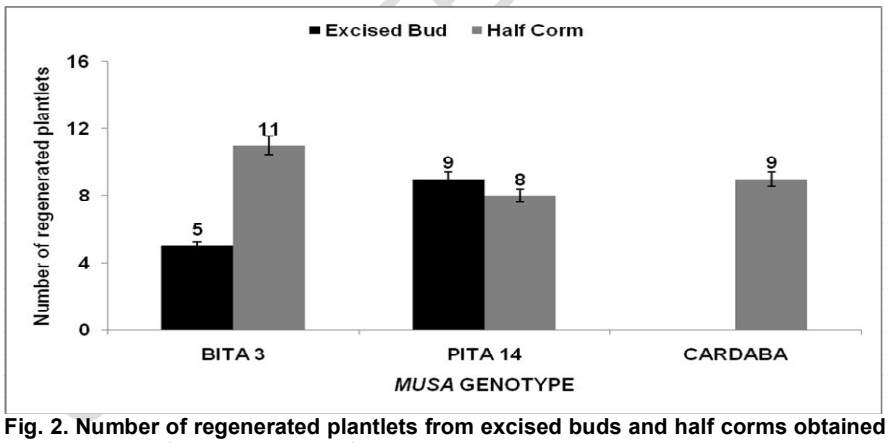

Fig. 2. Number of regenerated plantlets from excised buds and half corms obtained at the pre-flowering stage of growth in 3 *Musa* genotypes

Plantlet Regeneration at Post Flowering Growth Stage

At post flowering growth stage, there was no significant difference (P = .05) in the number of plantlets produced by the excised buds and the half corms in the cooking banana hybrid (BITA 3) (Fig 3) as well as in the plantain hybrid (PITA 14). However there was a significant difference (P = .05) in the number of plantlets produced by the excised buds and the half corms in the cooking banana Cardaba

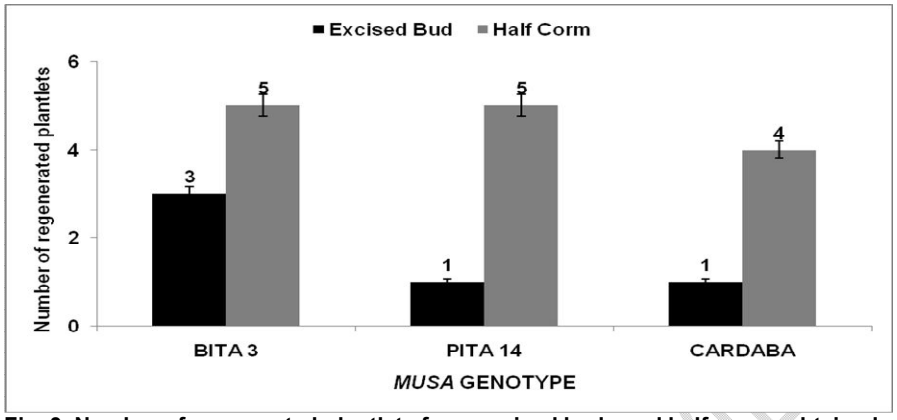

Fig. 3. Number of regenerated plantlets from excised buds and half corms obtained at the post-flowering stage of growth in 3 *Musa* genotypes

Plantlet Regeneration at Bunch Harvest Growth Stage

Excised buds (EB) obtained at bunch harvest stage in the cooking banana hybrid (BITA 3) produced significantly (P = .05) more (86% more) plantlets than its half corm (HC) counterpart (Fig.4). There was no significant difference (P = .05) in the number of plantlets produced by excised buds and half corm at this stage of growth in the plantain hybrid (PITA 14). The half corms produced significantly (P = .05) more (550% more) plantlets than excised buds in the cooking banana landrace Cardaba. This was the most productive physiological stage for using the half corm propagule in the cooking banana Cardaba

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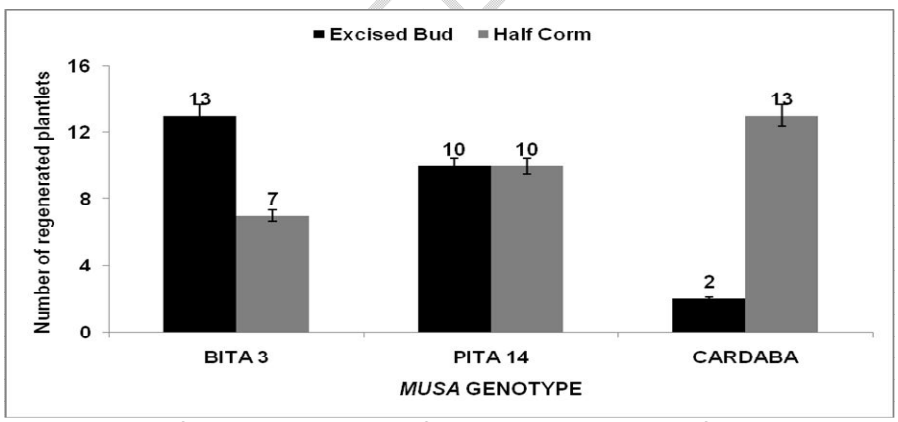

Fig. 4. Number of regenerated plantlets from excised buds and half corms obtained at the bunch harvest stage of growth in 3 *Musa* genotypes

Effects of Scarification on Excised Buds (EB) and Half Corms (HC)

Excised Buds

Scarification increased significantly (P = .05) by more than 4 times, the number of plantlets produced by excised buds (EB) in the cooking banana hybrid (BITA 3) and by approximately 3 times in the plantain hybrid (PITA 14) (Fig 5). In the cooking banana Cardaba, scarification

resulted in a significant (P = .05) increase by doubling the number of plantlets produced compared to non scarified buds.

Half Corms

Scarification increased significantly (P = .05) by more than double, the number of plantlets produced by half corms (HC) in the cooking banana hybrid (BITA 3) and by 50% in the plantain hybrid (PITA 14) compared to non scarified half corms (Fig 5). In the cooking banana Cardaba, scarification increased significantly (P = .05) the number of plantlets by 91% compared to non scarified half corms.

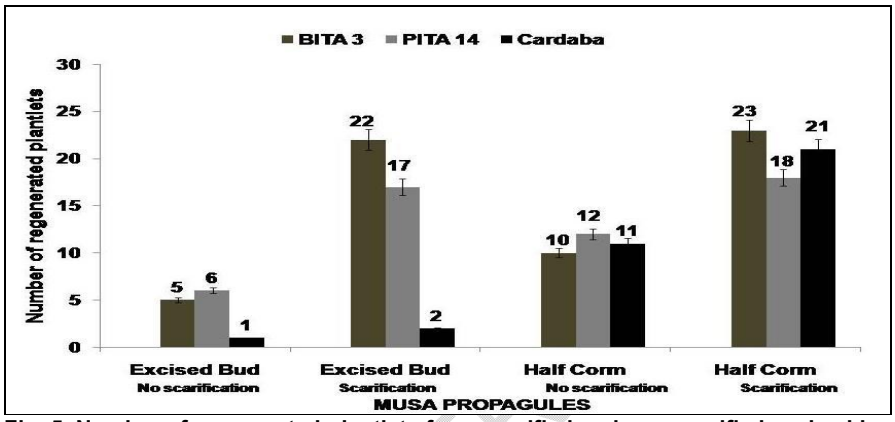

Fig. 5. Number of regenerated plantlets from scarified and non scarified excised buds and half corms obtained at the bunch harvest stage of growth in 3 *Musa* genotypes

4. DISCUSSION

***Musa* genotypes and Macro-propagation methods/ propagules**

Generally, hybrid cooking banana (BITA 3) produced significantly the highest number of plantlets from excised buds (EB) at both 6-month vegetative and bunch harvest stages of growth. However, at the pre-flowering stage, half corm produced the highest number of plantlets indicating at which stage to use each propagation method/ propagule. For hybrid plantain (PITA 14), excised buds and half corms produced the highest number of plantlets at bunch harvest stage of growth. Cooking banana, Cardaba, produced the highest number of plantlets from half corms obtained at harvest, followed by those obtained at pre-flowering and 6-month vegetative stages in that order. Of the 3 genotypes, significantly higher numbers of plantlets were obtained from the hybrids than from the cooking banana Cardaba. Generally excised buds were best for the hybrid cooking banana and half corm for cooking banana Cardaba while either of the propagules could be used for hybrid plantain. The higher number of plantlets obtained from hybrids suggests genetic improvement of the hybrids over the banana landrace Cardaba. Higher suckering of the hybrids over their plantain parents has been attributed to their ability to overcome apical dominance [31]. According to [11] sucker production and development are influenced by growth hormones produced by the mother plant, which is regulated by the Ad gene [31]. It could also be from hormonal changes which occur during the lifespan of any plant [32]. Besides the action of hormones, apical dominance may be influenced by the physiological stage of the plant which depends upon the source-sink relationship. The rate of regeneration is determined by the amount of assimilates from leaves to sink which in turn depend upon age and vigour of the plant [33]. The higher regeneration of the hybrids over the cooking banana landrace may also be due to the higher ploidy level of the hybrids. Higher plant ploidy could confer higher vigour resulting

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from higher sink accumulation and consequently result in a higher number of plantlets [34,35].

Scarification

Scarification of excised buds and half corms may have (a) triggered hormones that induced cell division, callus formation and elongation, (b) increased efficiency of uptake and translocation within the propagules and accumulation at the active sites and (c) may have removed any anatomical barrier limiting formation of plantlets causing higher regeneration of plantlets in both propagules [22,36,37,38].

4. CONCLUSION

This study found that macro-propagation of the hybrid plantain PITA 14 could be done using either excised buds or half corms at any physiological growth stage but ideally at bunch harvest stage for best results. In the cooking banana hybrid BITA 3, excised buds at the 6-month vegetative or bunch harvest stage proved optimal, while use of half corms is best at pre-flowering stage. In the cooking banana Cardaba, half corms at all physiological growth stages could be used although bunch harvest stage was the most productive. Scarification of excised buds and half corms increased number of plantlets in all genotypes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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