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Multiplication of Banana Vivo Plants by the PIF Method According to the Size of Bayonet Suckers

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Abstract

Banana plays an important role in food security. The lack of quality planting material is one of the main causes limiting the intensification of banana cultivation in Benin. In order to contribute to the improvement of plantain banana production through the use of healthy, homogeneous and vigorous banana vivo plants, this study was initiated to evaluate the influence of the size of plantain banana bayonet sucker bulbs on the initiation of banana vivo plants produced by the technique of plants derived from stem fragments (PIF). Thus, this study was conducted at the research station based in Niaouli, Municipality of Allada. In total, three (3) different sizes of bulbs (small, medium and large) of bayonet suckers of the yellow-pulp Aloga plantain banana variety were used for the production of vivo plants following the different stages of the PIF technique. The data collected during the experiment relate to the weight of bulbs; diameter of bulbs; sowing date before sowing of bayonet suckers and during weaning-on-weaning dates, the number of rank 1 suckers emitted per bulb, the number of rank 1 suckers reactivated; the number of rank 1 suckers weaned; the number of rank 2 suckers produced; the length and width of the median leaf; the number of leaves per plant and the height of plants. These data were entered and processed with Excel spreadsheet and Statistical Analysis System software version 9.2 (SAS v. 9.2) was then used for statistical anal-

yses. The results obtained show that there is a positive and significant correlation ($r = 0.26$; $p = 0.04$) between the size of bayonet suckers and the number of vivo plants emitted. Also, the weaning time influences the number of vivo plants emitted. Thus, a high number of vivo plants of the yellow-pulp Aloga variety is obtained with the use of large bulbs. This study opens a perspective to increase productivity in quality plantain banana vivo plants.

Keywords

Musa spp, Macro-Propagation, Explants, Fragments

1. Introduction

The cultivation of banana, particularly dessert banana and plantain varieties, holds considerable socio-economic importance, representing a source of income for millions of producers in tropical and subtropical regions. According to FAO (Food and Agriculture Organization of the United Nations), global banana production reached 153 million tons in 2020, making this crop one of the most consumed fruits in the world [1]. However, the multiplication of this plant, traditionally carried out through suckers, presents limitations in terms of genetic diversity and disease propagation, particularly fusarium and moko, which can compromise production [2]. Beninese producers experience difficulties due to the lack of quality planting material to facilitate production that will respect Codex standards.

Obtaining planting material is rare, due to the very nature of the plant, to produce sufficient numbers of suckers and the slowness of its development. It is therefore difficult to obtain directly from fields sufficient numbers of suckers because one of the characteristics of banana is its low propagation rate (2 - 3 suckers per plant) and especially poor-quality suckers are often parasitized by nematodes and weevils. This insufficiency of suckers during planting constitutes an obstacle to the intensification of this crop.

Faced with these challenges, the method of Plants Derived from Stem Fragments (PIF) stands out as an innovative solution. Developed by the African Research Center on Banana and Plantain (CARBAP), this technique improves multiplication practices by allowing rapid and efficient propagation of bananas from stem fragments. Indeed, unlike *in vitro* micropropagation, the PIF method uses local materials and presents a reduced cost, while making it possible to obtain healthy plants in only 3 to 4 months [3]. This method therefore proves particularly suited to small farmers who wish to increase their production without resorting to expensive inputs.

The PIF process begins with the collection of juvenile suckers, often called "bayonet suckers", which are characterized by pseudo-stems with a height varying between 50 and 150 cm. These suckers, presenting a richness in nutritive reserves, are taken and then trimmed to eliminate damaged parts, which promotes their reactivation [4]. The fragments thus obtained are then germinated in an adequate

substrate before being transplanted in nursery, where they benefit from a controlled environment to promote their growth.

The size of bayonet suckers constitutes a determining factor in the success of multiplication by the PIF method. Indeed, previous studies have shown that the initial size of plants influences their ability to produce suckers and their survival rate after transplantation [5]. However, data concerning the impact of this size on the multiplication of banana suckers by the PIF method remain limited.

Thus, this study proposes to explore the influence of the size of bayonet suckers on the multiplication of banana suckers by the PIF method. It aims to evaluate the effect of the size of bayonet sucker bulbs on the number of vivo plants produced, their vigor and their survival rate. The results of this research will contribute not only to the optimization of the PIF method, but also to a better understanding of banana multiplication practices, thus promoting sustainable production resilient to environmental and phytosanitary threats.

2. Study Area

The study was conducted at the experimental station of the South Agricultural Research Center (CRA-Sud) located in the village of Niaouli, District of Attogon, 12 km north of Allada and 70 km north of Cotonou. It is located at an altitude of 105˚, longitude 2˚19' East, latitude 6˚12' North. This experimental site is characterized by ferralitic soils, called "terre de barre" which covers ¾ of the center's areas and hydromorphic soils. The research station is characterized by a maritime sub-equatorial climate and an average rainfall of 1200 mm spread over 7 months in two rainy seasons (mid-April to mid-July and mid-September to mid-November) with maximum precipitation in June and October and minimum in August. The average temperature is around 27˚C [6].

3. Materials and Methods

The plant material used in this study consists of bayonet suckers (explants) having tapered leaves and bulbs of three (3) different sizes (small, medium and large) well developed from the yellow-pulp Aloga plantain banana variety. The vivo plants were obtained by the technique of Plants Derived from Stem Fragments (PIF). The multiplication of vivo plants from the PIF technique allows, under the particular conditions created in vivo, to regenerate young plants from a stem fragment (bayonet sucker) or to provoke proliferations from which numerous young plants will develop.

Thus, the bayonet suckers were trimmed, decorticated, coated in a solution of aqueous extract of neem leaves and spread on pallets for 72 hours. Then, these previously weighed suckers were scarified and sown in germination boxes containing wet sawdust that had been previously solar-sterilized by exposure to direct sunlight for seven consecutive days to reduce pathogen load and covered with transparent cloth. A first copious watering was done 24 hours after sowing the explants in the germination box. They were then watered every three days as needed until the initiation of the first shoots (rank 1 suckers) in the 3rd week. Rank

1 suckers refer to the primary shoots that emerge directly from the scarified apical meristem of the original bayonet sucker bulb. These rank 1 suckers were then re-activated, *i.e.*, their apical dome was carefully removed (scarified again) to break apical dominance and stimulate the development of secondary buds, which give rise to rank 2 suckers. These rank 2 suckers emitted at the three-leaf stage and having roots with a minimum of three primary roots each exceeding 2 cm in length were weaned in polyethylene bags containing an adequate substrate. These plants are then placed under shade for regular maintenance following good practices.

3.1. Experimental Design

The trials were conducted following a split plot design with three replications and three (3) factors (small, medium and large). The main plot factor was the weaning time, and the sub-plot factor was the size of the bayonet sucker bulbs. The plot unit consists of ten (10) bulbs repeated three (3) times according to the size of bayonet suckers of the yellow-pulp Aloga banana variety.

3.2. Determination of Parameters and Data Collection

Prior to sowing bayonet suckers, data relating to bulb weight; bulb diameter; sowing date and initiation date (plantlet emergence). Then, weaning dates, the number of rank 1 suckers emitted per bulb, the number of rank 1 suckers reactivated; the number of rank 1 suckers weaned; the number of rank 2 suckers produced; the length and width of the median leaf; the number of leaves per plant and the height of plants are the data collected at the time of weaning. The numbers of suckers were counted while measurements of length, diameter and height were taken with a centimeter.

3.3. Statistical Analysis

Microsoft Excel software was used for data entry and processing. Statistical Analysis System software version 9.2 (SAS v. 9.2) was then used for statistical analyses. These analyses essentially consisted of analyses of variance (one and two factors). Data on initiation rates underwent a two-factor analysis of variance (Weaning Time and Size of explants) while preliminary data and those collected on vivo plants underwent a one-factor analysis of variance (Weaning Times or weaning time). To obtain normal distributions (hypothesis of analysis of variance), the values of leaf numbers and vivo plants were transformed to log10 (n) [7], n being the actual value. The mean values were then compared to each other using the Student Newman-Keuls test at the 5% threshold (probability level). Correlations were also performed using MINITAB 14 software. The determination of Pearson's correlation coefficient was made between the circumference of bayonet suckers and the numbers of vivo plants.

4. Results

4.1. Preliminary Data on Bayonet Suckers

Table 1 presents preliminary data on bayonet suckers. A very highly significant

difference ($p < 0.001$) was observed between the different sizes of bayonet suckers experimented. The values of parameters of large bayonet suckers exceed those of small vivo plants by 34% to 160% (Table 1) depending on the parameters measured. Consequently, large bayonet suckers are the heaviest and most vigorous. Overall, coefficients of variation (CV) vary from 7% to 30% (Table 1).

Table 1. Preliminary data on bayonet suckers (explants).

Vivo plant size	Explant weight (g)		Explant circumference (cm)		Explant size (cm)	
	Mean ± Standard error	CV (%)	Mean ± Standard error	CV (%)	Mean ± Standard error	CV (%)
Small	246.55 ± 8.52^{c}	18.93	24.57 ± 0.49^{c}	11.14	23.17 ± 0.41^{c}	9.76
Medium	362.71 ± 7.52^{b}	11.35	28.40 ± 0.38^{b}	7.26	26.0 ± 0.31^{b}	6.55
Large	733.88 ± 37.49^{a}	27.98	35.40 ± 0.79^{a}	12.16	31.10 ± 0.70^{a}	12.36
F-value	126.64		89.73		63.83	
Probability	< 0.001***		<0.001***		<0.001***	

***: $P < 0.001$. Means followed by the same alphabetic letter are not significantly different ($P > 0.05$) according to the Student Newman-Keuls test.

4.2. Effect of Weaning Time and Size of Bayonet Suckers and Their Interaction on the Number of Vivo Plants Emitted or Emerged at Initiation

Table 2 presents the results of the two-factor analysis of variance performed on the number of vivo plants emerged at initiation. The analysis reveals that there is a very highly significant difference between weaning times regarding the number of vivo plants emitted. However, no significant difference ($p > 0.05$) is noted regarding the size of bayonet suckers. Similarly, the Moment × Size of bayonet sucker interaction had no significant effect ($p > 0.05$) on the number of vivo plants emerged. This indicates that the initial emergence (initiation) of suckers was not significantly influenced by bulb size. However, the significant advantage of large bulbs reported in the conclusion manifests primarily during subsequent proliferation and weaning phases, as reflected in the significant positive correlation ($r = 0.26$; $p = 0.04$) between bulb size and final number of emitted vivo plants, and in

Table 2. Result of variance analysis performed on the number of vivo plants obtained at initiation.

Source of variation	Degrees of freedom	Number of emerged suckers (F-value)
Weaning time	1	18.01***
Explant size	2	2.58 ns
Weaning time * Explant size	2	2.03 ns

ns: $P > 0.05$; ***: $P < 0.001$.

the superior performance of plants derived from large bulbs at weaning.

Figure 1 presents the results of the Student Newman Keuls test performed on the numbers of vivo plants obtained at the end of initiation of bayonet suckers. Student Newman Keuls test revealed no significant difference ($p > 0.05$) between the different sizes of bayonet suckers regarding the number of suckers obtained at the end of initiation regardless of weaning time. However, the number of vivo plants obtained at the 1st weaning (5.56 ± 0.49) is lower than those obtained before weaning (10.21 ± 1.09). In general, the number of suckers obtained at the first weaning decreased by 84% compared to before weaning.

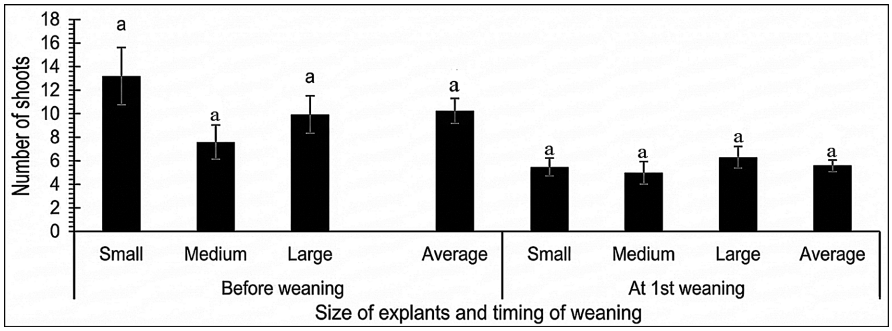

Figure 1. Results of Student Newman Keuls test performed on the numbers of vivo plants obtained at the end of initiation. Error bars represent standard errors. Bars bearing the same letter and for the same factor are not significantly different according to the Student Newman Keuls test ($P > 0.05$).

4.3. Effect of Weaning Time on Number and Length of Median Leaf

Analysis of variance and the Student Newman Keuls test reveal a very significant difference ($p < 0.01$ to $p < 0.001$) between weaning times (Table 3) regarding the numbers of leaves and the length of the median leaf of examined vivo plants. The results of the Student Newman Keuls test revealed that the numbers of leaves and median leaf lengths examined at the first weaning were significantly better ($p < 0.05$) than those obtained at the second weaning (Table 3).

Table 3. Number of leaves and median leaf length (mean ± standard error) at first and second weaning.

Weaning time	Number of leaves	Length of examined median leaf (cm)
1st weaning	4.44 ± 0.26[a]	25.33 ± 2.38[a]
2nd weaning	3.74 ± 0.15[b]	15.77 ± 1.60[b]
F-value	6.26	11.95
Probability	0.01**	0.001***

**: $P < 0.01$ ***: $P < 0.001$. Means followed by the same letter within a column are not significantly different ($P > 0.05$) according to the Student Newman Keuls test.

4.4. Effect of Weaning Time on Plant Height

Figure 2 presents the results of the Student Newman Keuls test performed on

plant height. Analysis of the figure reveals that at the second weaning, plant size regressed. In other words, plants obtained at the first weaning were taller ($p < 0.05$) than those obtained at the 2nd weaning (Figure 2).

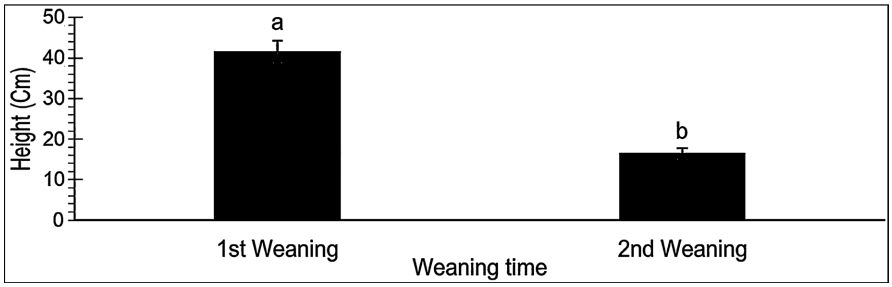

Figure 2. Results of Student Newman Keuls test performed on plant height. Error bars represent standard errors. Bars bearing the same letter are not significantly different according to the Student Newman Keuls test ($P > 0.05$).

4.5. Correlation between Circumference of Bayonet Sucker Bulbs and Number of Vivo Plants

The values of circumference (diameter) of bulbs of sown bayonet suckers and the number of emitted vivo plants were related through a Pearson correlation study. The analysis performed revealed that there is a positive and significant correlation ($r = 0.26$; $p = 0.04$) between the circumference of bayonet suckers and the number of emitted vivo plants. It follows that the number of emitted vivo plants is a function of the size of bayonet suckers. A large size of bayonet suckers therefore induces a high number of vivo plants.

5. Discussion

The different results after analysis at the end of this study allow us to say that the PIF technique contributed to obtaining a high number of vivo plants using bayonet suckers of the yellow pulp plantain variety Aloga. Indeed, banana vivo plants produced using the Plants Issued from Stem Fragment (PIF) technique allow for obtaining healthy, homogeneous suckers in sufficient quantity [8]. The use of this type of homogeneous plant material generates grouped harvests which lead to increased producer income, extension of the banana plantation's life cycle, and thus sustainable agriculture.

The bulb size of bayonet suckers positively influences the number of vivo plants produced by the PIF technique. Thus, through this study, the average number of suckers emitted, particularly at the second weaning, by large bulbs using the stem fragment technique is significantly the highest compared to those of small and medium sizes. This can be attributed to the greater carbohydrate and nutrient reserves stored in larger bulbs. These reserves provide sustained energy and building blocks for the sequential initiation and development of multiple meristems (rank 1 and rank 2 suckers) over the extended propagation period, leading to higher final plant counts and enhanced vigor. These results corroborate those obtained by Koua *et al.* [9] and Mageki *et al.* [10]; who reported that this number is in the

range of 10 - 100 seedlings depending on the tested cultivar. In contrast, this result is higher than the number (6 - 9 seedlings) obtained by Mensah *et al.* [11].

The initiation time of vivo plants observed in this study may be linked to the mastery of the production technique. According to Boye *et al.* [12], recovery times may be due to the content of nutrient reserves stored in the bulb.

Regarding the weaning time observed during this study, regardless of the bulb size of the bayonet suckers, the vivo plants were weaned within a short time interval (2 to 3 months), compared to the long weaning time (2 - 5 months) reported by the work of Dkomeku *et al.* [13]. These results show that the yellow pulp plantain variety Aloga used has the capacity to produce vivo plants in a short time, which is favorable for plant production as it helps avoid potential bulb rot.

In general, weaning time influenced the number of leaves, the length of the median leaf, and the height of the produced vivo plants. Thus, the results related to the number of leaves and length of the median leaf examined at the first weaning exceeded those of the second weaning by 19% to 61%, while the regression of heights reached 150% between the first and second weaning. These variations may be due to the quality of the substrate used for seeding the bayonet suckers, whose richness in fertilizing elements progressively decreased.

6. Conclusion

The execution of this study showed that bayonet suckers of the yellow pulp plantain variety Aloga cultivated in Benin have a good aptitude for the production of quality vivo plants using the plant production technique by stem fragmentation (PIF). Thus, a significant quantity of vivo plants is obtained using the large bulbs of these suckers. In light of the results obtained, banana sector stakeholders, particularly nurserymen, will be made aware of the production of quality planting material using the PIF technique from large bayonet sucker bulbs in order to have a significant number of banana vivo plants available for all those wishing to establish banana plantations.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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