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Macro-propagation of Dessert Bananas (Dankodu and Tsikodu) and Plantain (Savé) (Musa Spp.) by PIF Technique in Togo, West Africa

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Abstract

Banana is an important crop in many countries of the intertropical zone. The major problem in increasing production is the lack of healthy planting material. The classic discards often used by producers, are in small quantities and carry diseases germs. *In vitro* culture and other macropropagation methods such as PIF technique are being developed to produce healthy plant material for planting. *In vitro* culture is more expensive and slow compared to PIF technique which is cheaper and faster hence its adoption in this study. The general objective of this study is to help support the dessert banana and plantain banana sector in Togo. It is more specifically; to know the latency time (TL), to assess the rate of bud break at forty (40) days (TD); know the time necessary to wean the first shoot in each explant (TS), know the total number of young plants weaned (S) at 150 days after planting in sawdust; know the number of days between the appearance of the first shoot and the achievement of the first weaning (TS-TL); then compare the success percentages (PR) of rearing these young plants in the nursery after weaning. The varieties selected for the study are Savé (plantain), Dankodu (dessert) and Tsikodu (dessert). PIF technique was used to produce the healthy planting material. After experiment, the TL parameter is on average 2 weeks (14 days) for Savé and Tsikodu varieties and 3 weeks (21 days) for Dankodu variety. The TS parameter is 4 weeks (28 days) for Savé and Tsikodu varieties and 6 weeks (42 days) for Dankodu variety. For the TS-TL parameter, it is 2 weeks (14 days) for Savé and Tsikodu varieties and 3 weeks (21 days) for Dankodu variety. For S parameter, Plantain banana Savé is more prolific with 13 young plants per explant while it is 3 young plants per explant for Dankodu and Tsikodu varieties. For TD parameter, Savé variety has 100% while Tsikodu (66.66%), Dankodu (55%). For PR parameter, Dankodu variety is the best with 90%, Tsikodu (76.19%) and Savé (77.03%). PIF technique can be used to propagate bananas in general, but it needs to be improved for dessert bananas in particular.

Keywords

Banana, Dessert, Plantain, PIF, Togo

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1. Introduction

Banana (Musa spp.) is a fruit plant of great nutritional, economic and socio-cultural importance. From the genus Musa and belonging to the Musaceae family, it is widely cultivated in tropical regions of the world. Bananas occupy the fourth place in the human diet after rice, wheat and corn [1]. Bananas are the main source of food, employment and income in most of its production areas [2]. In Ivory Coast, plantains are used to make foods like Foutou, Aloco and

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boiled plantain. During the ripening of plantains, the mineral contents (Ca, Mg, P, K and Na) remain stable when those of reducing sugars, total sugars, proteins and lipids increase [3].

World production of sweet bananas and plantains was 153,160,139 tonnes in 2017; these products are grown on an area of 11,160,253 ha with a yield of 273,127 tonnes / ha. Togolese production of these fruits is 27,279 tonnes. Their worldwide export has a value of 12,138,404,000 US dollars [4]. The Litimé plain is the main banana production basin in Togo [5] with an annual production of 17,000 tonnes, ie 70% of national production [6]. However, there are other secondary basins such as Akata, Kpadapé, Agomé, Amou, Agou [7–9]. However, this production remains insufficient due to demographic change. The current plantain and dessert banana farms are insufficient to meet the needs of the population due to lack of good quality plant material. The discards used are often carriers of diseases germs and are not homogeneous [10, 11]. These diseases and pests disseminated by suckers contaminated by mother plants, are sources of reduced yield, longevity of plantations and also their disappearance [12–15].

The banana sucker capacity is on average three (3) suckers per year depending on agro-climatic conditions and cultural practices. The mother plants do not give enough suckers to meet the needs of producers [16–18]. Indeed, despite its importance for development, farmers not only do not find the number of suckers necessary for replanting, but also the classic suckers used are often infested. Pitekelabou and collaborators reported in 2018, that the Plants derived from Stems Fragments (PIF) technique allows to obtain a multiplication rate of twelve (12) to sixty (60) shoots per explant, depending on the varieties of plantains, for four (4) to five (5) months. This technique is suitable for producers because it requires few resources [19]. Studies have shown that there is no difference in agronomic characteristics between *in vitro* plants and banana plants resulting from the PIF technique, hence it is important to adopt this technique for the production of healthy banana planting material because of its simplicity, low cost and speed [20].

Research must therefore provide farmers with healthy plants, sources of good yields. Following the above, PIF technique can be applied to cultivars from Togo for their dissemination to the rest of the banana and plantain production areas of the country. The high multiplication rate of banana using this technique allows young plants to be obtained fairly quickly and on a massive scale from the clones chosen from producers, thus making healthy planting material available.

It was found that the fruits of the banana varieties dessert Tsikodu and Dankodu, then those of the plantain variety Savé, are found on the Togolese market, but these varieties are neglected by the producers, which leads to a low production at because of the lack of discards. All the above justifies the choice made on these three varieties for the multiplication of suckers by PIF technique.

The general objective of this study is to help support the dessert banana and plantain crops in Togo. It is more specifically; to know the latency time (TL) or time required for the appearance of the first shoot by PIF technique, then evaluate the rate of bud break at forty (40) days (TD) of each variety; know the time necessary to wean the first shoot in each explant (TS), know the total number of young plants weaned (S) at 150 days after planting in sawdust; know the number of days between the appearance of the first shoot and the achievement of the first weaning (TS-TL); then compare the success percentages (PR) of re aring these young plants in the nursery after weaning.

2. Material and Methods

2.1. Plant Material

The plant material consisted of discards of two varieties of dessert bananas (Dankodu and Tsikodu) and of a variety of plantain (Savé). The discards of the Savé plantain were provided by the producers of Yoto, while the dessert bananas (Dankodu and Tsikodu) come from Agou. The discards constitute the stem fragments used here in the PIF technique (Plants Derived from Stem Fragments) (Figure 1). Each fragment has several latent buds that must be activated by lifting the apical dominance, this is the basic principle on which the PIF technique is based. This lifting occurs following the destruction of the apical meristem. Twenty (20) suckers were used for each of the three varieties.

Figure 1. Dankodu variety bayonets suckers used.

2.2. Technical Material

To carry out this work, the materials were used, the dibble to collect the discards in the field, the machete and knives to cut the plant material. The germinator built in cement bricks

measuring 3.8m in Length, 1.5m in Width and 0.5m in height, covered with transparent plastic together constitute the propagator (Figure 2). Clear plastic was used in this case to create the greenhouse effect, letting in the daylight necessary for photosynthesis of the shoots. The substrate used for the soilless culture was sawdust.

The thermometer and hygrometer were used to assess temperature and humidity, respectively, to check the experimental conditions; the planted explants were labeled with the variety name, sowing date and sequence number. A garden hose was used to water the crop with tap water. The 0.5l black nursery plastic containing the potting soil were used for the acclimatization of the weaned young plants. Banko Plus fungicide was used to disinfect all the explants.

Figure 2. Propagator used for the PIF technique (3.8m x 1.5m x 0.5m).

2.3. Methods

The PIF technique (Plants Derived From Stem Fragments) was used in the experiment. In fact, this technique was developed by Kwa in 2003 in Cameroon and its basic principle is the lifting of apical dominance [21].

The preparation of the collected fragments began by washing the discards with tap water, the roots were then removed using knives. Clear trimming was performed; it consisted in peeling the stem of the discards down to 3 to 5mm or exceptionally up to 1 cm deep to avoid the possible presence of nematodes. The shelling consisted of removing three to four leaf sheaths, taking care to leave 2 mm of sheath above the edge of the corm while the pseudo-stem of the discards was reduced to (5 or 10) mm above the last visible node of the discards stem. Two to three cross incisions were made in the center of the implant to destroy the apical meristem and lift its dominance. The explants were left to dry for 24 hours under shade after disinfection with Banko Plus fungicide (550g / l of Chlorothalonil + 100g / l of Carbendazim) at a rate of 2ml / l of water, for 30 minutes. The explants were placed in sprout on a layer of sawdust of about 10 cm, then covered with sawdust with a layer of 3 to 5 cm; the first watering was carried out 24 hours after planting in sawdust (Figure 3).

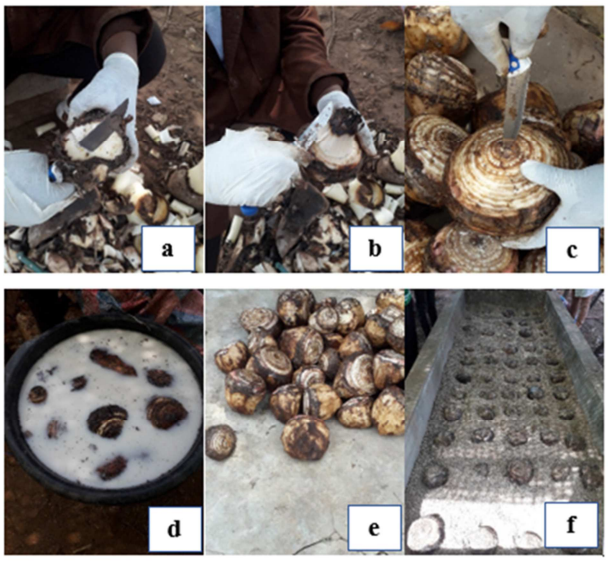

Figure 3. The different stages of the PIF technique.

a-Trimming of discards; b-Shelling of discards; c-Destruction of the apical meristem; d-Disinfection of the explants with Banko Plus Fungicide; e-Drying of explants after disinfection, f-Planting of explants in sawdust.

One or two watering per week were made depending on the humidity of the sawdust in the germinator. Temperatures and hygrometries were recorded both in the enclosure of the propagator and under shade. The average temperature inside the hotbeds was 40.13°C ± 5.05; the average internal humidity was 85.75% ± 9.63. The experiment took place during the rainy season from March to August 2020, with an average outdoor temperature and humidity of 36.75 C ± 3.92 and 63% ± 6.39 respectively. Cultures are made under shade, and the propagators, which are made up of seedbeds made of cement bricks, were covered with transparent plastics (Figure 2).

Weaning was done as soon as the plantlets had two to three leaves. The weaned young plants were transplanted onto potting soil contained in the 0.5l black nursery plastic. The water supply to the weaned young plants was made taking into account the weather conditions and the humidity of the substrate.

During the experiment, biotic parameters such as:

The time required (in days) to obtain the first shoot, or

Latency (TL),

The 40-day bud break rate (TD),

The time required to wean the first shoot in each explant (TS)

The total number of young plants weaned at 150 days after planting in sawdust, noted S,

The number of days between the appearance of the first shoot

and the obtaining of the first weaning (TS-TL)

The percentage of success (PR) of young plants weaned and acclimatized; was calculated at 150 days after planting the explants in sawdust. The formula used is as follows:

PR (%)=(Number of live young plants/Number of weaned young plants)x 100

The burst rate (TD) is calculated as follows:

TD (%)=(Number of explants having broken at 40 days/Total number of explants planted) x 100

Likewise, the abiotic parameters, namely the internal temperature and hygrometry of the hotbeds were monitored twice a week and at 3 p.m. GMT.

Statistical analyzes were performed using R software. Separation of means was performed with Tukey's test at the significance level of 5%.

3. Results

The results of Table 1 and Table 2 were obtained after an experiment conducted at the Togolese Institute for Agronomic Research (ITRA) in Lomé Cacaveli. This experiment was made from March to August 2020 during the rainy season. The average temperature inside the propagator was 40.13°C; while the internal humidity was 85.75%. The environmental conditions of the culture site were 36.75°C and 63% outside the propagator. Temperatures between the inside and the outside of the propagator range from -1 to 6°C. Twenty explants per variety were tested. Each explant constituting a repetition. The means of each column, in each table, assigned the same letter are not significantly different according to the Tukey test at the 5% level.

Table 1. Characterization of two varieties of dessert bananas (Dankodu and Tsikodu) and a variety of plantain (Savé), compared to the PIF technique.

Variety	TL (days)	TS (days)	TS-TL (days)	S (Shoots number/explant)
Savé	16.65±1.84 a	26.5±3.66a	9.95±4.04a	12.90±6.75b
Dankodu	21.35±8.02b	44.7±21.51b	23.35±20.44b	3.25±2.99a
Tsikodu	13.10±2.83a	27.5±2.56a	14.40±4.74ab	2.50±2.40a

For the TL parameter, there is no significant difference between the means of the plantain variety Savé and the dessert banana variety Tsikodu (p> 0.05). Indeed, these varieties take an average of two weeks (14 days) to grow in sawdust. There is a very significant difference between these two varieties and Dankodu dessert banana variety (p <0.01). The Dankodu variety has a latency time of three weeks (21 days) therefore longer than the Savé and Tsikodu varieties which grow after two weeks (14 days). In other words, the Savé and Tsikodu varieties grow faster than the Dankodu variety (Table 1).

For the TS parameter, there is also no significant difference between the Savé and Tsikodu varieties (p> 0.05); on the other hand, there is a very significant difference between these two varieties and the Dankodu variety (p <0.01). The first shoots of the Savé and Tsikodu varieties are weaned on average four weeks (28 days) after planting the explants in sawdust; while the first shoots of the Dankodu variety are weaned late on average at six weeks (42 days) after planting the explants in sawdust (Table 1). These results can be explained by the weak growth of the shoots of the Dankodu variety (Figure 4)

For the TS-TL parameter, there is no significant difference between the Savé and Tsikodu varieties (p> 0.05); likewise there is no significant difference between the Dankodu variety and the Tsikodu variety (p> 0.05); on the other hand, there is a very significant difference between the Savé variety and the Dankodu variety (p <0.01). Indeed, the number of days between the emergence of the first shoots and their weaning is shorter in the Savé and Tsikodu varieties, that is to say that after the emergence of the shoots it takes on average two weeks (14 days) to do weaning. This time is longer in the Dankodu variety which is on average three weeks (21 days) (Table 1).

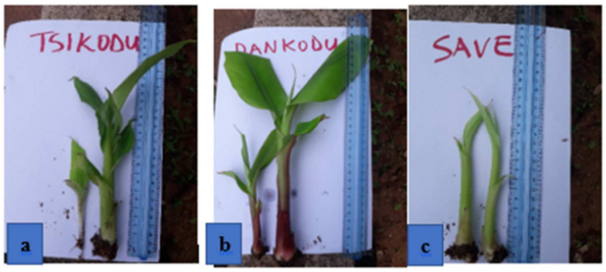

Figure 4. Weaning of young plants.

a- Two young plants of Tsikodu just after weaning b- Two young plants of Dankodu just after weaning; c-Two young Savé plants just after weaning.

For the parameter S, there is no significant difference between the Dankodu and Tsikodu varieties (p> 0.05); on the other hand, there is a very significant difference between these two varieties and the Savé variety (p <0.01). The observation is that the Savé plantain variety is more prolific with an average of thirteen (13) young plants weaned per explant planted, while the dessert banana varieties Dankodu and Tsikodu are less prolific with an average of three (3) young plants weaned. by explant (Table 1). This result confirms the fact that plantain varieties are more prolific than

dessert banana varieties. The Savé variety gives four (4) times more shoots than the Tsikodu and Dankodu varieties.

Table 2. Comparison of bud break rates (TD) and percentages of success (PR), dessert banana plants (Dankodu and Tsikodu) and a plantain variety (Savé).

Variety	Dankodu	Tsikodu	Savé
TD (%)	55	66.66	100
PR (%)	90	76.19	77.03

TD: Bud burst rate at 40 days or the number of explants germinated at 40 days
PR: Success rate of weaned plants 150 days after sowing the explants in sawdust

Considering the budding rate (TD), among the three varieties tested, the best performing is Savé with a rate of 100%; the others have a lower rate compared to this one but their rates remain higher than 50%. For this parameter, the Tsikodu variety is the best (66.66%) compared to the Dankodu variety (55%) among the dessert banana varieties tested.

For the seedling success rate (PR) parameter, the Dankodu dessert banana variety is the best variety, with a success rate of 90%. The best variety among dessert bananas is still Dankodu with a success rate of 90%. The success rates of the Tsikodu and Savé varieties are less than 80% but all are greater than 75%. Four months after weaning, the weaned young plants are ready to be planted in the field (Figure 5).

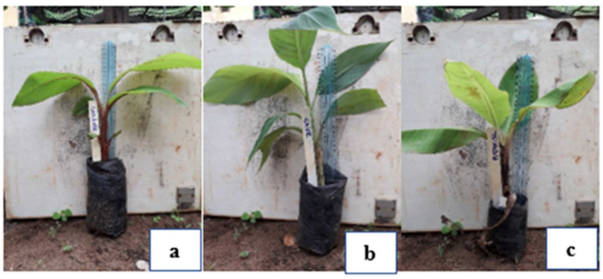

Figure 5. Four month old banana young plants ready for the field a-Dankodu; b-Savé; c-Tsikodu.

The results obtained for the bud break rate (TD) can be explained by the fact that the Savé variety is more apt to grow roots which are responsible for drawing nutrients from the sawdust to nourish the buds which will quickly emerge. in the form of young shoots. All of the above helped many explants to germinate quickly resulting in the high rate of 100% for this variety (Table).

Compared to the success rate (PR), the Dankodu variety is the best with a rate of 90%; this can be explained by the fact that during weaning it was found that the pseudo-stem of the weaned seedling of Dankodu is more robust than those of other varieties which are very tapering. The size of the pseudo-stem also directly induces the size of the corm or true stem of the young weaned plant; it should be remembered that this corm comes from the mother implant on which the weaning was done. It is through the corm that water exchanges take place just after weaning between the substrate and the young plant. This water absorption provides nutrients in the form of raw sap, which promotes the upright growth of the young plant, leading to photosynthesis which will subsequently lead to root growth. Thus, a very small corm does not offer a large surface for water absorption, which leads to the death of the young plant; all this explains the low success rates recorded at Tsikodu and Savé (Figure 4). In addition, most of the young plants weaned from these varieties do not have roots because they are found in the heart of the mother plant, this would explain the low success rate among them.

4. Discussion

The study of the macropropagation of the dessert banana cultivars Dankodu and Tsikodu and of the plantain variety Savé made it possible to characterize each of them in relation to their multiplication by the PIF technique. The Dankodu variety has a latency time of three weeks (21 days) therefore longer than the Savé and Tsikodu varieties which grow after two weeks (14 days). In other words, the Savé and Tsikodu varieties grow faster than the Dankodu variety, this can be explained by the fact that the emergence of apical dominance is faster in these first two varieties. For the Dankodu dessert banana variety, our results are similar to those of Kwa in 2003, but they are contrary to those of this author when it comes to the Savé and Tsikodu varieties; this difference can be explained by the genetics of these varieties which allow rapid emergence of shoots [21].

The first shoots of the Savé and Tsikodu varieties are weaned on average four weeks (28 days) after planting the explants in sawdust; while the first shoots of the Dankodu variety are weaned late on average six weeks (42 days) after planting the explants in sawdust; this fact is the corollary of the delay in the lifting of apical dominance in this variety. Despite the destruction of the apical meristem, if the phytohormonal ratio Auxins / Cytokinins remains high, it will be difficult to the axillary buds to grow. For it, to have early shoot proliferation, a low concentration of Indole 3-acetic acid (AIA) and a high concentration of 6-benzylaminopurine (2 or 4 mg / l BAP) are required [22, 23].

During the experiment, it was found that the number of days between the emergence of the first shoots and their weaning is shorter in the Savé and Tsikodu varieties, that is, after the emergence shoots it takes an average of two weeks (14 days)

to wean. This time is on the other hand longer in the Dankodu variety which is on average three weeks (21 days), this can be explained by the slowness of the development of the young shoots in this variety. This physiological phenomenon is explained by the fact that after the destruction of the apical meristem, the hormonal balance auxins / gibberellins is not in favor of gibberellic acid (GA3) which is responsible for the development in height of the shoots [24, 25].

For the S parameter, the results indicate that the plantain variety Savé is more prolific which have an average of thirteen (13) young plants weaned per explant planted, while the dessert banana varieties Dankodu and Tsikodu are less prolific with an average of three (3) young plants weaned by explant. The proliferation of shoots depends on the physiological state of the discards used, their health status, the environment and also the genetics of the variety. *Ex-vitro* multiplication studies have shown that genotypes often comprising the "B" genome (case of plantains) are more prolific than genotypes of the "AAA" group (case of dessert bananas) [26]. The multiplication ratio by the PIF technique recorded in the Savé variety is similar to that of Pitekelabou and collaborators in 2018, because this variety is a plantain like the varieties Apim, Agbavé and Kadaga [19]. For the dessert banana varieties Tsikodu and Dankodu, our results are contrary to those of Pitékélabou and collaborators (2018), because they are not from the same genetic group; on the other hand, these results are similar to those of Bodjona and collaborators in 2017, because this author worked on the varieties Adokpa and Fokona which are also varieties of dessert bananas [27].

Considering the budding rate (TD), among the three varieties tested, the best performing is Savé with a rate of 100%; the other varieties have a rate of 66.66% for the Tsikodu variety and 55% for the Dankodu variety. This result can be explained by the fact that the explants of the Savé variety quickly grow roots, which made it possible to draw nutrients from the sawdust and promote the rapid budding of all the explants 40 days after planting. For the Savé variety, our results are similar to those of Kwa in 2003 because this variety is a plantain like the French clair, French sombre, Kelong Mekuitou, Bâtard and Mbouroukou N° 1 varieties tested by this author. For the Tsikodu and Dankodu varieties, our results are contrary to those of this author who found a bud burst of 10% with the Grande naine variety which is a dessert banana variety; this can be explained by a difference in genotypes between the Togolese varieties and this one.

For the seedling success rate (PR) parameter, the Dankodu dessert banana variety is the best variety, with a success rate of 90%. The other varieties, Tsikodu and Savé, have respectively a success rate equal to 76.19% and 77.03%. These rates, although high, do not reach 95% and this can be explained by the fact that most of the young plants weaned from the PIF technique do not have roots compared to the weaned *in vitro* plants which have enough roots that are necessary to the survival of the plant [28]. This result can also be explained by the fact that the root system is the link between the plant and the soil. It is responsible for the absorption of water and nutrients, serves as an anchor, it is the place of synthesis and storage of certain phytohormones necessary for the development of the plant [29].

5. Conclusion

This study made it possible to characterize the Savé plantain variety and the Dankodu and Tsikodu dessert banana varieties. This experiment proved that the Dankodu variety has a longer latency time, 3 week (21days) than that of the Tsikodu and Savé varieties, 2 weeks (14days). Likewise, the Dankodu variety is weaned on average 6 weeks (42 days) after planting in sawdust. The Savé plantain variety is more prolific with 13 young plants weaned per explant planted. The dessert banana varieties are four times less prolific than the Savé variety; that is the reason why, the Togo Research Institutes must improve their multiplication by applying high doses of growth regulators(Cytokinins) such as 6-benzylaminopurine (BAP). In fact, this growth regulator, applied in place of the apical meristem after its destruction, would promote the proliferation of shoots from latent axillary buds, often inhibited by the apical dominance on the mother plant. The percentage of success of young plants in the nursery can be improved; by watering the plants once or twice a week, and by watering the soil in the nursery shade every day in order to maintain high humidity in the immediate environment of the weaned plants without roots, these conditions are necessary for their survival.

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