Bioscience and Bioengineering
Vol. 4, No. 2, 2018, pp. 23-28
http://www.aiscience.org/journal/bio
ISSN: 2381-7690 (Print); ISSN: 2381-7704 (Online)

Macro-Propagation of *Ananas comosus* (L.) Merr. (Bromeliaceae) by PIF Technique in Togo, West Africa

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Abstract

Pineapple is a fruit consumed in Togo. Two varieties: Brazza and Smooth Cayenne are grown in the south of the country in the Plateaux and Maritime regions; the fruits are exported to the big cities of which Lome the capital. The finding is that crowns are rejected when there is a shortage of planting material. This study has as a general objective the contribution to the production of pineapple in Togo and more specifically; to evaluate the success rates of the treatments used to make crown PIF technique, to analyze the number of rejections produced by explant and treatment, to evaluate the percentage of survival after acclimatization of young plants without roots and young plants with roots. PIF technique has been used to propagate seedlings. After experimentation, 100% of the explants broke up and gave 1 to 5 young plants each for the T1 treatment (apical meristem destroyed without division of the explant), the two (2) treatments T2 and T3 where the explants were divided longitudinally in two (2), all the explants rotted. The survival rate of rootless weaner seedlings is 63.63% and those with roots are 90.32%. Despite the low proliferation rate, the PIF technique can be used to produce healthy planting material, but it remains to be improved.

Keywords

Pineapple, Brazza, Lome, Togo, PIF

Received: June 26, 2018 / Accepted: July 23, 2018 / Published online: August 31, 2018

1. Introduction

Pineapple (Pineapple comosus) of the family Bromeliaceae, is known primarily for its edible fruit and also cultivated for its richness in Bromelain, which is an enzyme. This enzyme has many industrial uses ranging from the tenderizing of meat tanning leathers, through the stabilization of latex paints. In addition, from the leaves of certain varieties selected for this purpose, fibers are obtained for making coding, nets, and fine papers and textiles. It is grown in almost all tropical regions of the world [1].

This crop is generally characterized in Africa and in Togo in particular, mainly because of its low productivity. This is the result of the many constraints among which, the insufficiency

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and the difficulty of acquisition of planting material. This constraint is considered the most important because it can hinder the expansion and improvement of pineapple. The fact is that the spread of pineapple is by rejection and has drawbacks including: slow (the number of rejections per plant being limited), the heterogeneity of propagation material and finally the weight and volume of the material propagation; which consequently makes it expensive to transport. Propagation material is heavy and bulky, so transport is expensive [2].

In Togo, Pineapple is grown west of the maritime region and in the southern Plateaux region with rainfall ranging from 900 to 1400 mm / year. This farming is done by smallholders because of lack of land, financial problems and ignorance about the importance of pineapple. In monoculture, the pineapple plantation ranges from 35 000 plants / ha to 40 000 plants / ha and in association is 16 000 plants / hectare. The varieties grown are Cayenne Smooth and Brazza. Depending on the size, the selling price of pineapple varies from 150 to 225 FCFA [3]. Although pineapple production in Togo in 2014 is 1828 tons and then increases (22% increases) over the last ten years, it is not very significant [4]. The pineapple crop is facing several diseases including Phytophtora which is a major fungal pathogen.

In Togo, it has been observed that pineapple crowns are discarded on the dumps by the sellers of these fruits whereas for intensive cultivation, a lot of healthy rejections are needed. It is therefore essential to value these crowns that seem to be useless. The PIF technique would be the solution to this problem, but the Togolese research structures have not yet experienced it; all this motivates this study.

Only one type of discard should be planted throughout the field in order to have a uniform and moderately uniform fruit crop throughout the field [5].

Although the multiplication rate by explant "*in vitro*" is impressive and incomparably higher compared to other techniques, the authors recognize the extreme slowness of the acclimation phase. On Reunion Island, the acclimatization of vitro plants of 1-2 g of Queen Cultivar lasted 10 months to obtain seedlings of 120-130 g [6]. There is also a vitro-plant effect; the first plants resulting from *in vitro* culture may have aberrant behavior (eg poor quality fruit). It may take several cycles before these characteristics of the variety stabilize [7].

The technique of rapid multiplication of plants (macro propagation) by stem fragment (PIF) will allow to obtain several plants (rejects) that are healthy, moderately homogeneous (clones) and will fully retain the characteristics of the mother plant (best yield). Thus, this technique applied to pineapple is a new way of mass production of plants because of its speed, adaptability and low investment cost.

The production time of conventional rejects has the disadvantage of being long; three to seven months after the harvest of the fruits. This work may, indeed, be necessary to obtain planting material within a period of time. For example, the Plants Shrub Macropropagation (PIF) technique allows latent buds to be activated to regenerate large quantities of healthy plants in relatively short periods of time that can be adjusted to planting times. The general objective of this study is the contribution to the production of Pineapple in Togo and more specifically to evaluate the success rates of the treatments used to make the PIF technique of crowns, to analyze the number of rejections produced by explant and by treatment, evaluate the percentage of survival after acclimatization of seedlings without roots and seedlings with roots. This research has for interest the reduction of insalubrity in the city of Lome (figure 1) and also the provision of Togolese farmers, healthy material of pineapple plantation.

Figure 1. Crowns of Pineapple rejected on the dumps (Lome city, Togo).

2. Material and Methods

2.1. Plant Material

The crowns procured by the itinerant pineapple sellers were used during this experiment. The so-called Brazza variety called sugar loaf was used. The fruit of this variety has a

conical shape, a green skin and a white and sweet flesh (Figure 2).

Figure 2. Pineapple fruit of the Brazza variety.

2.2. Technical Material

To perform this work, materials were used namely knives for cutting plant material; baskets covered with transparent plastic together constitute propagators or germiners. The transparent plastic was used in this case to create the greenhouse effect, leaving the daylight necessary for photosynthesis of the shoots. The substrate used for above-ground cultivation was sawdust. The thermometer and the hygrometer were used respectively to take the temperature and the hygrometry, to check the experimental conditions. A garden hose was used for this purpose. The 0.5-liter black plastic bags containing the potting soil were used for the acclimation of weaned vivoplants. Banko Plus Fungicide has been used to disinfect all explants.

2.3. Methods

PIF (Plants from Stem Fragments) was used in the experiment. Indeed this technique was developed by Kwa in 2003 in Cameroon and has as a basic principle the lifting of apical dominance; by the destruction of the apical meristem [2]. The preparation of the recovered crowns began with the trimming by removing the rest of the fruit at the base of each crown using knives. The shelling consisted of removing with the hands one by one all the leaves of the crown going from the base to the summit. Three treatments were performed on the trimmed and shelled explants. Treatment T1: Two to three crossed incisions were made in the center of the top of the explant and it was planted in sawdust; T2 treatment: the trimmed and shelled explant was divided longitudinally into two (2), passing through the apex and then buried in sawdust at a depth of 2 to 5 cm; treatment T 3: the explant trimmed and shelled was divided longitudinally into two (2), passing through the apex and then deposited on the sawdust with the cutting plane in contact therewith. For each treatment, ten (10) explants were used with three (3) repetitions, thus a total of thirty (30) observations per treatment. The randomly arranged explants were dried for 2 hours under shading after disinfection with Banko Plus Fungicide (550 g / l Chlorothalonil + 100 g / l Carbendazime) at 2 ml / l water for 30 minutes.

The first watering was done just after planting in sawdust. One or two water supplies per week have been made depending on the moisture content of the sawdust in the propagator. Temperatures and hygrometry were also noted both in the basket enclosure and in the shade after every two (2) days throughout the three month trial. In the morning between 7am and 8am, the average temperature under the shade around the propagator was 29°C; the average external hygrometry was 89%. The average temperature in the propagator was 30°C; the average internal hygrometry was 97%. In the afternoon between 1pm and 2pm the average temperature under shade around the propagator was 33.36°C; the average external hygrometry was 71%. The average temperature in the propagator was 33.17°C; the average internal hygrometry was 97%. Generally under shade, the daily thermal amplitude was 5°C and the hygrometric amplitude was 18%; on the other hand inside the basket, the thermal amplitude was 4°C and the hygrometric amplitude was 8%. Excel and R software were used for data analysis.

3. Results

T2 and T3 explants have all rotten; except those of the T1 treatment. For T1 treatment, the explants broke out one (1) week after planting (Figure 3); the first weaning took place one (1) month after planting (Figure 4). It should be noted that not all weaned plants had roots. Three (3) weanings were done at tree times after each month because the shoots don't have the same age (Figure 5). At weaning, the average number of leaves (NF) per young pineapple plant was 9 ± 2; the mean size (T) was 7.6 ± 1.8 cm and the average number of roots (NR) was 4 ± 3 (Figure 6). Each explant yielded an

average of 1.35 ± 1.05 shoots during the experiment. Five months after weaning, the young plants are ready for the field (Figure 7). During weaning, all the weaned young plants did not have roots, so they were separated from the rootless plants. After acclimatization, the survival rate of seedlings was 63.63% while the rooted plants had a survival rate of 90.32%. The position of the bud which is at the origin of the young shoot explains the presence or absence of root. In fact, the buds that are closer to the world than to the roots did not have roots.

Figure 3. A pineapple explant having broken.

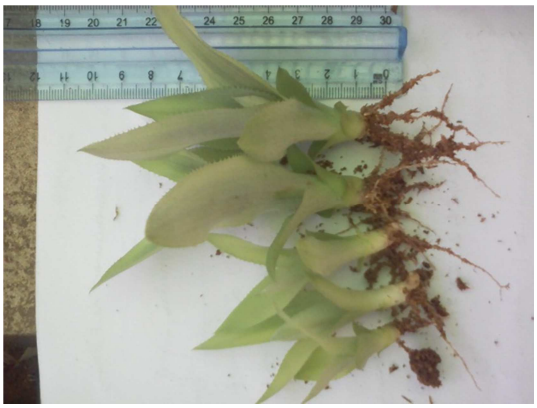

Figure 4. Weaned Pineapple Seedlings.

Figure 5. Young shoots not the same age for weaning.

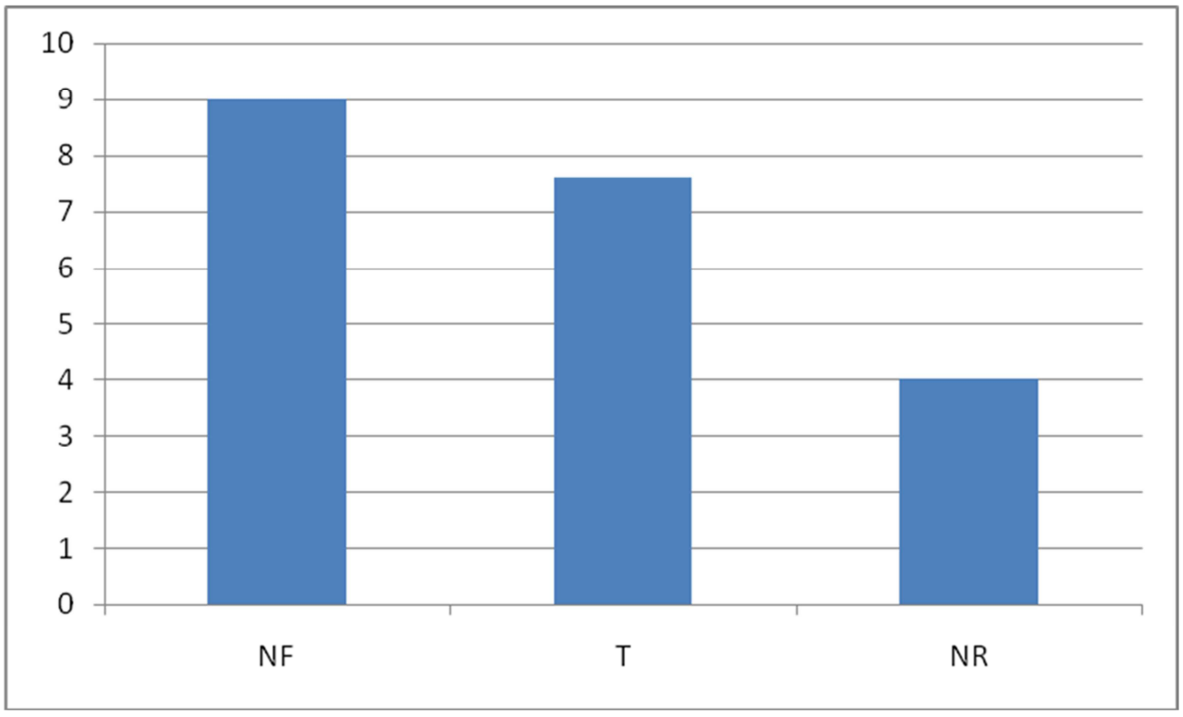

Figure 6. Young shoot growth parameters at weaning (Average number of leaves per young weaned pineapple plant (NF) Average size (T) and average number of roots (NR).

Figure 7. Young pineapple plant ready for the field.

4. Discussion

Brazza pineapple seedlings, weaned from T1 treatment, had an average of 4 leaves. The number of seedlings weaned per explant seeded ranged from 1 to 5, which is 6 times less than the expected result. The work of Fitchet and van de Venter in 1988 on the smooth Cayenne variety; show that there is no need for a greenhouse or chemical treatment to stimulate shoot growth to mass produce pineapple planting material. According to these authors; it is possible to obtain 24 seedlings from a single crown for seven (7) months of production [8]. They proved that the more the explant from the crown is divided into four sections, the greater the proliferation of shoots; moreover, the sections of the upper half are slow to bud and give less seedlings to wean relative to the sections of the lower part of the crown. The latter quickly break down and give more young plants to wean. These results are not consistent with our results.

The T_2 and T_3 explant rot indicate that the longitudinal section caused a massive influx of water into the explants leading to decay during watering. This fact proves that the pineapple crowns cut longitudinally do not limit the turgor of the cells, but rather allow a massive entry of water inside the explant because it is more concentrated in mineral salts, which leads at the cellular burst thus the death of the explant. In contrast, the junction between the fruit and the crown constitutes a natural barrier against the excessive entry of water into the explants, which explains the 100% survival of the explants.

The work of Thiemele and coworkers in 2015 carried out on the smooth Cayenne varieties, MD2 and H4, show that using pineapple discarded stems from aged plants or mother plants from an already harvested plantation; there is no problem of rot by making a longitudinal cut [9]. This work is contrary to our results, which can be explained by the origin and age of the explant. The older the explant, the more the cutting plane resists the entry of water into the explant. During our experiment, each explant gave at the end 1 to 5 weaned young plants, these results are contrary to those of Thiemele and *al.* in 2015 who found 10 to 15 plants per seeded explant. The low rate of regeneration of the explants from the crowns indicates that although there are about 30 buds on the shell crown, the destruction of the apex by a cross incision above the explant, could not reverse the hormonal balance in favor of cytokinins compared with auxins to allow shoot proliferation. Agogbua et *al*. (2011) proved that the more the crown is divided into several pieces, the least is the apical dominance and therefore the decrease of the auxin inhibition effect on the explants, hence the emergence of the buds as well as the Proliferation of shoots to wean [10]. This work on the smooth Cayenne variety is consistent with our results because we obtained very few seedlings compared to the expected results. Indeed, these authors proved that by dividing the crowns into four, the shoots were more numerous than dividing them in two. With the same number of explants seeded for all treatments at the start of their experimentation. Crowns divided into 4 had the largest number of weaned suckers (543) six months after planting; followed by crowns divided into 2 (375) and fewer were undivided crowns with excised meristem (166). Mukendi (2014) indicates that the crowns used as explants in PIF macropropagation give a lower number of shoots than other explants such as the happa, the air cayeu and the underground cayeu [7]. This author found nevertheless an average number of shoots per crown explant, which is 9.45, higher than what was found during our experiment which is 1.35 per seeded explant.

5. Conclusion

The PIF technique applied to the Brazza variety in Togo gave very few shoots to wean. The number of weaner seedlings averaging 1.35 ± 1.05 is very small compared to the number of seedlings expected to be seedlings of 30 seedlings per explant seeded. This experience nevertheless made it possible to see the shortcomings of the different treatments applied

and to correct them in future studies. This test, although it did not provide a high proliferation rate, still makes it possible to value the pineapple crowns after consumption of the fruits; to provide healthy pineapple planting material and contribute to the sanitation of the city of Lome the capital of Togo. This technique remains to be improved. In the perspectives it is desirable to consider acting on the abiotic conditions namely watering, temperature, hygrometry, the substrate to see their effects. For the biotic factors to be handled, it is a question of testing the smooth cayenne variety cultivated in Togo, other types of rejects such as the bulbille, the happa, the cayeu of stump and the air cayeu as well as the old stem after harvesting.

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