OPEN ACCESS
Scholars Bulletin
Abbreviated Key Title: Sch Bull
ISSN 2412-9771 (Print) |ISSN 2412-897X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsbulletin.com/

Subject Category: Bio Sciences

Macro-propagation and Micro-propagation of BBTV-Free Plants in Kisangani, DR Congo

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| **Received:** 01.05.2019 | **Accepted:** 08.05.2019 | **Published:** 19.05.2019
DOI:10.21276/sb.2019.5.5.1

Abstract

The Banana Bunchy Top Disease (BBTD), caused by the Banana Bunchy Top Virus (BBTV), is one of the important banana diseases in the Democratic Republic of Congo. It drastically reduces the production and diversity of bananas. This study focused on the production of banana and plantain planting materials free of BBTV from plants infected by micro-propagation and macro-propagation. 15 suckers of cultivars Litete [plantain (*Musa* AAB), French type)], Libanga Likale [plantain (*Musa* AAB) False Horn type)] and Bluggoe (*Musa* ABB) were used for micro-propagation and 15 others for macro-propagation. These suckers were collected from banana mats with stages 4 or 5 of BBTD symptoms. The Murashige and Skoog (MS) medium augmented with 30 g glucose, vitamins, 1 µM of Indole Acetic Acid (IAA) and 10µM of 6-Benzyl aminopurine (BAP) was used for micro-propagation. The plants resulting from stem fragments was used for macro-propagation. After 5 subcultures in micro-propagation, the sanitation rate was 76.6% for Litete, 66.6% for Libanga Likale and 76.6% for Bluggoe. After macro-propagation, the rate was 27.5% for Litete, 6.6% for Libanga Likale and 73.3% for Bluggoe. These results indicate that the proliferation rate increases the chance to clean up infected planting material explaining why macro-propagation is less effcient than micro-propagation.

Keywords: BBTV, banana and plantain, clean planting materials, micro-propagation, and macro-propagation.

INTRODUCTION

The rapid production of planting material is one of the main concerns of many banana research programs [1]. Different vegetative propagation techniques have been developed *In vitro* to produce massive quantities of plants: micropropagation from meristems cultured in agar or liquid medium, somatic embryogenesis, embryogenic cell suspensions, etc [2].

However, despite the successes obtained with these methods to multiply the banana plants used in industrial plantations, these *In vitro* techniques are not adapted to small producers in southern countries who, like plantain producers, are regularly confronted with the problem of the availability of discards to create, replant or extend their banana plantations.

Some in vivo nursery propagation techniques have increased the multiplication rate of banana in the field [3].

Although accessible to farmers, many of these in vivo methods present the risk of multiplying material contaminated by nematodes or weevils for example if certain precautions are not taken during the establishment of the multiplication plot: application of nematicides and insecticides on the plots used for these multiplications or choices for these operations of a soil that is sanitized or free from specific parasites of banana plants.

In addition, the production time of the rejects has the disadvantage of being long; six to twelve months may be needed to obtain planting material [4].

On the other hand, many buds formed on mother stock remain unexploited [4]. To reduce these problems and to optimize the exploitation of the banana seed potential, a new rapid multiplication technique has been developed by the African Center for Banana and Plantain Research in Cameroon (CARBAP). This is the "technique of plants derived from stem fragments"

called PIF technique. The use of banana stem fragments activates latent buds to regenerate large numbers of healthy plants in relatively short time periods and adjustable to planting times.

However, the Banana Bunchy Top Disease (BBTD), which is one of the most devastating diseases in banana and plantain crops, sometimes results in 100% yield loss [5]. It is among the top 10 viruses worldwide in terms of economic impact [6]. Its causative agent is the Banana Bunchy Top Virus (BBTV), a Babuvirus genus belonging to the family Nanoviridae, whose genome is composed of a circular single-stranded DNA multiple segments [7].

In sub-Saharan Africa, BBTV was reported for the first time in the Democratic Republic of Congo (DRC) in the 1950s [8]; then, spread throughout the country [9]. Recently, 16 BBTV isolates from tshopo and South Kivu (Northeast and central DRC) were compared in a study of the global distribution of BBTV, revealing a large human contribution to long-range dispersal events [10]. In the DRC, BBTV is present in all of its 11 former provinces [8, 9, 11].

MATERIAL AND METHODS

Field of Study

This work was done in the city of Kisangani in the Democratic Republic of Congo; it is the chief town of the Tshopo province and is located in the part of the Congolese central basin at 0° 31'North and 25° 11'E at an altitude of 396m. In the Kisangani region, rainfall is abundant but irregularly distributed over the year; the annualaverage rainfall calculated for a period of 50years (1956 to 2005), shows 1724 mm, for an average annual temperature of 25.3 ° C, the monthly precipitation height is greater than 60 mm [12].

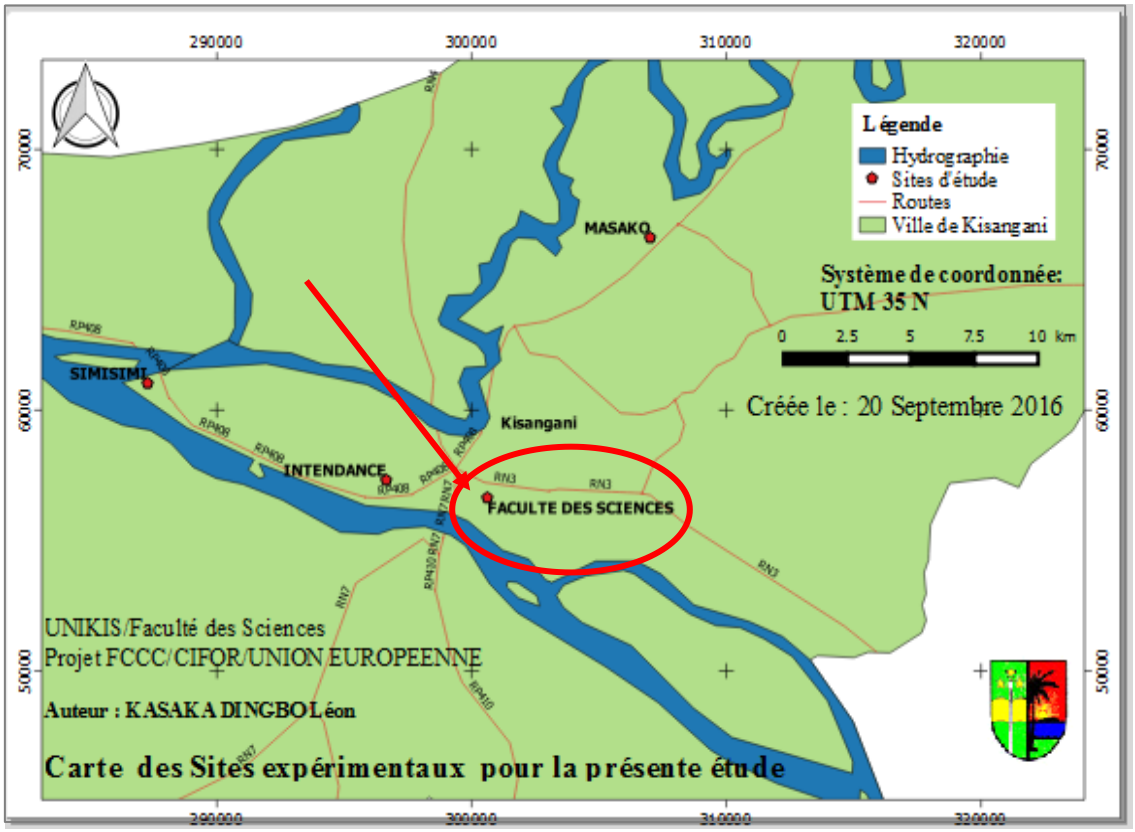

Fig-1: Map of Kisangani City showing the location of experimental sites

Plant Material

The plant material used consisted mainly of young rejects (30-40 cm in height) of three cultivars harvested on the banana trees of the AAB genomic group, particularly the cultivars Litete (plantain (*Musa* AAB), french type), Libanga Likale [plantain (*Musa* AAB) false horn type)] and Bluggoe (*Musa* ABB). The harvest of these ill discharges took place in the city of Kisangani (DRC) and its surroundings based on the visual symptoms of BBTD. A total of 30 infected suckers were collected of which 15 for Litete, Libanga Likale and Bluggoe were put in microproparation and 15 others in macro-propagation. The collected waste was tested at TAS-ELISA using the AGDIA commercial kit (Agdia-Biofords, Evry, France) at the *In vitro* laboratory of plants, Faculty of Science, University of Kisangani (UNIKIS) to confirm the presence of BBTV. The severity of the disease was observed using a scale of 0-5 (0: Absence of symptoms, 1: presence of streaks on the leaf, 2: presence of streaks on the pseudostem, 3: Discoloration of the leaf keeping its normal size, 4: reduction in leaf size and 5: bushy

appearance at the top or Bunchy top) [12]. Only releases with the advanced stages of BBTV (4 and 5) were collected.

The laboratory culture of explants taken from young rejects was carried out in the ready environment containing the mineral salts [13]. this medium was enriched with 30g / l of sucrose, 2 g / l of gelite, nicotic acid (0.5mg / l), pyridoxine (0.4mg / l), thiamine (0.5mg) and 2mg / l glycine and added growth regulator 10 .mu.M of 6-benzylaminopurine (BAP) and 1 .mu.M indole acetic acid (AIA). Each cultivar was inoculated in 5 tubes and after subcultures performed at the interval of one month each time.

The cultivation in the laboratory was carried out under aseptic conditions, protected from all contamination in order to obtain a large number of healthy and fertile vitroplants. The method used is that described by [14-16] in micropropagation and macro-propagation, the method used is that of PIF (Plants issued from stem fragments).

The vitro plants obtained from the micropropagation were acclimated to the screenhouse for two months until the seedlings reached a size of 20 cm. Afterwards, these vitro plants and the seedlings obtained from the macro-propagation were in turn tested with TAS-ELISA to check their immunoenzymological state.

To analyze the data from this research, Microsoft Excel and Software R (3.1.3) were used to find all the results of this study.

RESULTS

In this chapter, the results of TAS ELISA immunoassay before and after *In vitro* culture are presented, as well as the macro-propagation of samples from three banana cultivars, Litete, Libanga Likale and Bluggoe of this study. This chapter also gives the evolution of the average number of buds for each cultivar *In vitro* as well as macro-propagation.

Serological status of banana cultivars before *In vitro* culture and macro-propagation

The serological status of samples of each variety is shown in Table-1 for *In vitro* culture below.

Table-1: Serological status of banana cultivars before *In vitro* culture and macro-propagation

Cultivars	Genotypes	Number of samples	Resultats TAS ELISA	
			Positive	Négatives
Litete	AAB	5	5	0
Libanga likale	AAB	5	5	0
Bluggoe	ABB	5	5	0

The results in Table-1 above show that all banana samples with level 4 and 5 disease were 100% positive for the TAS ELISA test for all varieties.

Rate of Sanitation

TAS ELISA test on plants obtained after *In vitro* culture and in macro-propagation. Tables 2 and 3 respectively present the results in relation to the rate of purification of the banana plants studied virosed by BBTV by *In vitro* culture and by macro-propagation.

Table-2: Remediation rate of Libanga Likale, Litete and Bluggoe after *In vitro* culture

Cultivars	Plants tested	Plants positives	Plants negatives	Remediation rate (%)
Libanga Likale	30	10	20	66,6
Litete	30	8	22	73,3
Bluggoe	30	7	23	76,6
Total	**90**	**25**	**65**	**72 ,2**

The results in Table-2 show that *In vitro* culture was able to clean up 76.6% of BBTV-infected Bluggoe plants; 73.3% of Litete; 66.6% of Libanga Likale. Overall, this technique was used to remediate 72.2% of the cultivar plants studied.

Table-3: Remediation rate of Libanga Likale, Litete and Bluggoe after macro-propagation

Cultivars	Plants tested	Plants positives	Plants negatives	Remediation rate (%)
Libanga Likale	30	28	2	6,6
Litete	29	21	8	27,5
Bluggoe	30	22	8	73,3
Total	**89**	**71**	**18**	**20,2**

The results in Table-3 reveal that the remediation rate of Bluggoe plants is 73.3%; 27.5% of Litete and 6.6% for Libanga Likale.

Fig-2: Healthy seedlings in micro and macro-propagation

DISCUSSION

This chapter relates to the discussion of the main results obtained according to the applied methodology. It revolves around the serological status of banana cultivars before *In vitro* culture and macro-propagation, and the TAS ELISA test on plants obtained after *In vitro* culture and in macro-propagation.

TAS ELISA test on plants obtained after *In vitro* culture and in macro-propagation

The results on the TAS ELISA test of the plants obtained after the *In vitro* culture and the macro-propagation (Tables 2 and 3) respectively show the rate of purification of the viranized banana plants studied. *In vitro* culture was used to remediate 72.2% of the studied cultivar plants while the macro-propagation cleaned up 20.2% of the cultivar plants studied.

However, sanitation in 1952 by Morel and Martin who, by taking meristematic points viral dalhias to reproduce dalhias genetically similar to parents, but free of viruses, have managed to eliminate the mosaic of dalhias and the spottedwilt virus.

The [17] in Gembloux, looking for "resistant" cells in the "green islands" of Chinese cabbage leaves infected with yellow turnip mosaic, obtained regenerations, some of which, 3 out of 18 plants, revealed healthy, but unfortunately not resistant. Sweet, J. B *et al*., [18] obtained a high level of purification for the "Nepo" viruses (RRV = Raspberry ringspot, AMV = Arabis mosaic virus) by coupling thermotherapy and meristem culture, whereas meristem culture alone is sufficient to eliminate cucumber mosaic (CMV).

Mosella, L. C. H *et al*., [19] obtains 57% removal of N.R.S.V (Sharka necrotic ringspot virus) and 72% for Sharka with 0.4-0.8mm explants. Moreover, it is still using the meristem culture that Wang and Hu in 1980, report the elimination of more than 70 known viruses in more than 40 different species [20].

Boxus, P. H [21] On *In vitro* woody cultivation at the service of the phytopathologist has shown that the rate of sanitation is generally a function of the size of the sample. Navarro and his team obtained 100% remediation when the meristems collected consisted of only two foliar blanks (0.1-0.15mm).

Comparing our results with those of [22] who found 37.9% of seedlings regenerated from proliferating meristems tested negative for ELISA; we note that the *In vitro* culture technique gave the higher percentage while the macro-propagation gave a slightly lower percentage. Indeed, in the case of our study, by comparing the overall rate of sanitation obtained by the *In vitro* culture and the macro-propagation, it follows that the sanitation rate is high in the first technique than in the second one. . Indeed, in the *In vitro* culture, small explants are used, whereas in the macro-propagation, large explants are used. However, in sanitation, the larger the size of the explant, the lower the sanitation rate. Conversely, the smaller the explant size, the higher the sanitation rate.

CONCLUSION

The comparative study of the rate of purification of banana plants virosed by BBTV by *In vitro* culture and by macro-propagation is the subject of this research. This sanitation has used the technique of *In vitro* culture and that of macro-propagation. To do this, we collected the banana samples specifically the cultivars Libanga Likale, Litete and Bluggoe. These samples were harvested in Kisangani City and

surrounding areas on plants with BBTD severity levels 4 and 5. Plants with positive TAS-ELISA samples were harvested and cultured. The seedlings obtained from these cultures were tested again by the TAS-ELISA test to confirm their sanitation rates.

The results obtained showed that prior to *In vitro* culture and macro-propagation, the TAS-ELISA enzyme immunoassay revealed that all Banana plants with Banana Bunchy Top Disease (BBTD) levels 4 and 5 were positive on the test.

All these results show that both techniques can be used not only in the multiplication of banana planting material, but also in the sanitation of their plants infected with BBTV. It should be noted, however, that *In vitro* breeding and sanitation is the best technique because it has improved to 72.2%, while the macro-propagation has only cleaned up 20.2%.

ACKNOWLEDGMENTS

We sincerely thank the VLIR-UOS Project (Belgium), the VLIR-UOS-CIFOR-EU Partnership and Bioversity International for Technical Capacity Building of the Laboratory of *In vitro* Culture of the Faculty of Science of Kisangani University, which allowed the realization of this work.

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