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Sanitation of *Musa* AAA, AAB and ABB Infected By Banana Bunchy Top Virus (BBTV) By *In vitro* Culture, Kisangani, DR congo

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Abstract

The *in vitro* culture of meristeme according to several researchers is regarded as a means of cleansing the seedlings virus diseases. This work of tests of cleansing of banana by *in vitro* culture concerned three cultivars of banana: Bluggoe (*Musa* ABB), Prata (*Musa* AAB), Yangambi Km 5 (*Musa* AAA) infected by Banana Bunchy Top Virus (BBTV). Before *in vitro* culture, the immuno enzymologic test TAS-ELISA (Triple Antibody Sandwish Enzym-Linked Immuno Sorbing Assay) revealed that the totality of the seedlings of banana having 4 and 5 levels of Banana Bunchy Top Disease (BBTD) symptom were positive with the test. The setting in culture and three successive subcultures of the meristemes gave a median number of moves by going tube from 1.8 to 25.7 buds for Bluggoe, 1.5 to 26.8 buds for Prata and 1.7 to 28.5 buds per tube for Yangambi Km 5. These results prove that *in vitro* culture allows obtaining a large number of plants at a reduced time. After *in vitro* culture, the plants of banana resulting from this culture are cleansed with a percentage of 65.22 % for the cultivar Yangambi Km5 followed by Bluggoe with 71.40 % and Prata with 72.72 %. The *in vitro* culture thus remains a suitable technique for the cleansing of banana virus disease and can allow obtaining a large number of bananas without virus.

Keywords: BBTV, *in vitro* tissue culture, plantains.

INTRODUCTION

The Banana Bunchy Top Disease (BBTD) is one of the most devastating diseases in banana and plantain, sometimes causing 100 % yield losses [1]. About 20 virus species belonging to five families have been reported to infect banana and plantain worldwide [2]. The most economically important viruses of banana are Banana bunchy top virus (BBTV, genus Babuvirus, family Nanoviridae), several species of Banana streak viruses (BSVs, genus Badnavirus, family Caulimoviridae) and Banana bract mosaic virus (BBrMV, genus Potyvirus, family Potyviridae). Of minor significance are Abaca bunchy top virus (ABTV, genus Babuvirus), abaca mosaic disease caused by a distinct strain of Sugarcane mosaic virus (SCMV) designated as SCMV-Ab (genus Potyvirus), Banana mild mosaic virus, and Banana virus X (BVX) both unassigned members in the family Betaflexiviridae, and Cucumber mosaic virus (CMV, genus Cucumovirus, family Bromoviridae).

Banana is the fourth largest agricultural product in terms of world production after rice, wheat and maize. It ranks first in fruit production, with just over 145 million tonnes produced in 2011 worldwide [3].

In sub-Saharan Africa, BBTV was first reported in the Democratic Republic of Congo (DR Congo) in the 1950s [2]. It has spread throughout the country [4]. Recently, 16 BBTV isolates from the former Orientale and South Kivu provinces (North-east and central DR Congo) were compared as part of a global distribution study of BBTV, revealing a large human contribution to its dispersal over long distances [5]. In DR Congo, BBTV is present in all its 11 old provinces [2, 4, 6].

In the Democratic Republic of Congo (DRC), plantain is a staple food for people in the north-east and central parts of the country. About 70% of the banana production is consumed directly by local producers, rural, 30% represents the commercial part and the set of

losses recorded in packaging of the products after its harvest [7].

However, heavy parasitic threats currently weigh on the production of this crop. Among these threats, fungal diseases, bacterial diseases, nematodes, viral diseases and insects are reported in banana growing areas [8] and have a considerable impact on production. To this end, it is reported that viral diseases occupy the first place in the drastic fall in yields of banana cultivation.

Farmers collect suckers from infected symptomless plants to establish new fields thereby spreading further the disease and encountering heavy yield losses. There is thus a clear need to provide farmers with virus-free planting material.

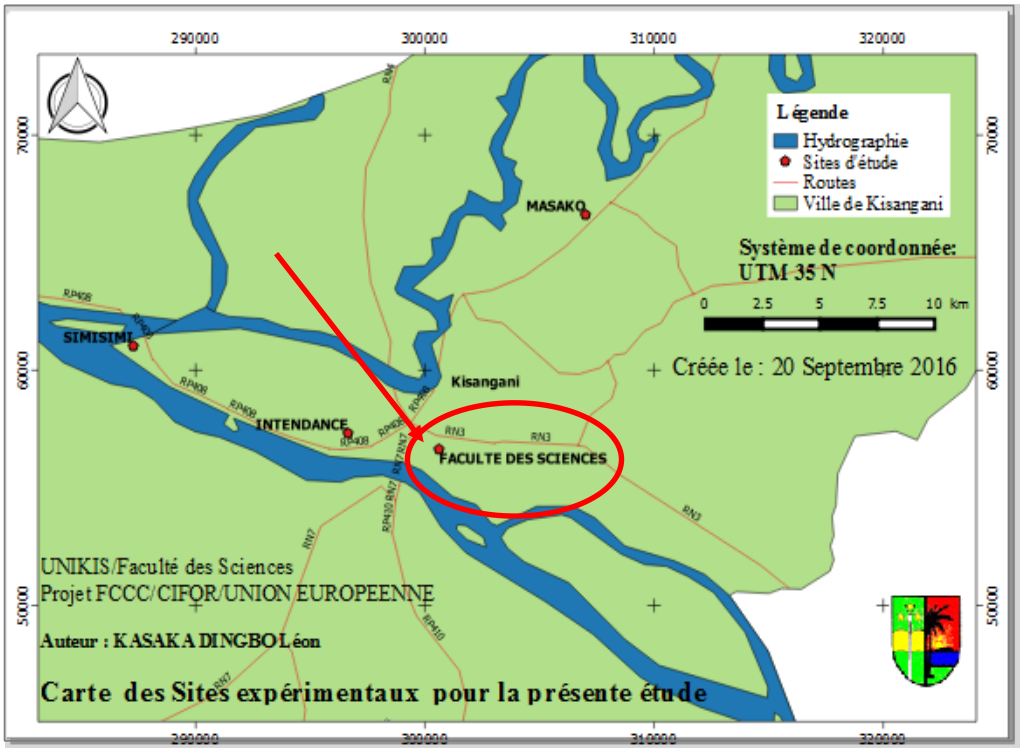

Fig-1: Map of Kisangani City showing the location of experimental sites

Plant Material

The plant material used in this study of BBTV-infected banana sanitation trials by *in vitro* culture consisted of banana plants belonging to the following cultivars: Bluggoe (*Musa* ABB), Prata (*Musa* AAB) and Yangambi km 5 (*Musa* AAA).

Samples were harvested in Kisangani City and surrounding areas. Sampling for the TAS-ELISA was done on the first leaves after the cigar of the plants that showed the BBTD symptoms of a level 4 and 5 severity. Only the bulbs of the plants that revealed BBTV seropositivity were collected and cultured *in vitro* in the Genetics, Plant Breeding and Biotechnology Laboratory of the Faculty of Science, University of Kisangani for possible remediation. The banana plants from this culture are tested by the TAS-ELISA to imbibe their health status.

The Excel program made it possible to analyze the results obtained during the *in vitro* culture. The Anova test evaluated the difference in the evolution of bud numbers between three banana cultivars that were the subject of this study, and the Tukey test was used to check whether the difference between these two banana varieties was significant.

RESULTS AND DISCUSSION

This chapter essentially presents the results and discussions of TAS ELISA immunoenzymological test before and after *in vitro* culture of samples of three banana cultivars Bluggoe (*Musa* ABB), Prata (*Musa* AAB) and Yangambi Km 5 (*Musa* AAA). is the subject of this study. This chapter also gives the evolution of number of buds for each cultivar during three subcultures.

Serological status of banana cultivars before *in vitro* culture

The serological status of the samples of each variety is given in Table 1 below. These samples had symptom levels 4 and 5 of BBTD disease.

Table-1: Serological status of banana cultivars before *in vitro* culture

Cultivars	Genotypes	Number of samples	TAS ELISA results	
			Positives	Negatives
Bluggoe	ABB	10	10	0
Prata	AAB	10	10	0
Yangambi Km 5	AAA	10	10	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.

Evolution of cultivars according to different subcultures

Figure-2 below shows the evolution of many of the buds for each cultivar in three successive subcultures.

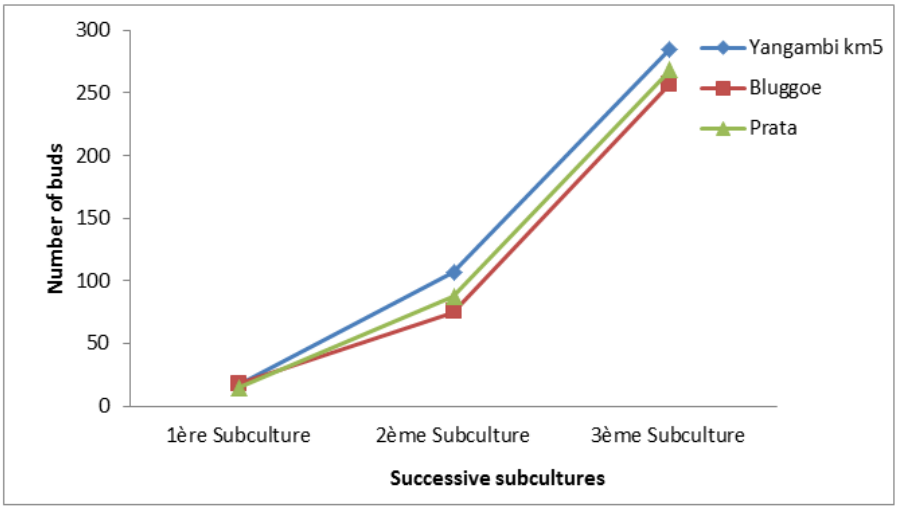

Fig-2: Evolution of cultivars according to different subcultures

It appears from Figure-2 that the number of bud increases with the number of subcultures, with Yangambi Km 5 followed by Prata and Bluggoe. We believe that this difference in the evolution of many of the buds during the different subcultures is due to the ability of each cultivar to proliferate.

Our results corroborate those of [9], who in his study on the morphological characterization and *in vitro* multiplication rate of three banana cultivars (*Musa* AAB) showed that the total number of buds varies according to number of subcultures and type of cultivar.

Table-2: Serological status of different banana plantain cultivars by TAS ELISA after proliferation and regeneration

Cultivars	Plants tested	Plants positives	Plants negatives	Remediation rate (%)
Bluggoe	7	2	5	71,43
Prata	11	3	8	72,72
Yangambi Km 5	23	8	15	65.22

Table-2 shows that the *in vitro* culture of virulent banana plants has cleaned the latter at a rate of 65.22% for the cultivar Yangambi Km 5 followed by Bluggoe with 71.43% and Prata with 72, 72% of remediation.

The [10] study of the banana plant (Libanga likale, Litete) viral assay of BBTV by *in vitro* culture shows that after *in vitro* culture (proliferation of meristematic buds and regeneration), On the 100% analyzed samples, 75% of Litetese cultivars and 66.7% of Libanga likale were found to be free from the virus. On the other hand, the 25% of Litete cultivar and 33.3% of Libanga likale cultivars showed the persistence of the virus by always being positive with the TAS ELISA.

Remediation in 1952 by [11] who, by taking meristematic points of viral dalhias in order to reproduce dalhias genetically similar to parents, but free of viruses, have succeeded in this way in eliminating mosaic of dalhias and spottedwilt virus. And later still using the meristem culture, [12] in 1980, report the elimination of viruses in more than 70 known virus plants in more than 40 different species [13].

CONCLUSION

The objective of this study is to clean the banana trees infected by one of the most devastating viruses of this crop and which is at the base of the fall of its production. This remediation used the *in vitro* culture technique of apical meristem of banana under controlled conditions on a base medium of Murashige and Skoog.

To do this, we collected the banana samples precisely the following cultivars: Bluggoe (*Musa* ABB), Prata (*Musa* AAB), Yangambi Km 5 (*Musa* AAA). These samples were collected in Kisangani town and surrounding area on the plants showing the symptoms of level four (reduced size of discolored leaves) and level five (bushy appearance at the top Bunchy Top) of the disease through the scale of listing of BBTD. Plants with ELISA positive samples were harvested and cultured. The seedlings obtained from this crop were tested again to confirm their sanitation. The evolution of many of the buds during three subcultures was followed for each bulb.

The results obtained showed that:

Prior to *in vitro* culture, the enzyme immunoassay TAS-ELISA (Triple Antibody Sandwich Enzyme-Linked Immuno Sorbent Assay) revealed that all banana plants with Banana Bunchy Top Disease (BBTD) levels 4 and 5 were test positive.

Cultivation and the three successive subcultures of meristems yielded an average number of buds per tube ranging from 1.8 to 25.7 buds for Bluggoe, 1.5 to 26.8 buds for Prata and 1.7 to 28 buds. 5 buds per tube for Yangambi Km5.

After *in vitro* culture, the banana seedlings from this crop are sanitized to 65.22% for the Yangambi Km5 cultivar followed by Bluggoe with 71.4% and Prata with 72.72%. *In vitro* culture therefore remains an appropriate technique for the sanitation of virulent banana and can allow to obtain a large number of banana without viruses.

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