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Screening for Black Sigatoka Disease of Banana (Musa Spp.) in Togo, West Africa

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Abstract

Black leaf streak disease (BLSD) or Black sigatoka disease of banana is very destructive and reduces yield. Most of the studies done on its pathogen *Mycosphaerella fijiensis* are done in the field and often influenced by natural environmental conditions. The objective of this study is to select cultivars under controlled conditions in the greenhouse. Artificial inoculation is done with a concentration of 15mg/ml of the mycelium inoculum of the pathogen and the propagator was used as a greenhouse in this experiment. The selection is made on local varieties of economic interest of dessert bananas (Adokpa, Fokona, Kaveguê, Tsikodu, Dankodu), plantains (Agbavé, Kadaga, Apim, Savé) and partially resistant controls from IITA to namely FHIA-01 and Yangambi Km 5. The inoculum is applied to the dorsal surface of the first three open leaves of each plant of each variety. The plants are evaluated at 30 days after Inoculation (DAI) and at 60 DAI. The control variety FHIA-01 is resistant, the local variety Savé is susceptible, the control variety Yangambi Km 5 and the other local varieties are partially resistant. The 15mg/ml inoculum of the mycelium and the propagator used as a greenhouse are very useful for the selection of varieties under controlled conditions. The susceptible varity Savé can be improved by stimulating its natural defense by elicitor.

Keywords

Black Sigatoka, *Mycosphaerella fijiensis,* Banana, Plantain, Togo

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1. Introduction

Black Sigatoka is a disease of banana (Musa Spp.) caused by the pathogen *Mycosphaerella fijensis* (M. Morelet) is the most costly and destructive leaf spot disease of banana affecting all cultivars leaves belonging to the AAA genotype (case of dessert bananas) and the AAB genotype (case of plantains) [1–3]. Since it was first reported in 1963 in Fiji Island in the southern Pacific, black leaf streak disease has spread to different banana growing areas [4]. West Africa and particularly Togo is part of the black leaf streak disease belt [5]. Black leaf streak disease (BLSD) of bananas is a major constraint in humid tropics where bananas are a staple food crop [6 –9]. It attacks the leaves and causes a reduction in the photosynthetic activity of the plant; leading to lower yields. This disease would induce yield losses of 76% and reduction in fruit quality, especially during the second production cycle [1, 8, 10–16]. Studies carried out in the field to reduce crop losses due to this banana disease are carried out in West Africa and more in Ivory Coast [15, 17–20]

Most of the selections of bananas for the response of the fungus *Mycosphaerella fijiensis* are made in the fields and are often influenced by natural growing conditions. This selection is very complicated despite the advances in *in vitro*

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culture and macropropagation techniques which produce many plants for field essays. To resolve this problem, it is important to make the selection in the greenhouse in order to better control the environmental conditions of the crops during the selection. In Togo, the disease was discovered in 1990, but no study on variety selection has been made, hence the need to carry out this study by inoculations in the greenhouse of varieties of economic interest, in particular dessert banana varieties (Adokpa, Fokona, Kaveguê, Tsikodu, Dankodu) and plantains (Agbavé, Kadaga, Apim, Savé). The general objective of this study is to improve the yield of local varieties of dessert bananas and plantains. Its specific objective is to artificially inoculate local varieties in the greenhouse with the inoculum of 15mg/ml of *Mycosphaerella fijiensis*, then evaluate at 30 days and 60 days after inoculation (DAI), the inoculated varieties; then classify the varieties tested into susceptible, partially resistant and resistant varieties.

2. Material and Methods

2.1. Plant Material

Local varieties of dessert bananas (Adokpa, Fokona, Kaveguê, Tsikodu, Dankodu) and plantains (Agbavé, Kadaga, Apim, Savé) were used for the experiment (Figure 1). The young plants used were produced according to the PIF technique [21]. The dessert banana varieties (FHIA-01 and Yangambi Km 5) which were used as partially resistant control varieties [8, 22] come from the International Institute of Tropical Agriculture (IITA) and produced by the *in vitro* culture technique. The fungus *Mycosphaerella fijiensis* was used to prepare 15mg/ml inoculum according to Kumakech and collaborators [23] (Figure 2).

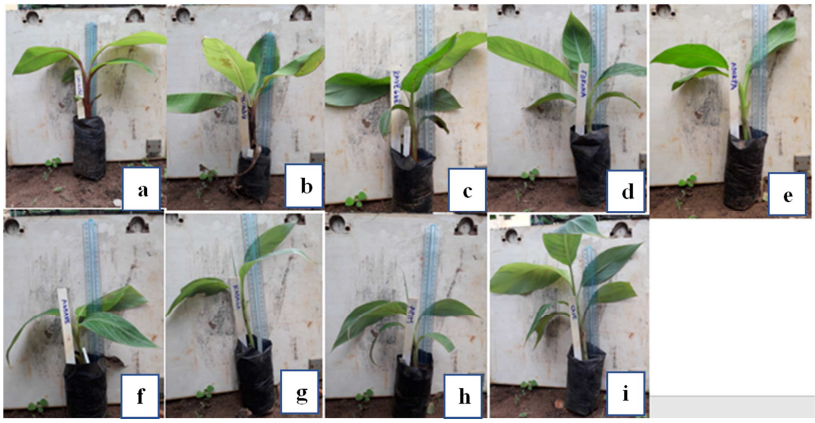

Figure 1. Young plants of local cultivars of dessert bananas aged four months (a-Dankodu, b-Tsikodu, c-Kaveguê, d-Fokona, e-Adokpa); young plants of local cultivars of plantains four months old (f-Agbavé, g-Kadaga, h-Apim, i-Savé/

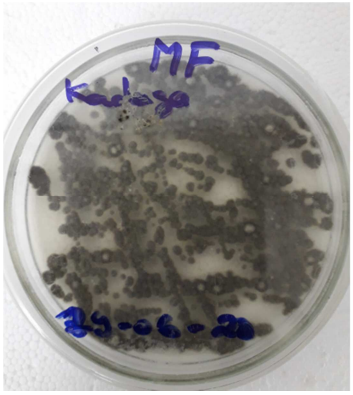

Figure 2. *Mycosphaerella fijiensis* strain used for the experiment named KADAGA CRAL MF.

2.2. Technical Material

Distilled water, beakers, test tubes, balance and stirrer were used in the preparation of the inoculum. The painter's brush was used to inoculate the young plants of local dessert bananas and local plantains as well as the controls.

2.3. Methods

Four (4) months old plants was used for the experiment, five (5) local varieties of dessert bananas (Adokpa, Fokona, Kaveguê, Tsikodu, Dankodu) and four (4) local varieties of plantains (Agbavé, Kadaga, Apim, Savé), as well as the partially resistant control varieties, four (4) months old also were inoculated with the inoculum (15mg/ml) of the pathogen of black leaf streak disease (*Mycosphaerella fijiensis*) in the greenhouse of the former site of the Agronomic Research Center of Littoral (CRAL) of the Togolese Institute for Agronomic Research (ITRA) in Lomé-Cacaveli. The strain of the fungus *Mycosphaerella fijiensis* used was named KADAGA CRAL MF because it comes from the Kadaga variety cultivated at the former CRAL site. For artificial greenhouse inoculation, the 15 mg/ml dose of the pathogen *Mycosphaerella fijiensis* inoculum is applied to the dorsal surface of the first three (3) leaves open using a painter's brush. For each variety, three (3) young plants were used for the experiment, and three (3) leaves of each plant were inoculated,

or nine (9) observations per variety. Each plant was one repetition, or three repetitions per variety. The inoculated plants are incubated in a propagator serving as a greenhouse, made of cement, topped with a wooden frame and covered with transparent plastic. The temperature ranged from 28°C to 31°C and the relative humidity inside was maintained by two buckets of water of five liters each; it varied from 98% to 100% (Figure 3). Plant evaluations was done eight (8) weeks after inoculation [23]. A scale of 0 to 5 developed by Fullerton and Olsen [24] was used for the phytopathological assessment (Table 1). For data analysis, data from the three inoculated leaves of each plant is recorded and the average of the values was assigned to each plant; similarly, the average of the values of the three inoculated plants of the same variety was calculated using the R software in order to assign a stage to each variety to classify the genotypes.

Table 1. Scale used to evaluate symptom development and the classification of genotypes according to the stage of symptom development.

Stage	Description
0	Leaf symptoms mostly abscent
1	Reddish flecks on lower leaf surface. No symptoms on the upper surface
2	Regular or irregular reddish circular spots on the lower leaf surface. No symptoms on the upper surface
3	Regular or diffuse light brown circular spots on the upper leaf surface
4	Black or brown circular spots, possibly with a yellow halo or chlorosis of adjacent tissues, on the upper leaf surface Areas of green tissue sometimes present
5	Black spots with dry centre of grey colour. Leaf completely necrotic, sometimes hanging down.
Classification of genotypes according to symptom development Resistant: stages 0-1 Partialy resistant: stage 2-3 Susceptible: stage 4-5 0-5 Stage of black sigatoka disease symptom development as described by Fullerton and Olsen [24] used by Kumakech and collaborators [23]	

Figure 3. A- Inoculation of dessert banana and plantain plants (Musa Spp.); B- Inoculated banana plants, in a propagator serving as a greenhouse. C- Propagator serving as a greenhouse covered with transparent plastic.

3. Results

The results obtained thirty (30) days after inoculation (DAI) and 60 days after inoculation (DAI) with the pathogen *Mycosphaerella fijiensis* at a concentration (15 mg/ml) are shown in the table below (Table 2). The various observations made during the experiment showed the different stages of black leaf streak disease of bananas on the inoculated leaves. Leaves coded 0 do not have black leaf streak disease symptoms. The leaves coded 1 have the symptoms of stage 1, those coded 2 have the symptoms of stages 2; those coded 3 have symptoms of stages 3, those which are coded 4 have symptoms of stages 4 and finally those which are coded 5 have symptoms of stages 5 (Figure 4). The local cultivars of dessert bananas (Adokpa, Fokona, Kaveguê, Tsikodu and Dankodu) and plantains (Agbavé, Kadaga, Apim) are characterized by the presence of stage symptoms 2 to 30 days after inoculation (DAI), against the local plantain Savé presented symptoms of stages 4. The control varieties FHIA-01 on the other hand did not present symptoms at 30 days after inoculation; witness Yangambi Km 5 the second partially control presented stage 1 symptoms. At 60 DAI, the local varieties of dessert bananas and plantains presented stage 3 except the Savé plantain variety which presented stage 5; on the other hand, the control variety FHIA-01 presented stage 1 and the control variety Yangambi Km 5 presented stage 2. According to the classification of Fullerton and Olsen [24]; the local varieties of dessert bananas (Adokpa, Fokona, Kaveguê, Tsikodu and Dankodu) and plantains (Agbavé, Kadaga, Apim) are all classified varieties partially resistant to black leaf streak disease in Togo; on the other hand, the Savé plantain variety is classified as a variety susceptible to black leaf streak disease. Our results show that the control variety FHIA-01 is resistant to black leaf streak disease and the control variety Yangambi Km 5 is partially

resistant (Figure 5). These results can be explained by the fact that the defense reaction against infections was set up early in the local varieties of dessert bananas and plantains except in the Savé variety, which explains its sensitivity characterized by a high stage of symptoms at 30 DAI and at 60 DAI. The partially resistant control variety FHIA-01 according to Mourichon, Carlier and Fouré [25], has been shown to be resistant in our experiment, this can be explained by the strain used or by the fact that its plants were obtained from *in vitro* culture.

Figure 4. Symptom development stages of banana black leaf streak (Musa Spp.) in greenhouse from 0 to 5.

Table 2. Greenhouse reaction of 11 cultivars of bananas (Musa spp.) To artificial inoculation with 15 mg/ml of Mycosphaerella fijiensis inoculum.

Cultivar	Symptom development stage		Greenhouse reaction
	30 DAI	60 DAI	
Agbavé	2	3	Partially resistant
Apim	2	3	Partially resistant
Kadaga	2	3	Partially resistant
Tsikodu	2	3	Partially resistant
Kaveguê	2	3	Partially resistant
Fokona	2	3	Partially resistant
Adokpa	2	3	Partially resistant
Dankodu	2	3	Partially resistant
Savé	4	5	Susceptible
FHIA-01 (Control)	0	1	Resistant
Yangambi Km 5 (Control)	1	2	Partially resistant

DAI: Days After Inoculation. The varieties FHIA-01 and Yangambi Km5 was used as partially resistant control [8, 25]

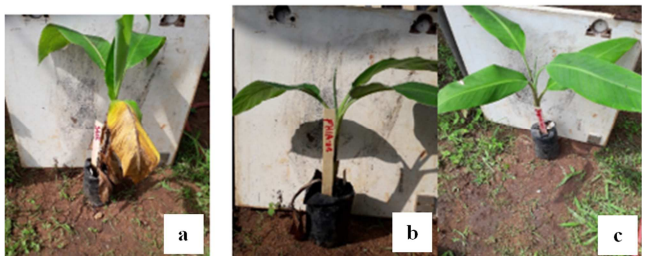

Figure 5. a- Susceptible local variety Savé b- Resistant control variety FHIA-01 c-Partially resistant control variety Yangambi Km 5.

4. Discussion

The mycelium of the one month old fungus *Mycosphaerella fijiensis* was weighed to prepare the inoculum which was used for the greenhouse screening experiment of susceptible, partially resistant and resistant banana varieties. The weighed mycelium was fragmented using the stirrer for about 3 minutes and was used for the preparation of the inoculum useful for artificial inoculation as opposed to the method of quantification by hemacymeter. This mycelium weighing method adopted for inoculation of banana leaves made it possible to confirm the partial resistance of the control variety Yangambi Km 5 and showed that the control FHIA-01 is resistant to the KADAGA CRAL MF strain under our experiment conditions. The development of black streak disease symptoms corresponds well to the descriptions of Fullerton and Olsen [24]. This method enabled us to select the sensitive Togolese variety called Savé which is a plantain; on the other hand, the other varieties of dessert bananas (Adokpa, Fokona, Kaveguê, Tsikodu and Dankodu) and the varieties of plantains (Agbavé, Kadaga, Apim) have been shown to be partially resistant to black leaf streak disease. Our results are similar to those of these authors in that the development of banana leaf streak disease is comparable, which means that the greenhouse conditions are the same.

A significant difference was observed between the Savé variety and the other local varieties four weeks after inoculation. The other varieties of dessert bananas (Adokpa, Fokona, Kaveguê, Tsikodu and Dankodu) and plantains (Agbavé, Kadaga and Apim) have already been shown to be partially resistant like the control variety Yangambi Km 5 which is partially resistant [8] (Table 2). The susceptible Savé variety from Togo is comparable to the susceptible Nfuuka banana variety from the mountains of East Africa as they both have a stage 4 to 30 DAI and stage 5 to 60 DAI [23]. This author with an inoculum of 15mg/ml and on 8 varieties tested, he found 6/8 susceptible varieties, 1/8 partially resistant and 1/8 resistant; these results are contrary to our results which are presented as follows; out of 11 varieties tested, there is 1/11 resistant which is the variety FHIA-01; Sensitive 1/11 which is the Savé variety and the other varieties represented by 9/11 are partially resistant, 8 of which are Togolese varieties. This comparison indicates that there are more susceptible varieties of bananas in East Africa than in West Africa.

The development of symptoms in different varieties depends on the growing conditions [2, 26]. These results are similar to our results.

The 4 month old plants used for our experiment are suitable for greenhouse selection of bananas to the sensitivity of the fungus *Mycosphaerella fijiensis*; this method is similar to that of Gauhl, Pasberg-Gauhl and Jones [27].

5. Conclusion

The 15mg/ml dose of the inoculum of the fungus *Mycosphaerella fijiensis*, as well as the propagator serving as a greenhouse with 10 liters of water to create a high humidity, made it possible to select the local varieties and to classify them into tolerant and sensitive varieties. This method allows the development of black leaf streak disease symptoms in inoculated plants under temperature conditions ranging from 28-31°C and high humidity ranging from 90% to 100%. This approach made it possible to identify the sensitive variety from Togo, which is the Savé plantain, the other local varieties are partially resistant. In the future, to fight against the drop in yield due to black leaf streak disease, producers must be supplied with young dessert banana plants (Adokpa, Fokona, Kaveguê, Tsikodu and Dankodu) and plantains (Agbavé, Kadada and Apim) by the simplified macropropagation technique called the PIF technique. The sensitive Savé variety must be improved by new techniques that respect human health and the environment, such as the stimulation of natural defenses by elicitors.

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