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REACTION OF BANANA GERMPLASM TO INOCULATION WITH *Xanthomonas campestris* pv *musacearum*

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ABSTRACT

Pot trial to evaluate the reaction of Uganda's local banana germplasm to artificial inoculation with *Xanthomonas campestris* pv *musacearum (Xcm)* was established in 2004 and 2005 in Mukono district, Uganda. *X. campestris* pv *musacearumcauses* infection causes premature fruit ripening and leaf wilting, killing every affected plant hence leading to total yield loss. The disease is managed through cultural practices but it was thought that use of resistant varieties would provide a better management option. Potted plants in a farmer's field were therefore inoculated with a bacterial suspension containing 1×10^8 bacterial cells/ mL of Xcm. 1 mL of bacterial suspension was injected into the petiole of the youngest open leaf using a hypodermic needle. Twenty five plants of each of the 42 banana genotypes were involved in the study. Wilt disease developed in the three months old banana plantlets of all local germplasm tested except for *Musa balbsiana*.

Key Words: Genotypes, *Musa* sp., Uganda

RÉSUMÉ

Les essais de pot ont évalués les réactions des germplasmes des variétés locales (Ougandaise) de la banane à l'inoculation artificielle avec *Xanthomonas compestris* p.v *musacearum* étaient établies en 2004 et 2005 dans le district de Mukono en Ouganda. Les infections aux *Xanthomonas compestris* p.v *musacearum* causent un murussement prématuré des fruits et le déclenchement des feuilles, tuant chaque plante affectée et ainsi contribuant à une perte totale du rendement. La maladie est contrôle à travers des pratiques culturales mais il était pensé que l'utilisation des variétés résistantes incitera des bonnes options de gestion. Les plantes dans les pots sur les fermes des paysans étaient alors inoculées avec une bactérie en suspension contenant 1×10^8 cellules des bactéries/mL de Xcm. Un mL de bactérie en suspension était injecté dans un pétiole de la plus jeune feuille en utilisant une aiguille. Vingt cinq plantes de chacun de 42 génotypes de banane étaient considérées dans cette étude. La maladie de alanguissement développée sur une plantule de la banane de trois mois des germplasmes locaux testes a l'exception de *Musa balbsiana*.

Mots Clés: Genotypes, *Musa* sp., Ouganda

INTRODUCTION

Banana (*Musa* sp.) in Uganda has been reported to be affected by a wilt disease caused by the bacterium *Xanthomonas campestris* pv *musacearum* since 2001 (Tushemereirwe *et al.*, 2003, 2004). This bacterium was also first reported causing wilt in a close relative of Banana *Ensete ventricosum* in Ethiopia (Yirgou and Bradbury, 1968). The disease is thought to affect all cultivated banana types in Uganda, but more severely to the ABB types (Psang awak, Bluggoe and Ndiizi). It

leads to total yield losses to effected plants as it causes the fruits to ripen prematurely, the whole plants to wilt and die.

In affected fruited plants, the disease is noted as male buds wilting and drying, premature un-even fruit ripening and leaf wilting. In the non fruited affected plants, leaves wilt starting with the young ones and sometimes may develop scalded patches within the lamina. Yellow bacterial ooze is seen to flow out of the vascular bundles of the affected plants when cut. The affected fruits show rusty brown stains when sliced (Tushemereirwe *et al.*, 2003, 2004), dry rot and are inedible.

The disease and the pathogen are thought to be transmitted from flower to flower by contaminated insects off oozing male buds. Transmission of banana bacterial diseases especially Moko disease caused by *Ralstonia solanacearum biovar 2* via the inflorescences by insects has been reported in Asia. (Buddenhagen, 1962). Xcm is also transmitted on cutting tools in sap (Yirgou and Bradbury, 1974).

Disease management options practiced by farmers include male bud removal by breaking them with a forked stick, sterilisation of cutting tools in fire or diluted Sodium hypochloride and destruction of infected plants. If they are not practiced perfectly, disease development, outbreak and spread can progress. It was thought that resistant varieties would provide a better disease management option. The objective of this study was to screen local germplasm reaction to Xcm infection.

MATERIALS AND METHODS

A pot trial was set up in a farmer's field in Mukono district of Uganda late 2004 through early 2005. Mukono is a disease hotspot and was the first district where the disease was reported. Mukono is located in the central part of Uganda on a high plateau, 1060-1220m above sea level within 0° 30'N-1° 00'N and 32° 30'E-33° 00'E. It is a warm (average temp 25 °C and max temp 29° C) humid area that receives over 1500mm rainfall per annum in two seasons (March-May and September-November).

Thirty plants of each of the 42 genotypes were introduced as corms into pots containing sterile soil. They were allowed to establish for 3 months before inoculation. Twenty five plants (5 plants by 5 replicates) were inoculated with *Xcm* while five plants (1 plant by 5 replicates) were inoculated with sterile water. The test germplasm included 5 natural diploids (Psang lilin, *M. balbsiana*, Cultivar rose, Calcutta4 and kisubi), 15 natural triploids (Kibuzi, Mpologoma, Nfuuka, Kabuchuragye, Nante, Mbwazirume, Kisansa, Yangambi KM5, Kabula, Psangawak, Plantain, Ndiizi, Kikundi, Bluggoe, Psang cylan), 10 hybrid diploids (TMB2X8075-7, TMB2X6142, TMB2X7197-2, TMB2X5105, TMB2X9128, SH3142, SH3217, OPP861, SH3362 and SH3217,) and 12 hybrid tetraploids (FHIA 02, FHIA 01, 660K-1, 246K, 466K-1, 466K-3, 1201K-1, FHIA 03, FHIA 23, FHIA 17, Cultivar force, FHIA 25).

Flesh culture cells were harvested into sterile distilled water and adjusted to 1×10^8cfus/mL by dilution to a visibly cloudy suspension. The plants were inoculated by drawing 1 mL of bacterial suspension into a hypodermic syringe and needle then injected into the petiole of the youngest open leaf. The inoculated site was covered with transparent polythene for 12 hours to maintain humid conditions.

Plants incubated under field conditions and were monitored for 8 weeks. Weekly observations were made and data was recorded on disease incubation on each plant of each cultivar. Disease severity was also assessed on individual plants using the following scale (Winstead and Kelman 1952): 0; no symptoms, 1; 1 inoculated leaf wilted, 2; 2-3 leaves wilted, 3; = 4 leaves wilted, 4; all leaves wilted and 5; plant dead. Each plant was run on this severity scale and disease index for each genotype calculated as:

$$\text{Disease index} = [(0xa + 1xb + 2xc + 3xd + 4xe + 5xf)/(nx5)]\,100;$$

Where n is the total number of plants for a given genotype; 0, 1 ..... 5 is disease severity scale and a, b .... f are the respective number of plants in each severity scale for the given genotype.

Since plants under control treatment did not wilt, the disease index means for the various genotypes were analysed on one way ANOVA using General linear model procedure of SAS (SAS Institute Inc., 1997) after angular transformation (arc sine transformation) using the formula below. Transformed disease index =

$100 \times \sin^{-1}\sqrt{[(a+0.5)/100 \times 22/28]}$; Where 'a' is a given disease index. The disease incubation means separation was compared using Fishers' Protected least significant Different test.

RESULTS

Two weeks after inoculation, some of the plants started showing leaf wilting symptoms. Symptoms were first noted on the inoculated leaves. At least one plant from each of the tested genotypes exhibited leaf wilting and yellow/ brown internal vascular streaking. *Xcm* was recovered from these wilted test plants except in *Musa balbisiana*. Most of the plants that developed symptoms eventually died. Most plants died within the 5th week after inoculation. The genotypes generally showed variability in disease incubation period and in overall disease indices as assessed eight weeks after inoculation. Generally the most severely affected genotypes also had a shorter disease incubation period (Table 1).

TABLE 1. Relative severity of banana bacterial wilt in various banana genotypes, 8 weeks after inoculation with Xcm

Genotypes	Mean disease incubation (weeks)	Arc sine transformed mean disease indices	Bacterial recovery
M. balbsiana	8 ± 0.1a	5.6 ± 0.00d	No recovery
TMB2X 6142	5 ± 0.7b	73.6 ± 7.98c	Recovered
Psang cylan	5 ± 0.6bc	65.7 ± 10.23c	Recovered
FHIA 25	4 ± 0.4bcd	89.4 ± 1.25ab	Recovered
FHIA 02	4 ± 0.4cd	83.2 ± 3.49ab	Recovered
TMB2X 9128	4 ± 0.7cde	82.9 ± 7.77ab	Recovered
FHIA 01	4 ± 0.3def	88.2 ± 2.49ab	Recovered
FHIA 03	3 ± 0.2efg	90.7 ± 0.00a	Recovered
OPP861	3 ± 0.4e-h	86.5 ± 4.14ab	Recovered
FHIA 17	3 ± 0.2e-h	90.7 ± 0.00a	Recovered
Kisansa	3 ± 0.3e-h	90.7 ± 0.00a	Recovered
246K	3 ± 0.1e-h	90.7 ± 0.00a	Recovered
kikundi	3 ± 0.1e-h	90.7 ± 0.00a	Recovered
466K-3	3 ± 0.2e-i	88.2 ± 2.49ab	Recovered
Nfuuka	3 ± 0.4f-j	88.2 ± 2.49ab	Recovered
Calcutta4	3 ± 0.2f-j	90.7 ± 0.00a	Recovered
kisubi	3 ± 0.2f-j	90.7 ± 0.00a	Recovered
TMB2X 5105	3 ± 0.4f-j	88.2 ± 2.49ab	Recovered
Plantain	3 ± 0.2g-j	90.7 ± 0.00a	Recovered
Ndiizi	3 ± 0.1g-j	90.7 ± 0.00a	Recovered
TMB2X 7197-2	3 ± 0.3g-j	90.7 ± 0.00a	Recovered
Cultivar force	3 ± 0.4g-j	81.9 ± 3.60b	Recovered
1201K-1	3 ± 0.2g-j	90.7 ± 0.00a	Recovered
466K-1	3 ± 0.1g-j	90.7 ± 0.00a	Recovered
660K-1	3 ± 0.3g-j	90.7 ± 0.00a	Recovered
Yangambi KM5	3 ± 0.2g-j	90.7 ± 0.00a	Recovered
TMB2X 075-7	3 ± 0.2g-j	90.7 ± 0.00a	Recovered
Kabula	3 ± 0.1g-j	90.7 ± 0.00a	Recovered
Mbwazirume	3 ± 0.1g-j	90.7 ± 0.00a	Recovered
Mpologoma	3 ± 0.1g-j	90.7 ± 0.00a	Recovered
Psang lilin	3 ± 0.2 g-j	88.2 ± 2.49ab	Recovered
Bluggoe	3 ± 0.1 g-j	90.7 ± 0.00a	Recovered
SH 3217	3 ± 0.2 g-j	90.7 ± 0.00a	Recovered
SH 3142	3 ± 0.2 g-j	90.7 ± 0.00a	Recovered
SH 3362	3 ± 0.3 g-j	90.7 ± 0.00a	Recovered
SH 3217	2 ± 0.1 g-j	90.7 ± 0.00a	Recovered
FHIA 23	2 ± 0.1 g-j	90.7 ± 0.00a	Recovered
Kibuzi	2 ± 0.1hij	90.7 ± 0.00a	Recovered
Nante	2 ± 0.1ij	90.7 ± 0.00a	Recovered
Cultivar rose	2 ± 0.1j	90.7 ± 0.00a	Recovered
Kabuchuragye	3 ± 0.1j	90.7 ± 0.00a	Recovered
Psang awak	2 ± 0.1j	90.7 ± 0.00a	Recovered

Means in columns with the same letter are not significantly different ($P \leq 0.05$) by LSD

Disease did not successfully develop in plants of one genotype *M. balbisiana* despite development of leaf wilt-like symptoms and internal vascular streaking within the 8th week. In some genotypes (TMB2X 6142, Psang cylan, FHIA 25, FHIA 02, TMB2X 9128 and FHIA 01) disease incubated longer (4-5 weeks) and their overall disease indices after 8 weeks varied from 65-90%. Most genotypes(FHIA 03, OPP861, FHIA 17, Kisansa, 246K, kikundi, 466K-3, Nfuuka, Calcutta-4, kisubi, TMB2X 5105, Plantain, Ndiizi, TMB2X 7197-2, Cultivar force, 1201K-1, 466K-1, 660K-1, Yangambi KM5, TMB2X 8075-7, Kabula, Mbwazirume, Mpologoma, Psang lilin, Bluggoe, SH 3217, SH 3142 and SH 3362) showed symptoms within 3 weeks after inoculation and their disease indices after 8 weeks varied from 88 -100 %. Other genotypes (SH 3217, FHIA 23, Kibuzi, Nante, Cultivar rose, Kabuchuragye and Psang awak) showed symptoms quite early (within 2 weeks after inoculation) and their disease index after 8 weeks was 100%.

DISCUSSION

Generally all the tested 10 diploid hybrids, 12 tetraploid hybrids, 15 natural triploids and 4 out of 5 Natural diploids showed susceptible reaction to *Xanthomonas campestris* pv *musacearum*. On the other hand the natural wild type, *Musa balbsiana* (Plate 1) showed some resistance reaction.

Genotypes with low disease incubation period (2- 4weeks) and high disease index (75-100%) were considered highly susceptible to the pathogen under this method of inoculation. These include; SH 3217, FHIA 23, Kibuzi, Nante, Cultivar rose, Kabuchuragye , Psang awak, FHIA 03, OPP861, FHIA 17, Kisansa, 246K, kikundi, 466K-3, Nfuuka, Calcutta4, kisubi, TMB2X 5105, Plantain, Ndiizi, TMB2X 7197-2, Cultivar force, 1201K-1, 466K-1, 660K-1, Yangambi KM5, TMB2X 8075-7, Kabula, Mbwazirume, Mpologoma, Psang lilin, Bluggoe, SH 3217, SH 3142, SH 3362, FHIA 25, FHIA 02, TMB2X 9128 and FHIA 01. Those genotypes with high disease incubation period (5 weeks) and low disease index (1 - 74%) were considered partially susceptible. These include; TMB2X 6142 and Psang cylan. Those genotypes that did not successfully develop disease are considered resistant. These only include; *M. balbsiana*.

All the natural East Africa highland banana genotypes included here (Nfuuka, Nante, Kisansa, Mbwazirume, kabula, Mpologoma,

Plate 1. Surviving *M balbsiana* plants (a) among other dead genotypes (b) and (c), at 8 weeks after inoculation with Xcm.

Kabuchuragye and Kibuzi) are highly susceptible. They have been drawn as representatives from each of the clone sets as categorised by Karamura (1998). This suggests that genotypes within these clone sets that have not been included in this study are not likely to react any differently. All the hybrids of East African highland bananas used in this study (1201K-1, 246K, 466K-3, 466K-1and 660K-1) are also highly susceptible. Plants from these genotypes (natural East African highland bananas and their hybrids) developed symptoms within 2-3 weeks after inoculation and all the affected plants completely died (Table 1). Field observations have always indicated that natural infection is less encountered in the natural East African highland banana genotypes as compared to the natural ABBs (Bluggoe, Psang awak and Ndiizi) (Eden-Green, 2004). The results in table 1 however indicate similar disease severity amongst natural ABBs and the natural East African highland bananas. The observed field differences amongst these genotypes are likely to be due to other mechanisms of resistance/ disease escape which need to be explored.

The FHIA hybrids included in this study (FHIA 25, FHIA 02, FHIA 01, FHIA 23, FHIA 03 and FHIA 17) are all highly susceptible to Xcm under artificial inoculation.

According to Agrios (1997), disease development is affected by the age of the host plant. This may suggest the reactions reported in this study apply to 3 months old plants. It is not known if the same genotypes when studied at advanced development stages will react the same way.

In conclusion, resistance to *Xcm* is very limited within the banana germplasm locally available within Uganda. This preliminary study suggests its existence in a wild type, *Musa balbsiana* but is yet to be utilized. It is recommended that all the genotypes including *Musa balbsiana* should be evaluated at their advanced stage of development. These genotypes should be subjected to natural infection to find their reaction. Foreign germplasm should also be obtained and screened for resistance to Xcm.

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