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Full Length Research Paper

Relative susceptibility of banana cultivars to *Xanthomonas campestris* pv. *musacearum*

L. Tripathi* and J. N. Tripathi

International Institute of Tropical Agriculture, P. O. Box 7878, Kampala, Uganda.

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The banana Xanthomonas wilt (BXW) is the most devastating disease of banana in Great Lakes region of East and Central Africa. The pathogen kills plants quickly and spreads rapidly over a large area in a short time making the disease one of the most dreaded in banana. The disease affects almost all varieties of commonly grown banana cultivars. Some knowledge of the relative susceptibility of banana cultivars would be extremely useful and could be a basis for management strategies for BXW. Ten banana cultivars were evaluated for their relative susceptibility to *Xanthomonas campestris* pv. *musacearum*. All the ten cultivars were tested by injecting bacterial inoculum in pseudostem of *in vitro* plantlets as well as potted plants. The various banana cultivars showed significant differences in susceptibility to *Xanthomonas campestris* pv. *musacearum*. The beer banana cultivar 'Pisang Awak' and dessert cultivars 'Dwarf Cavendish', 'Giant Cavendish', and 'FHIA-17' were found to be highly susceptible, where as East African Highland banana cultivar 'Nakitembe' was found to be least susceptible. The other cultivars 'Mpologoma', 'Mbwazirume', 'Sukali Ndiizi' and 'FHIA-25', were found to be susceptible. Diploid parent '*Musa balbisiana*' was found to be resistant. This study clearly highlights the need for development of new resistant cultivars for BXW disease control, as all the commercial cultivars are susceptible.

Key words: Banana *Xanthomonas* wilt, artificial inoculation, Pisang Awak, Nakitembe, Cavendish.

INTRODUCTION

The banana Xanthomonas wilt (BXW) disease caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (Tushemereirwe et al., 2004) endangers the livelihood of millions of farmers in the Great Lakes region of Eastern and Central Africa. The disease was first reported about 40 years ago in Ethiopia on *Ensete*, which is closely related to banana (Yirgou et al., 1968). Outside Ethiopia, BXW was first identified in Uganda in 2001 and now has spread in epiphytotic proportions to almost all major banana producing districts of the country (Tripathi et al., 2009). The disease has also been reported in Democratic Republic of Congo (Ndungo et al., 2006), Rwanda (Reeder et al., 2007), Kenya, Tanzania and Burundi (Carter et al., 2009). Economic impact of the disease is manifested as result of absolute yield loss or reduced bunch weights, and death of the mother plant and suckers that help in subsequent ratoon plant production cycles. Infected fields cannot be replanted with banana for at least 6 - 8 months due to soil borne inoculum of the pathogen.

Affected banana plants develop symptoms characterized by a progressive yellowing and wilting of leaves, uneven and premature ripening of fruit with sections showing unique yellowish blotches in the pulp and dark brown placental scars (Tripathi et al., 2009; Figure 1). Symptoms on floral parts include wilting of bracts, shriveling and rotting of the male buds, and yellow-brown flower stalks (Figure 1). Cross sections of diseased pseudostems reveal yellowish bacterial ooze (Figure 1). Yellow or brown streaks occur in the vascular tissues of infected plants. Eventually, infected plants wither and the plant rots. BXW has many similarities to bacterial wilts of banana in other parts of the world (Moko, blood and bugtok diseases) that are caused by *Ralstonia* and closely related organisms (Thwaites et al., 2000). Once these pathogens have become established, disease control is very difficult and eradication impossible (Eden-Green, 2004).

*Corresponding author. E-mail: l.tripathi@cgiar.org. Tel: 256-414-285060. Fax: 256-414-285079.

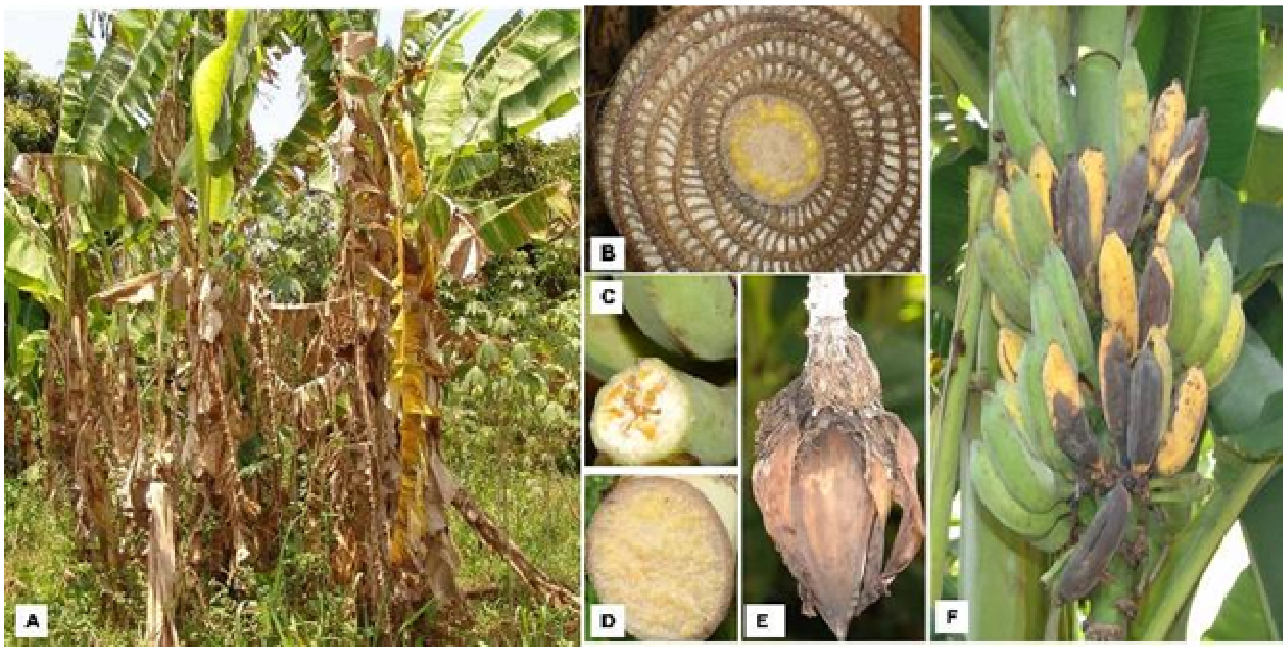

Figure 1. Symptoms of Xanthomonas wilt disease; A: Banana plantation damaged by wilt, B: Yellow ooze from cut pseudostem, C: Yellowish blotches and ooze in the pulp of fruits, D: Yellow ooze from rachis, E: Wilting and shriveling of male bud, F: Premature ripening of fruits.

Use of disease-resistant cultivars has been an effective and economically viable strategy for integrated management of major diseases in numerous crops. This strategy could be used to control BXW if resistant cultivars could be identified or developed. The disease attacks almost all cultivars of banana including both East African Highland bananas and exotic dessert and beer bananas (Tushemereirwe et al., 2003; Ssekiwoko et al., 2006). High levels of cell-mediated resistance to *X. campestris* pv. *musacearum* have not been identified in any banana cultivars. Evaluating material in the fields is laborious and hampered by the variations in environmental conditions that exist at the test site, including temperature, moisture, and non-uniform distribution of pathogen throughout the experimental site. Screening of germplasm requires a reliable and rapid screening method to unambiguously discriminate resistant and susceptible cultivars. Early evaluation under controlled conditions using artificial inoculation of *in vitro* plantlets has been reported recently for screening banana cultivars for resistance to BXW (Tripathi et al., 2008).

The banana cultivars grown in the Great Lakes region in-clude East African Highland banana subgroup (AAA-EA genome) used to make 'matooke' (steamed and mashed fruit) and beer; brewing cultivar 'Pisang Awak' (ABB genome); the dessert cultivars 'Sukali Ndiizi' (AAB genome) and the Cavendish subgroup (AAA genome); plantains ('Gonja', AAB genome) and hybrid cultivars. Some knowledge of the relative susceptibility of commonly grown banana cultivars would be extremely useful and could be a basis for management strategies for BXW. This article describes the evaluation of commonly grown banana cultivars for relative susceptibility to *X. campestris* pv. *musacearum* using *in vitro* and potted plants under controlled conditions.

MATERIALS AND METHODS

Plant materials

Ten banana cultivars were selected with diverse genetic constitution and ploidy levels (Table 1). All these cultivars were observed to have differential responses to BXW disease in the fields. The *in vitro* plantlets were regenerated through micropropagation using apical shoot tips (Tripathi et al., 2003). The rooted plantlets with 3-4 leaves were used for *in vitro* screening experiments.

For *in vivo* screening experiments the rooted plantlets were transferred to sterile soil in plastic pots for acclimatization. The plants were maintained in a humid and shady environment for 12-15 days and then transferred to a screen house for 8 weeks.

Bacterial culture for artificial inoculation

X. campestris pv. *musacearum* was isolated from the infected plants on semi-selective medium YTSA-CC (1% yeast extract, 1% tryptone, 1% sucrose, 1.5% agar, 150 mg l^{-1} cycloheximide and 50 mg l^{-1} cephalexin; Tripathi et al., 2007). The isolate was characterized by appearance of yellow mucoid colonies and pathogenicity test. The cultures were maintained on YTSA medium (1% yeast extract, 1% tryptone, 1% sucrose and 1.5% agar) at 4°C.

A single bacterial colony was inoculated into 25 ml of YTS medium (1% yeast extract, 1% tryptone and 1% sucrose) and cultured at 28°C with shaking at 150 rpm for 48 h. The bacterial culture was centrifuged at 5000 rpm for 5 min and pellet was

Table 1. List of banana cultivars evaluated for relative susceptibility to *Xanthomonas campestris* pv. *musacearum.*

Cultivar	Genome	Genetic Group
Dwarf Cavendish	AAA	Dessert banana
Giant Cavendish	AAA	Dessert banana
FHIA-17	AAAA	Hybrid, dessert banana
FHIA-25	AABB	Hybrid, cooking banana
Mbwazirume	AAA-EA	East African Highland Bananas – Nakitembe clone set, cooking banana
Mpologoma	AAA-EA	East African Highland Bananas – Musakala clone set, cooking banana
Musa balbisiana	BB	Fertile diploid parent
Nakitembe	AAA-EA	East African Highland Bananas – Nakitembe clone set, cooking banana
Pisang Awak (Kayinja)	ABB	Bluggoes, beer banana
Sukali Ndiizi (Apple banana)	AAB	Ney Poovan, dessert banana

resuspended in sterile double distilled water. The optical density (OD 600 nm) of the bacterial suspension was checked and bacterial concentration was adjusted to 10^8 cfu ml^{-1} with sterile water. Fresh inoculum was used for all the experiments in order to have high virulent potential of the pathogen.

Evaluation of banana cultivars using *in vitro* plantlets

Ten banana cultivars were tested for relative susceptibility to *X. cam-pestris* pv. *musacearum* by injecting inoculum in pseudostem of *in vitro* plantlets as described by Tripathi et al. (2008). Ten plantlets of each banana cultivar were inoculated, where as control plantlets were injected with sterile water.

The pathogenic bacteria were re-isolated from wilted plants and identified as *X. campestris* pv *musacearum* on the basis of their characteristic morphology as yellowish, mucoid and circular colonies on YTSA-CC semi-selective medium.

Evaluation of banana cultivars using potted plants

All the ten banana cultivars were tested for relative susceptibility to *X. campestris* pv. *musacearum* by injecting inoculum in pseudostem of potted plants. Ten week old tissue culture grown plants transferred into sterile soil in plastic pots were used for inoculation.

Disease rating

Plants were assessed every day for 8 weeks for disease symptoms with preliminary symptoms as chlorosis or necrosis of leaves and finally as complete wilting of plants. Wilt incidence was measured based on number of wilted plants and total number of plants inoculated. The relative susceptibility of cultivars to BXW was evaluated 8 weeks after inoculation based on wilt incidence and the following disease rating scale; Highly Susceptible (HS) - 90 - 100% plants wilted, Susceptible (S) - 50 - 89% plants wilted, Least Susceptible (LS) - less than 50% plants wilted, Resistant (R) - none of the plants wilted.

Experimental design and statistical analysis

For each experiment, ten plants per cultivar were used for artificial inoculation. The trial was repeated in three separate experiments. Ten plants of each cultivars inoculated with sterile water were used as control. The means and standard errors were calculated and analyzed using SAS Software V8 (SAS, 2000).

RESULTS AND DISCUSSION

Evaluation of banana cultivars using *in vitro* plantlets

Ten banana cultivars were evaluated for relative susceptibility to *X. campestris* pv. *musacearum* using rapid screening procedure (Tripathi et al., 2008). In this technique *in vitro* plantlets were used for artificial inoculation. Evaluation of germplasm for resistance to several plant diseases using similar rapid screening methods has been previously reported for several crops (Denman et al., 2005; Kull et al., 2003; Onyeka et al., 2005). Similar early evaluation methods have also been developed for screening *Musa* spp. for resistance to the fungal disease, black leaf streak (Twizeyimana et al., 2007).

All the cultivars showed symptoms of chlorosis or necrosis on leaves of the inoculated plants, whereas control plantlets inoculated with water did not show any symptoms (Figure 2). Symptom development after artificial inoculation was similar to those observed in young plants following natural infection in field. The incubation period for the appearance of first visible symptoms varied significantly ($P < 0.0001$) among the various cultivars (Table 2). The banana cultivar 'Pisang Awak' showed symptoms earliest in all 100% of plants tested, while *M. balbisiana* showed localized symptoms in only 20% of plants tested. The youngest leaf of *M. balbisiana* showed localized chlorosis and necrosis, possibly due to a hypersensitive reaction and all of the plants recovered and thereafter appeared healthy and no bacteria was recovered from these plants. The hypersensitive reaction is characterized by the rapid death of individual plant cells which come into contact with pathogenic bacteria, and is generally associated with disease resistance of whole plant to the pathogen (Kiraly, 1980; Klement and Goodman, 1967).

The cultivars 'Mbwazirume', 'Mpologoma', 'Sukali Ndiizi', 'Dwarf Cavendish', 'Giant Cavendish', 'FHIA-17' and 'FHIA-25' showed same type of response and first symptoms were observed 12-14 days after inoculation. Cultivar 'Nakitembe' showed symptoms 18-19 days after inoculation. Plants of all the tested cultivars except for *M.*

Figure 2. Comparison between different banana cultivars for complete wilting, 8 weeks after inoculation with Xanthomonas *campestris* pv. *musacearum* using the *in vitro* plantlets; A- Pisang Awak, B-Dwarf Cavendish, C-Giant Cavendish, D- Mpologoma, E- Mbwazirume, F- Sukali Ndizi, G- FHIA-17, H- FHIA-25, I-Nakitembe, J-*Musa balbisiana,* K- Control plant inoculated with water.

Table 2. Comparison of ten cultivars of banana for appearance of first disease symptoms, complete wilting of plants, and wilt incidence after inoculation with *Xanthomonas campestris* pv. *musacearum* using *in vitro* plants.

Cultivars	No. of days [xy] to appearance of symptom	No. days [xy] to wilting	Wilt Incidence [xy] (%)	Disease rating
Dwarf Cavendish	12.7 [b]	22.3 [a]	90.0 [b]	Highly Susceptible
Giant Cavendish	14.3 [c]	25.2 [a]	90.0 [b]	Highly Susceptible
FHIA-17	13.3 [c]	32.0 [b]	90.0 [b]	Highly Susceptible
FHIA-25	13.7 [c]	40.0 [c]	70.0 [d]	Susceptible
Mbwazirume	13.3 [c]	31.3 [b]	80.0 [c]	Susceptible
Mpologoma	12.0 [b]	31.3 [b]	83.3 [c]	Susceptible
M. balbisiana	21.0 [e]	- [e]	0 [f]	Resistance
Nakitembe	18.7 [d]	43.7 [d]	42.7 [e]	Least Susceptible
Pisang Awak	11.0 [a]	24.0 [a]	100.0 [a]	Highly Susceptible
Sukali Ndiizi	12.7 [b]	30.0 [b]	80.0 [c]	Susceptible

x: Mean of three replicates.
y: Means with the same letter are not significantly different within each column.
Wilt Incidence % = [No. of Plants wilted / total number of plants inoculated] x 100.

balbisiana wilted totally but the rate of wilting incidence varied. Incubation period for complete wilting and rate of wilt incidence varied significantly ($P < 0.0001$) with cultivars (Table 2). All the 100% plant of 'Pisang Awak' wilted and 90% plants of 'Dwarf Cavendish', 'Giant Cavendish' and 'FHIA-17' wilted completely. The cultivars 'Mbwazirume', 'Mpologoma' and 'Sukali Ndiizi' showed complete wilting in about 80-83% of plants, whereas 'FHIA-25' showed wilting in about 70% of plants. In cultivar 'Nakitembe', some of the plants showing initial symptom of necrosis recovered and only about 43% plants showed complete wilting after 42 - 44 days, where as none of the

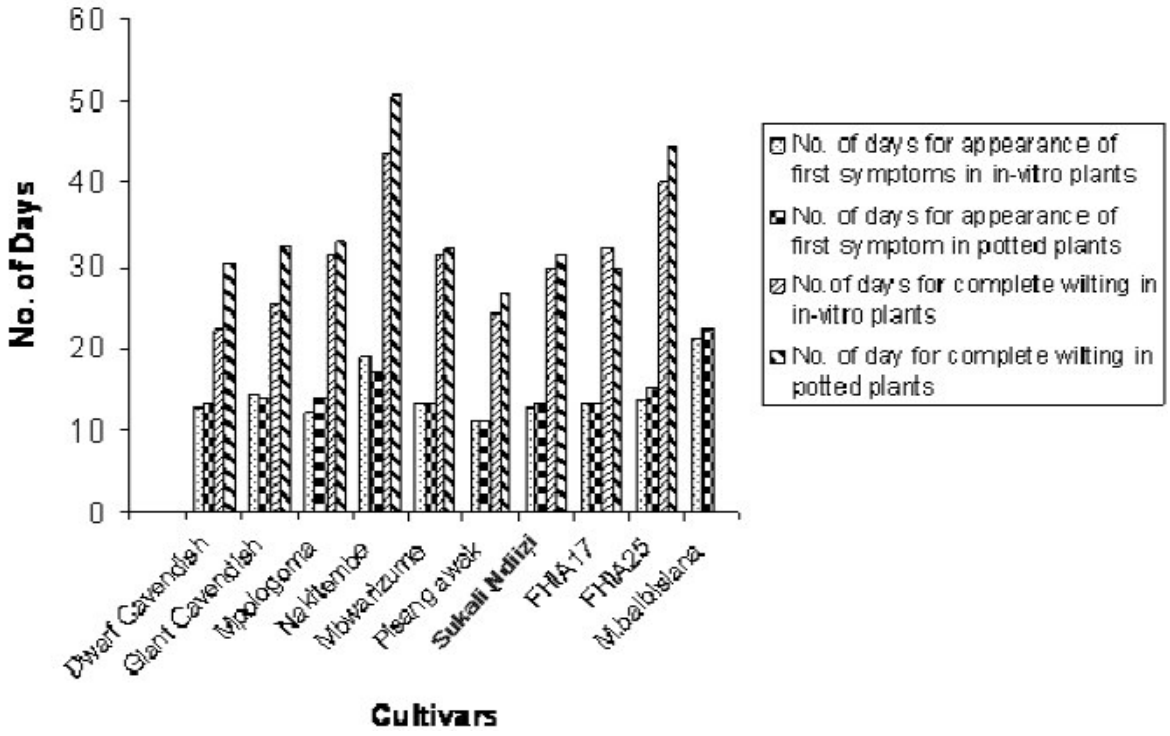

Figure 3. Comparison of time period mean number of days between inoculation and appearance of disease symptoms, and complete wilting of plants in ten banana cultivars inoculated with *Xanthomonas campestris* pv. *musacearum* using the *in vitro* and potted plants.

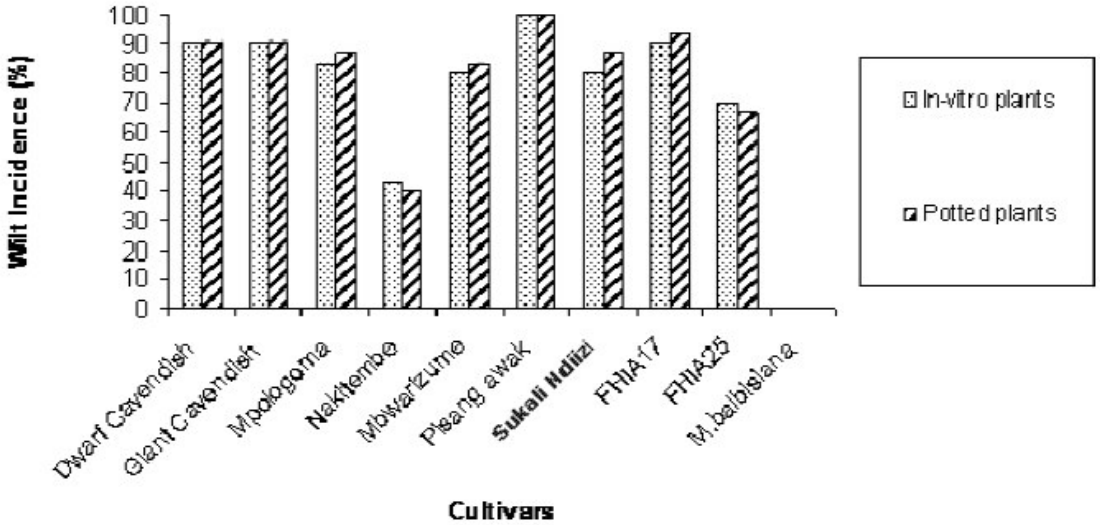

Figure 4. Wilt incidence in ten banana cultivars inoculated with *Xanthomonas campestris* pv. *musacearum* using *in vitro* and potted plants.

plants of cultivar *M. balbisiana* showed wilting.

The bacteria were recovered from all the wilted plants and yellow mucoid colonies of appeared on semi-selective medium confirming that symptoms were due to the *X. campestris* pv. *musacearum* infection.

Evaluation of banana cultivars using potted plants

The response of all ten cultivars tested varied in a manner which was very similar to results produced by the *in vitro* screening procedure (Figures 3 and 4). The

Table 3. Comparison of ten cultivars of banana for appearance of first disease symptoms, complete wilting of plants, and wilt incidence after inoculation with *Xanthomonas campestris* pv. *musacearum* using potted plants.

Cultivars	No. of days [xy] to appearance of symptom	No. days [xy] to wilting	Wilt Incidence[xy] (%)	Disease rating
Dwarf Cavendish	13.3 [b]	30.3 [b]	90.7[a]	Highly Susceptible
Giant Cavendish	14.0 [b]	32.3 [c]	90.3 [a]	Highly Susceptible
FHIA-17	13.3 [b]	30.0 [b]	93.3 [a]	Highly Susceptible
FHIA-25	15.0 [c]	44.7 [d]	66.7 [c]	Susceptible
Mbwazirume	13.3 [b]	32.0 [c]	83.3 [b]	Susceptible
Mpologoma	14.0 [b]	33.7 [c]	86.7 [b]	Susceptible
M. balbisiana	22.3 [e]	- [f]	0 [e]	Resistance
Nakitembe	17.0 [d]	50.7 [e]	40.0 [d]	Least susceptible
Pisang Awak	11.0[a]	26.3 [a]	100.0 [a]	Highly Susceptible
Sukali Ndiizi	13.3 [b]	31.3 [b]	86.7 [b]	Susceptible

x: Mean of three replicates.
y: Means with the same letter are not significantly different within each column.
Wilt Incidence % = [No. of Plants wilted / total number of plants inoculated] x 100.

incidence of disease, incubation period for the appearance of symptoms and complete wilting varied significantly ($P < 0.0001$) between cultivars (Table 3). The banana cultivar 'Pisang Awak' developed chlorotic or necrotic symptoms 11-12 days after inoculation and all plants wilted completely 26-28 days after inoculation. Cultivars 'Dwarf Cavendish', 'Giant Cavendish' and 'FHIA-17' showed symptoms after 13-14 days after inoculation and 90-93% plants wilted completely. Cultivars 'Mbwazirume', Mpologoma', and 'Sukali Ndiizi' developed chlorosis or necrosis 13-14 days after inoculation with complete wilting in about 83-86% of plants 30-33 days after inoculation. Cultivar 'FHIA-25' developed first symptoms 15 days after inoculation and showed complete wilting in about 66% of plants; whereas 'Nakitembe' showed necrosis 17 days after inoculation and complete wilting only in 40% of plants. *M. balbisiana* showed localized symptoms of chlorosis and necrosis in the youngest leaf of about 10% plants, but these plants recovered from disease and no complete wilting was observed. The banana cultivars tested using potted plants were classified to the same groups of susceptibility as previously using *in vitro* plantlets (Tables 2 and 3).

Disease rating

The response to artificial inoculation with *X. campestris* pv. *musacearum* varied between the cultivars of banana tested. The relative susceptibility of cultivars was evaluated based on the incidence of wilted plants both *in vitro* and potted plants. Disease development in the *in vitro* plants and potted plants were well correlated and cultivars were consistently ranked using two methods (Figures 3 and 4). The symptoms produced in the *in vitro* screening tests were very similar to the potted screening tests and represented those due to BXW under natural conditions in the field.

The commonly grown beer banana cultivar 'Pisang Awak' (known locally in Uganda as Kayinja) was found to be the highly susceptible with 100% wilt incidence. 'Pisang Awak' was reported to be the highly susceptible cultivar with natural infection in the fields (Eden-Green, 2004). Cultivars 'Dwarf Cavendish', 'Giant Cavendish', and 'FHIA-17' were also found to be highly susceptible with 90-93% wilt incidence. Cultivars 'Mbwazirume', 'Mpologoma', 'Sukali Ndiizi' and 'FHIA-25' were rated as susceptible to BXW. The cooking banana cultivar 'Nakitembe' showed wilt incidence of about 40-43% and was rated as least susceptible. The cultivar 'Nakitembe' could be deployed in the field through recommending it to the farmers to reduce the impact of BXW. It has been found to escape BXW in the field through the inflorescence because they have persistent male flowers and bracts (Figure 5), therefore the cushions on which insects could land and deposit the bacteria are not exposed. This characteristic allows the plants to escape insect-transmitted infections only, and might not possess cell-mediated resistance.

Only diploid parent *M. balbisiana* was rated as resistant as none of the plants completely wilted after inoculation. This may serve as an important source of resistance for breeding for new resistant cultivars.

The majority of the cultivars evaluated in this study and in the previous one (Ssekiwoko et al. 2006) were sus-ceptible or highly susceptible to *X. campestris* pv. *musacearum*. This highlights the need for development of new resistant cultivars for BXW disease control. Such need is particularly strong in beer type and dessert type bananas, as all the cultivars in these groups are highly susceptible.

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Figure 5. Nakitembe cultivar with inflorescence having persistent male flowers and bracts.

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