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# Banana field resistance to insect-vector transmission of bacterial wilt caused by *Xanthomonas campestris p.v musacearum*

**B. Mudonyi[1,2]*, L. Vanhaelewyn[3], G. Tusiime[4], F. Ssekiwoko[1], J.Kubiriba[1], W. K. Tushemereirwe[1] and C. M. Changa[1]**

[1]National Agricultural Research Laboratories P. O. Box 7065 Kampala, Uganda.
[2]Bukalasa Agricultural College, P.O. Box, 174, Wobulenzi, Uganda.
[3]Laboratory of Functional Plant Biology, Faculty of Sciences, Ghent University, KL. Ledeganckstraat 35, B-9000, Gent, Belgium.
[4]College of Agriculture and Environmental Sciences, Makerere University P. O Box 7062, Kampala, Uganda.

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**Banana, a major staple in East and Central Africa is constrained by banana Xanthomonas wilt (BXW) caused by *Xanthomonas campestris* pv*. musacearum* (Xcm). Xcm-infected plants are rapidly destroyed leading to 100% yield loss. Cultural controls are effective but laborious attracting laxity among farmers. This has led to epidemic resurgence in areas where BXW had been contained hence spread to new regions. Reliable control option would be planting Xcm-resistant varieties but extensive germplasm evaluation for their identification has not been conducted. Objective therefore was to determine existence of Xcm-resistance in banana by evaluating major banana cloneset representatives among indigenous cultivars plus introduced foreign Musa accessions. Potted plants were artificially inoculated with 0.5 ml ($10^8$CFU) of Xcm suspension. Promising selections from pot trial were later evaluated under natural transmission in field. Field trial plants were infected via insect vectors from spreader plants of highly susceptible cv Kayinja infected by spraying flowers with Xcm. Severity of Xcm-infection was semi-quantified using scales 1-5 and 0-5 for pot and field screening trials respectively. This enabled calculation of disease index as a measure of resistance for each genotype. High index implied highly susceptible banana genotype and low index resistant genotype. Findings 44 days after artificial inoculation showed wild banana *M. balbisiana* had 0.0 disease index thus highly resistant. All other banana genotypes tested under similar conditions had disease index of 100 thus susceptible. In field (insect vector transmission), disease index varied significantly among various genotypes evaluated, some susceptible while others; *M. balbisiana, Mbwazirume*, M9 and *M. Zebrina* resistant throughout 360 days of observation. We recommend that heritable traits that confer resistance in M*. balbisiana*, Mbwazirume, M9 and *M. zebrina* to Xcm be identified for utilization in genetic modification of farmer preferred bananas. Varieties Mbwazirume and M9 should be promoted for farmer growing to complement cultural controls against BXW.**

**Key words:** *Xanthomonas campestris pv. musacearum,* banana Xanthomonas wilt, banana.

## INTRODUCTION

Banana (*Musa* spp.) is an important staple food crop in Uganda where production is globally ranked second

largest after that of India (Biruma et al., 2007; Vurro et al., 2010). Since the emergence of Banana *Xanthomonas* wilt (BXW disease in 2001, it has devastated banana production undermining food and income security for more than 70% of Uganda's population that depend on banana industry (Tushemereirwe et al., 2004; Biruma et al., 2007). BXW that is caused by *Xanthomonas campestris pv. musacearum* (*Xcm*) (Yirgou and Bradbury, 1974) which leads to complete yield loss. Banana fruits on infected plants ripen prematurely; their pulp is hardened and/or rotten. Also suckers on mat of such plants that would give subsequent ratoon crop cycles and planting material needed for plantation expansion and /or establishing new ones also wilt and die (Smith *et al*., 2008). Unfortunately also the BXW that was initially reported since the 1960s to be confined in Ethiopia on enset (*Enset ventricosum*) a close relative of banana, (Shimelash et al., 2008) for unknown factors has spread rapidly throughout Eastern and Central Africa. Banana plantations in as far as Burundi, the Democratic Republic of Congo, Kenya, Rwanda, Tanzania and Uganda (Tushemereirwe et al., 2001; Ndungo et al., 2006; Biruma et al., 2007; Reeder et al., 2007; Carter et al., 2010) have been affected.

BXW symptoms appear on all plant parts and arise from internal blockage of the vascular tissue leading to incapacitated plant transport system. External symptoms involve initial drooping of leaves to entire plant wilting, death and eventual rotting of the entire stem (Karamura et al., 2008). There is mummification of the male bud characterized by fading of bract color from deep purple to dark brown or grey, shriveling of bracts and eventual drying up or rotting of the entire stalk. This is a typical characteristic of insect mediated (natural) transmission of *Xcm* (Karamura et al., 2008). Infection from the male bud of very susceptible varieties for example cultivar Pisang awak (Kayinja) (ABB) proceeds through the rachis, pseudostem down to the corm where it spreads to the adjoining suckers around the mother plant resulting in death of entire mat. However the other banana varieties; Bluggoe (ABB), Sukali Ndizi (AAB), East African highland banana (AAA) cultivars that have shriveled and less conspicuous flowers do not show this symptom. Infected fruits harden and develop a dark brown discoloration in the pulp. In addition, a cross-section through the fruit and pseudo stem reveals yellow pus-like bacterial ooze.

Recommended cultural practices are quite limited towards achieving long term acceptable control levels of BXW. These practices involve complete uprooting of diseased mats, burying of uprooted and chopped plant debris, use of clean garden tools and timely removal of male buds to prevent insect vector transmission (Ssekiwoko et al., 2010). Compliant farmers to BXW-control mobilization and sensitization campaigns have achieved 60-90% success (Kubiriba et al., 2012). These practices are quite laborious and are often associated with laxity tendencies among farmers. Consequently, there has been a repeat of epidemic resurgence in areas where it had been contained. The epidemic has also continued to advance into additional plantations in regions not previously affected by bacterial wilt. East African Highland Bananas (EAHBs) are the dominant cultivars grown by farmers in this region where BXW is endemic. EAHBs that are also clonally propagated aid dissemination of BXW through infected planting materials.

EAHBs that are triploids of AAA genotype presumably have a very narrow genetic base. They are very difficult to improve utilizing conventional breeding approaches because they are sterile and pathenocarpic. The practice of planting resistant banana varieties therefore seems to be nonrealistic BXW control option for the farmers in East Africa since all popular cultivars grown are unfortunately perceived to be highly susceptible to BXW. For example, Ssekiwoko et al. (2006) evaluated 42 indigenous EAHB cultivars and four wild banana relatives by artificial inoculation under screen house trial conditions. They reported that all these bananas except *M. balbisiana*, were susceptible to BXW. Since *M. balbisiana* is a diploid with BB genome, it is very interesting to establish whether presence of B genotype in banana triploids and tetraploids would consistently confer resistance to BXW. In present investigation the range of banana genotypes evaluated by artificial inoculation with *Xcm* under screen house trial conditions was broadened to include those that were not evaluated by Ssekiwoko et al. (2006). Introduced triploids and tetraploid bananas with B or BB genotypes available in the Uganda's National Banana germplasm collection that reportedly arose from hybridization between diploid species of *M. acuminata* (AA) and *M. balbisiana* (BB) were also evaluated. Finally, unlike for the study of Ssekiwoko et al. (2006) that was only via artificial inoculation of test banana plants under screen house conditions, representative banana genotype selections from those in the screen house trial were also further evaluated in the field for resistance to *Xcm* utilizing procedure of natural spread of *Xcm* by vector transmission.

## MATERIALS AND METHODS

### Banana genotypes evaluated in pot trial

To broaden the number of germplasm accessions evaluated for response to infection by *Xcm* under screen house conditions, 40

*Corresponding author. E-mail: bmudonyi@gmail.com

**Table 1.** List of banana genotypes evaluated for resistance to BXW in pot trial.

| Genome group | Name of genotype | Genotype category and ploidy level |
|---|---|---|
| A | Long tavoy | Foreign banana, Diploid (AA) |
| | *M. ornata* | Wild banana, Diploid (AA) |
| | *M. zebrina* | Wild banana, Diploid (AA) |
| | Pisang mas | Foreign banana, Diploid (AA) |
| | Pitu | Foreign banana, Diploid (AA) |
| | GCTC.V.215 | Foreign banana, Triploid (AAA) |
| | KM5 | Landrace, Triploid (AAA) |
| | M2 | Hybrid banana, Triploid (AAA) |
| | M9 | Hybrid banana, Triploid (AAA) |
| | Cavendish | Dessert banana, Triploid (AAA) |
| | Dumingi | Foreign banana, Triploid(AAA) |
| | Kafunze | Local banana, Triploid (AAA) |
| | Mbwazirume | Local banana, Triploid (AAA) |
| | Nakasabira | Local banana, Triploid (AAA) |
| | Pisang nangk | Foreign banana, Triploid (AAA) |
| | Red bogoya | Dessert banana, Triploid (AAA) |
| | Williams | Foreign banana, Triploid (AAA) |
| | IC2 | Foreign banana, Tetraploid (AAAA) |
| B | *M. balbsiana* | Wild banana, Diploid (BB) |
| AB | Amou | Foreign banana, Triploid (AAB) |
| | Pisang rajabul | Foreign banana, (AAB) |
| | burroCEMSA | Foreign banana , Triploid (ABB) |
| | ITC | Foreign banana, Triploid (ABB) |
| | Saba | Foreign banana, Tripliloid (ABB) |
| | P.A. 03.22 | Foreign banana, Tetraploid (AAAB) |
| | PV.03.44 | Foreign banana, Tetraploid (AAAB) |
| | FHIA3 | Foreign banana, Tetraploid (AABB) |

plants for each of the 25 banana genotypes presented in Table 1 that had not been previously screened (Ssekiwoko et al., 2006) for resistance to BXW were included in the current pot trial. In addition, two matooke hybrids, M2 and M9 developed utilizing conventional breeding approach by scientists of the Banana Research Programme, at NARL, Kawanda were included in this study. The wild relative of banana, *Musa balbisiana* and an East African.

Highland Banana cv Mbwazirime respectively resistant and susceptible to artificial inoculation with *Xcm* in the evaluation by Ssekiwoko et al. (2006) were included to act as reference checks for the current trial. In all, a total of 1000 plants that were regenerated from corms of suckers obtained from symptomless mother plants were established in plastic pots containing steam sterilized top loam soil. Emerging plants from corms were monitored through establishment for a period of at least 21-30 days to confirm their disease-free status. The experiment was laid down in a Completely Randomized Design (CRD) arrangement with four replications each comprising of ten plants per genotype in the trial.

### *Xcm* strain used for artificial inoculation of banana in pot trial

*Xcm* strain used for inoculation of banana test plants in the pot trial was isolated from infected banana cv Kayinja (Mudonyi et al., 2017). Then cultured and maintained by regularly sub-culturing on Yeast colonies characteristic of *Xanthomonas* were resuspended in sterile water in a bottle. The turbidity of the inoculum suspension of *Xcm* was adjusted using a spectrophotometer at an optical density of 600nm that corresponds to a concentration of $1x10^8$ CFU/ml.

### Artificial inoculation of banana plants in pot trial

Initially before inoculation of experimental plants, the pathogenicity of *Xcm* isolate from Kayinja was confirmed by infection of susceptible banana cultivar Kisansa (AAA) following procedure described by Ssekiwoko et al. (2006) and Mudonyi et al. (2017). These Kisanasa plants had been micropropagated in tissue culture, weaned in poly pots containing steam sterilized top loam soil and allowed to go through an acclimatization period of one month in the screen house. Infection of these potted plants were via injection into meristematic tissue within the petiole of topmost expanded youngest leaf, with 0.5ml of ($10^8$CFU) utilizing a hypodermic syringe and needle. The negative control plants were injected similarly with an equal volume of sterile water. After this initial pathogenicity test confirmation, similar approach was used to inoculate all pot trial test plants by injection with 0.5ml of the bacterial suspension. Artificial inoculation was carried out in the evening when ambient relative

humidity conditions were rising and temperature decline from an average day maximum of 30°C to avoid wound drying before bacterial multiplication and colonization of plant tissue (Karamura et al., 2008).

**Monitoring disease progress in pot trial**

Monitoring BXW progress on artificially inoculated pot trial plants maintained under screen house conditions lasted 60 days post inoculation. The screen house temperature conditions were at an average maximum of 28-30°C and minimum of 18-21°C. Monitoring was made twice a week and data was recorded on disease latency (incubation) period, disease incidence and disease severity. Disease severity was semi quantitatively estimated using a scale of 0-5 (where 0=no symptoms on all leaves, 1=one inoculated leaf wilted, 2=two to three leaves wilted, 3=four leaves wilted, 4=all leaves wilted and 5=plants dead; Winstead et al. 1952). Disease index for each genotype was determined using following formula;

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

Where, 0, 1, 2, 3, 4, 5 is disease severity scale for each respective plant a, b……f and n = total number of plants inoculated (25) for each genotype. This disease index was expressed as a percentage and therefore a very high index meant a highly susceptible banana genotype and low index meant resistant genotype (0% means resistant, 1- 40% moderately resistant, 41-59% susceptible, 60-100% highly susceptible).

**Field evaluation of selected *Musa* germplasm accessions**

***Banana genotypes evaluated in the field***

10 promising genotype selections from among those tested in the pot trial (Table 2) were advanced and further evaluated under natural field conditions. These plants were raised from corms and planted in holes measuring 45 x 45 cm wide and 60 cm deep and at a spacing of 3 m x 3 m. The field trial layout was a Randomized Complete Block Design (RCBD) with four blocks each comprising of 16 plants per genotype. A total of 640 test plants were planted in the trial. It was then surrounded with spreader row of plants of banana cultivar Kayinja that is highly susceptible to BXW.

***Field trial inoculation and management***

The spreader row plants that comprised of BXW- susceptible banana cv Kayinja were inoculated by spraying their male bud flowers and cushions with 0.5 ml of suspension *Xcm* strain. This strain was also the one that was used for inoculation of plants in pot trial evaluation. This *Xcm* strain was previously isolated from banana cv Kayinja. Field test plants were kept weed free by regular herbicide spray with Roundup and hand pulling around the plants. Tools for weeding, pruning and de-suckering were not used to avoid possible damage to suckers which would have potentially exposed plants to some other infections and to avoid spread of *Xcm* infection in the field trial via contaminated tools. Removal of male buds was also avoided to promote natural vector transmission of *Xcm* when moving from various male flowers of banana plants while foraging for nectar.

***Monitoring BXW progress in field trial***

Field trial plants were observed monthly for a period of one year and data collected on latency period, disease incidence and severity. Disease incidence was computed as a percentage of plants showing BXW characteristic symptoms for each cultivar per block. Disease severity was estimated utilizing modified 0-5 scale of Winstead et al. (1952a), (where 0=no symptoms, 1=inflorescence symptoms on the male bud, rachis and the bunch, 2=mother plant wilted, 3=mother plant dead, 4=one or more daughter plant(s) wilted, and 5=entire mat dead. Disease severity data was then used to calculate disease severity index (DI) for each genotype in the block utilizing formula described in the pot trial above.

**Statistical analysis of data collected from pot and field trials**

For both pot and field trials, disease indices were subjected to analysis of variance (ANOVA) using GenStat 12th Edition (VSN International Ltd, 2009). Means were separated using Fisher Least Significance difference at 5%.

## RESULTS

### Banana resistance to meristematic tissue injection with *Xcm* under screen house conditions

#### *Identity of Xcm for evaluation of banana resistance confirmed*

The strain of *Xcm* utilized in evaluation of banana resistance (Figure 2a) was found to induce wilt symptoms in all eight inoculated plants (100% incidence) of banana "cv" Kisansa within 14 days of pathogenicity confirmation test trial (Figure 2a). These infected plants wilted and eventually died by end of 32 days post-inoculation. Negative control reference test plants that were inoculated with sterile distilled water did not develop wilt symptoms (Figure 2b) during the same observation period. Cultural characteristics were established by re-isolation of causative bacteria onto YPGA (Figure 1). This pathogenic bacteria formed shiny, yellow, mucoid and smooth colonies on YPGA (Figure 2c). These colony characteristics on YPGA are typical of Xcm. After conducting these in vitro and pathogercity confirmatory tests, this Xcm strain was used for infection of all test plants in subsequent pot and field evaluation trials.

### Banana is susceptible to Xcm by artificial inoculation

All the banana genotypes that were evaluated under screen house conditions became infected by Xcm via artificial inoculation but the response to infection over time was highly variable (Table 3). For example, 12 of the genotypes evaluated exhibited the shortest latency period of 12 days. Also latency period was 14-16 days for 14 genotypes and 20 days for one genotype PV.03.44. M. balbisiana which is a diploid with B genome (Table 1) exhibited the longest latency period of 32 days. Further, whereas about 29% of inoculated plants of M. balbisiana

**Table 2.** List of selected banana genotypes evaluated for resistance to BXW in field trial.

| Genome group | Name of genotype | Genotype ploidy level |
|---|---|---|
| A | M. ornata | Diploid (AA) |
| | M. zebrina | Diploid (AA) |
| | M2 | Triploid (AAA) |
| | M9 | Triploid (AAA) |
| | KM5 | Triploid (AAA) |
| | Mbwazirume | Triploid (AAA) |
| B | *M. balbsiana* | Diploid (BB) |
| AB | BurroCEMSA | Triploid (ABB) |
| | Saba | Triploid (ABB) |
| | FHIA3 | Tetraploid (AABB) |

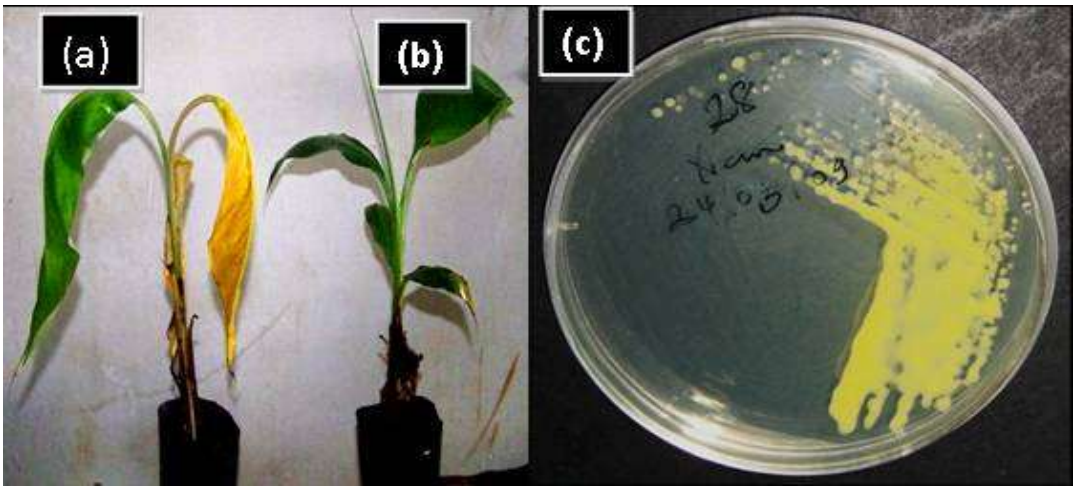


**Figure 1.** *Xcm* infected banana and colony characteristics on YPGA. Potted Kisansa banana plants inoculated with *Xcm* (a) and sterile water (b). Cultural appearance of pathogenic bacteria on YPGA (c) that was re-isolated from the symptomatic plant (a) was typical for *Xcm*.


**Figure 2.** Appearance of bacterial wilt symptoms on inflorescence of genotype Saba in the field trial. Symptom appearance on healthy-looking inflorescence (A) gradually progressed from wilting of male bud and rachis (B) to premature fruit ripening (C) and finally rotting of the entire.

showed bacterial wilt symptoms, all the plants of the other genotypes evaluated by artificial inoculation developed wilt symptoms both on inoculated and non inoculated leaves within 44 days after inoculation (Table 3). Wilt symptom manifestation by these other genotypes was neither linked to their ploidy level nor to their genome group (A, B or AB) as described in Table 1. Interestingly, wilt symptoms on the infected plants of M. balbisiana that is a diploid with BB genome were restricted to the inoculated leaf only and also these plants eventually outgrew such symptoms of infection 44 days after inoculation. On the other hand no such symptom recovery was observed for all the inoculated plants of the other genotypes evaluated by artificial inoculation. Also with exception of M. balbisiana, disease index at 26 days after inoculation of the different genotypes was highly variable and ranged from 40-83%. However at 44 days after inoculation the disease index for all these genotypes was not significantly different and ranged from 93-100% (Table 3).

Artificial inoculation of potted plants via injection with 0.5 ml ($10^8$CFU) of bacteria suspension into meristematic tissue within the petiole of youngest fully expanded leaf of each plant utilizing a hypodermic syringe and needle was performed in the evening when ambient temperature was declining to below 25˚C and relative humidity rising to above 80%.

### Persistent bract trait is associated with field resistance of banana to *Xcm*

Infection of banana genotype plants by *Xcm* in the field trial was achieved by spraying flowers of spreader plants of susceptible banana cultivar Pisang awak with bacterial suspension. Then infection by *Xcm* was transmitted naturally by foraging insect vectors from these inoculated spreader plants to the healthy test plants. Under these field trial conditions, initial manifestation of bacterial wilt symptoms varied significantly among the banana genotypes evaluated. For example, banana genotypes Saba (BBB) and BurroCEMSA (ABB) were the first to show symptoms of infection (Figure 2). Later four additional genotypes also showed wilt symptoms while another four genotypes did remain symptomless (Table 4). For those plants of genotypes that became infected by *Xcm* under field trial conditions, initial symptoms appeared on the healthy looking inflorescence (Figure 2A). Symptoms on the inflorescence began with wilting of the male bud followed by that of the rachis (Figure 2B), then premature ripening and rotting of fruits (Figure 2C, D), and finally death of the entire plant.

The infection by *Xcm* was transmitted naturally by foraging insect vectors from the inoculated spreader plants of susceptible cultivar Pisang awak to the healthy looking test plants of genotypes evaluated in the field trial.

Six genotypes that were evaluated in the field trial developed wilt symptoms and four did remain symptomless (Table 4). Notably, these six genotypes that wilted under field trial conditions of BXW spread have dehiscent male bud bracts and the four that remained symptomless have persistent male bud bracts (Figure 3).

This persistent bract trait on the inflorescence was apparently associated with banana field resistance to infection by *Xcm* via insect transmission.

The disease incidence and severity index for the six banana genotypes that became infected by *Xcm* under field conditions of disease spread varied significantly (Table 4, $p < 0.001$). The highest disease incidence of 65% and severity index of 63.5% was recorded for genotype Saba (ABB). In addition, disease incidence and severity index that varied from 14.6-55% and 13.7-44% respectively was recorded for the remaining five genotypes that became infected by *Xcm*. Finally, disease incidence and severity index of 0% (Table 4) was recorded for four genotypes inclusive of two cooking varieties, Mbwazirume (AAA) and recently released matooke hybrid M9 (AAA). These four genotypes that did not show symptoms of infection in the field trial had developed wilt symptoms within 12-32 days after artificial inoculation with *Xcm* under screen house trial conditions (Table 3). Additionally, it was only *Xcm*-infected *M. balbisiana* potted plants that were also able to recover fully from bacterial wilt symptoms developed 32 days after artificial inoculation.

Unlike for artificial inoculation in pot trial (Table 3) where all genotypes tested became infected by *Xcm*, four of the ten banana genotype selections that were advanced and evaluated under field conditions of bacterial wilt spread were not infected by *Xcm*. These interesting genotypes have persistent male bud bracts and include two important cooking banana varieties; recently released banana hybrid M9 and popular variety Mbwazirume.

## DISCUSSION

### Evaluation of banana for resistance to Xcm in the screen house

This study evaluated 4 indeginous banana cultivars, 2 banana hybrids, 3 wild relatives of banana and 17 introduced banana germplasm accessions for resistance towards Xcm infection by injection of inoculum into meristematic tissue of the leaf petiole. The technique was very effective since 100% of inoculated plants became infected (Table 3). Apparently, the inoculum was directly deposited in the main transport system enhancing inoculum delivery to all parts of the plant to cause infection. On the other hand, inoculum for the field trial plants was placed onto the flowers of spreader plants to enable easy access by foraging insects for transmission to healthy plants. All the genotypes evaluated in the screen house except M. balbisiana that is diploid with BB genotype succumbed to BXW via artificial inoculation.

**Table 3.** Variation in response of banana genotypes to infection by *Xcm* via artificial inoculation under screen house conditions at National Agricultural Research Laboratories, Kawanda.

| Genotype | Latency period | Mean incidence (%) | | Disease index (%) | |
|---|---|---|---|---|---|
| | | 26 dai | 44 dai | 26 dai | 44 dai |
| Pisang nangk | 12-22 | $100^{a}$ | $100^{a}$ | $83^{i}$ | $100^{a}$ |
| Red bogoya | 16-20 | $100^{a}$ | $100^{a}$ | $82^{hi}$ | $100^{a}$ |
| Dumingi | 16-22 | $100^{a}$ | $100^{a}$ | $82.5^{i}$ | $100^{a}$ |
| GCTC.V.215 | 12-22 | $100^{a}$ | $100^{a}$ | $75^{gh}$ | $100^{a}$ |
| Nakasabira | 16-22 | $97^{b}$ | $100^{a}$ | $73^{fgh}$ | $100^{a}$ |
| ITC | 12-20 | $100^{a}$ | $100^{a}$ | $69^{efg}$ | $100^{a}$ |
| Williams | 12-22 | $100^{a}$ | $100^{a}$ | $69^{efg}$ | $100^{a}$ |
| Pisang rajabul | 12-22 | $100^{a}$ | $100^{a}$ | $69^{efg}$ | $100^{a}$ |
| Pitu | 12-22 | $100^{a}$ | $100^{a}$ | $68^{defg}$ | $100^{a}$ |
| IC2 | 12-22 | $100^{a}$ | $100^{a}$ | $64^{cdef}$ | $100^{a}$ |
| Cavendish | 14-22 | $100^{a}$ | $100^{a}$ | $63^{cde}$ | $100^{a}$ |
| Long tavoy | 12-20 | $100^{a}$ | $100^{a}$ | $62^{c}$ | $100^{a}$ |
| Amou | 16-22 | $100^{a}$ | $100^{a}$ | $60^{cde}$ | $100^{a}$ |
| M2 | 14-22 | $100^{a}$ | $100^{a}$ | $60^{cde}$ | $100^{a}$ |
| M9 | 16-20 | $100^{a}$ | $100^{a}$ | $60^{cde}$ | $100^{a}$ |
| Saba | 16-22 | $100^{a}$ | $100^{a}$ | $59^{cd}$ | $100^{a}$ |
| Mbwazirume# | 16-22 | $100^{a}$ | $100^{a}$ | $59^{cd}$ | $100^{a}$ |
| KM5 | 12-22 | $100^{a}$ | $100^{a}$ | $58^{c}$ | $100^{a}$ |
| Kafunze | 12-22 | $100^{a}$ | $100^{a}$ | $58^{c}$ | $100^{a}$ |
| FHIA3 | 12-22 | $100^{a}$ | $100^{a}$ | $53^{bc}$ | $100^{a}$ |
| M. ornate | 12-26 | $100^{a}$ | $100^{a}$ | $53^{bc}$ | $93^{b}$ |
| burroCEMSA | 16-22 | $100^{a}$ | $100^{a}$ | $51^{b}$ | $100^{a}$ |
| M. zebrina | 16-22 | $91^{e}$ | $100^{a}$ | $51^{b}$ | $100^{a}$ |
| PV.03.44 | 20-22 | $100^{a}$ | $100^{a}$ | $47^{ab}$ | $100^{a}$ |
| Pisang mas | 14-26 | $94^{c}$ | $100^{a}$ | $47^{ab}$ | $100^{a}$ |
| P.A. 03.22 | 16-22 | $92^{d}$ | $100^{a}$ | $40^{a}$ | $100^{a}$ |
| M. balbsiana | 32 | $0^{f}$ | $28.6^{b}$ | 0j | $0.0^{c}$ |
| LSD(5%) | | 0.0464*** | 0.02575*** | 9.1*** | 0.932*** |
| CV% | | 10 | 5.4 | 30.1 | 2 |

Presence of B genotype in other bananas did not confer resistance to BXW. This result is in agreement with previous evaluation where all indigenous edible banana cultivars in East and Central Africa succumbed to the disease (Ssekiwoko et al., 2006; Michael et al., 2006). In this study, two East African highland banana cultivars Nakasabira and Kafunze that were not previously screened were evaluated and equally found susceptible, suggesting there is limited possibility that there is resistance among the East African highland bananas to BXW infection. Most of M. balbisiana plants consistently did not develop symptoms in this study. About one third of M. balbisiana plants developed symptoms that were restricted to only the inoculated leaf but these plants overgrew these symptoms by 32 days after inoculation. The result suggests that M. balbisiana is resistant to Xcm which also is in agreement with findings reported by Ssekiwoko et al. (2006) and Tripathi et al. (2008). M. balbisiana is thus so far, the only possible source of resistance to Xcm and could be used for breeding improvement of banana for resistance to Xcm following both conventional and genetic engineering approaches.

### Evaluation of banana for resistance to natural transmission of Xcm by insects in the field

Unlike for the result of artificial inoculation technique of injecting inoculum into meristematic tissue in the leaf petiole under screen house trial conditions additional banana genotypes to M. balbisiana showed field resistance to Xcm by insect transmission. Field resistant geneotypes included popular banana cultivar Mbwazirume, recently released banana hybrid M9, and genotype M.

**Table 4.** Variation in response of banana genotypes to infection by *Xcm* under field conditions for bacterial wilt spread. Wilt incidence and severity data was recorded until 360 days after inoculation of flowers of spreader-plants of susceptible banana cultivar Pisang awak (ABB) surrounding each experimental block that had been sprayed with bacterial suspension.

| Genotype | Disease incidence (%) | Disease severity index (%) |
|---|---|---|
| Saba (BBB) | 65.0$^{g}$ | 63.5$^{e}$ |
| BurroCEMSA (ABB) | 55.0$^{f}$ | 44.0$^{d}$ |
| M2 (AAA) | 40.0$^{e}$ | 38.5$^{cd}$ |
| Km5 (AAA) | 35.0$^{d}$ | 27.0$^{bc}$ |
| *M. ornata* (AA) | 32.5$^{c}$ | 30.5$^{cd}$ |
| FHIA3 (AABB) | 14.6$^{b}$ | 13.7$^{ab}$ |
| *M. balbsiana* (BB) | 0$^{a}$ | 0$^{a}$ |
| M9 (AAB) | 0$^{a}$ | 0$^{a}$ |
| Mbwazirume (AAA) | 0$^{a}$ | 0$^{a}$ |
| M. zebrina (AA) | 0$^{a}$ | 0$^{a}$ |
| LSD (5%) | 0.1555*** | 14.46*** |
| CV% | 150.5 | 155.5 |


**Figure 3.** Persistent (Top panel: A and B; Bottom panel: A, B and C) and dehiscent (Top panel: C) male bud bract traits of banana inflorescence. Banana hybrid M9 (Bottom panel: A), popular cultivar Mbwazirume (Bottom panel: B) and wild relative of banana *M. balbisiana* (Bottom panel: C) that have persistent male bud bracts also remained wilt symptomless throughout the period of observation in the field trial.

zebrina. These field resistant banana genotypes were observed to have persistent male bud bracts. In contrast, the other cultivars that succumbed to infection by Xcm in the field had dehiscent male bud bracts. The wet wounds left behind after dehiscence of male bud bracts observed on inflorescence of the banana genotypes that succumbed to Xcm infection in the field may have acted as entry points for Xcm carried around as body contaminate of insects foraging for pollen and nectar. Inflorescence of M9 and M. zebrina on the contrary were left with a dry wound after dehiscence of male bud bracts that may not be suitable entry points for spread of Xcm by foraging insects. Apparently, persistent bract trait is a preformed structure that may enable banana resist ingress of pathogenic bacteria as implied by description of Lucas (1998). Karamura et al. (2008) reported that certain bananas had persistent bracts and or dry wounds left after abscission of bracts and male flowers that enabled them resist natural infection by insects. Since these field resistant bananas with persistent bracts include popular cultivar Mbwazurime and recently released banana hybrid M9 as well as other edible banana cultivars grown by the farmers that are highly preferred by the consumers they may be planted by farmers and integrated in already recommended cultural practices being promoted for control of BXW. Finally, gene(s) controlling persistent bract trait in banana should be identified, isolated and utilized for genetic engineering improvement of farmer preferred Xcm-susceptible banana cultivars.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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