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FULL RESEARCH PAPER

A rapid technique for screening banana cultivars for resistance to *Xanthomonas* wilt

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Abstract The banana *Xanthomonas* wilt disease (BXW) has threatened the livelihood of millions of farmers in East Africa. Use of resistant varieties is the most cost-effective method of managing this bacterial disease. A reliable and rapid screening method is needed to select resistant banana varieties. An in vitro screening method was developed for early evaluation of *Xanthomonas* wilt resistance using small tissue culture-grown plantlets. Eight cultivars of banana were screened with sixteen isolates of *Xanthomonas campestris* pv. *musacearum* using this method. There were significant differences ($P<0.0001$) in susceptibility among the various banana cultivars tested, whereas no significant difference ($P=0.92$) in pathogenicity was observed between the pathogen isolates. The cv. Pisang Awak (Kayinja) was found to be highly susceptible and *Musa balbisiana* resistant. Nakitembe was found to be moderately resistant while cvs Mpologoma, Mbwazirume, Sukali Ndiizi, FHIA-17 and FHIA-25 were susceptible. The susceptibility of these cultivars was further tested in vivo by artificial inoculation of potted plants with similar results. This study shows that an in vitro screening test can serve as a convenient, cheap and rapid screening technique to discriminate BXW-resistant from BXW-susceptible banana cultivars.

Keywords Banana cultivars · Screening method · *Xanthomonas* wilt

Abbreviations

BAP	6-benzylaminopurine
BXW	banana *Xanthomonas* wilt
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
Xcm	*Xanthomonas campestris* pv. *musacearum*

Introduction

Bananas are the fourth most important food crop in the tropical and sub-tropical zones of the world. Annual banana production in the world is estimated at 104 million tons of which less than 10% enters the commercial market, suggesting that the crop is more important as food for local consumption than for export (FAOSTAT 2004). East Africa is the largest banana producing and consuming region in Africa with Uganda being the world's second leading producer with total production of about 10.5 million tons (FAOSTAT 2004). The banana *Xanthomonas* wilt (BXW) disease caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (*Xcm;* Tushemereirwe

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et al. 2004) threatens the livelihood of millions of farmers in East Africa. The disease was first identified in Uganda in 2001 and now has spread in epiphytotic proportions to almost all major banana producing districts of the country. The disease has also been reported in Democratic Republic of Congo (Ndungo et al. 2005), Rwanda (Svetlana Gantashova, Institut des Sciences Agronomiques du Rwanda, ISAR, Rwanda, pers. comm.), Tanzania (Mgenzi Byabachwezi, Agricultural Research and Development Institute Maruku, Tanzania, pers. comm.), Kenya and Burundi. BXW was first reported more than 30 years ago in Ethiopia on *Ensete* species, which are closely related to banana (Yirgou and Bradbury 1974). The disease attacks almost all varieties of commonly grown banana cultivars.

Xcm is a gram-negative, rod shaped bacterium belonging to *Xanthomonadaceae* and produces typical yellow, circular, mucoid, slimy colonies on nutrient agar and semi-selective medium YTSA-CC (Tripathi et al. 2007). Affected banana plants develop symptoms characterized by 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 (Tushemereirwe et al. 2004). Symptoms on floral parts include wilting of bracts, shrivelling and rotting of the male buds, with the flower stalks turning yellow-brown. Cross sections of diseased pseudostems reveal yellowish bacterial ooze (Tripathi 2005). Eventually, infected plants wither and the plant rots. Many sources of infection are known for BXW. The major sources of pathogen inoculum are infected banana plants, plant residues, contaminated soil and traded products. Field observations suggest that the primary means of disease spread is by insect transmission through the moist cushions exposed after male flower shed from the inflorescence. Other modes of pathogen spread are through the roots, infected plant materials or the use of contaminated tools (Eden-Green 2004).

Economic impact of the disease is manifested as absolute yield loss or reduced bunch weights, and death of the mother plant and suckers that help in subsequent ratoon plant production cycles. Diseased fields cannot be replanted with banana for a long time due to soil borne inoculum of the pathogen. BXW has many similarities to other bacterial wilts of banana (Moko, blood and bugtok diseases) that are caused by *Ralstonia* (formally *Pseudomonas solanacearum* spp.) and closely related organisms (Thwaites et al. 2000). Experience with these diseases shows that once they have become established in smallholder banana cropping systems, then control is very difficult and eradication effectively impossible (Eden-Green 2004).

Development or selection of resistant varieties is a useful and cost-effective method of managing bacterial diseases. Attempts to develop disease resistant varieties through conventional breeding require resistant donor parents but germplasm exhibiting resistance has not been identified against *Xcm*. Screening for resistance also requires a reliable and rapid screening method to unambiguously discriminate resistant and susceptible 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. Early evaluation under controlled conditions using artificial inoculation is an important requirement to screen large numbers of *Musa* spp. for resistance to BXW. This article describes a rapid method for evaluation of banana cultivars for resistance to *Xanthomonas* wilt using in vitro plantlets.

Materials and methods

Bacterial isolates

Sixteen isolates of *Xcm* collected from various locations in Uganda, for which Koch's postulates had been proven, were used in this study (Table 1). *Xcm* 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} cyclo-

Table 1 Details of location for 16 isolates of *X. campestris pv. musacearum* recovered from diseased banana in Uganda

Xcm isolates	Location	*Xcm* isolates	Location
001/gyz/05	Wakiso	045/kla/05	Kampala
002/wks/05	Wakiso	019/iga/05	Iganga
006/wks/05	Wakiso	023/kay/05	Kayonga
007/wks/05	Wakiso	003/muk/05	Mukono
036/lra/05	Lira	041/nsg/05	Nakasongola
038/lra/05	Lira	014/luw/05	Luwero
031/kib/05	Kibale	043/msd/05	Masindi
033/kib/05	Kibale	034/kibo/05	Kiboga

heximide and 50 mg l^{-1} cephalexin; Tripathi et al. 2007). The isolates were characterized by the appearance of yellow mucoid colonies and a pathogenicity test. The cultures were maintained on YTSA medium (1% yeast extract, 1% tryptone, 1% sucrose and 1.5% agar) at 4°C. For long term storage, bacterial isolates were preserved in glycerol (50% v/v in YTS; YTSA minus agar) at −80°C.

Plant materials

Eight banana cultivars were selected with diverse genetic constitution and ploidy levels (Table 2). All these cultivars were observed to have differential responses to BXW disease in the field (Eden-Green 2004; Ssekiwoko et al. 2006). The in vitro plantlets were regenerated through micropropagation using apical shoot tips (Tripathi et al. 2003). Apical shoot tips of the banana cultivars were cultured on regeneration medium (RM). The RM contained the macro- and micro- mineral salts and vitamins of MS (Murashige and Skoog 1962), myo-inositol (100 mg l^{-1}), sucrose (4% w/v), ascorbic acid (100 mg l^{-1}) and supplemented with 6-benzylaminopurine (BAP, 5 mg l^{-1}). The pH of the medium was adjusted to 5.8 and solidified with 0.2% (w/v) gelrite. The medium (15 ml) was dispensed into culture tubes (25×150 mm, Sigma-Aldrich) and autoclaved at 121°C and 103 Kpa for 20 min.

Table 2 Banana cultivars used to evaluate resistance to *Xanthomonas* wilt

Cultivars	Genome	Genetic group
Mpologoma	AAA-EA	East African Highland Bananas – Musakala clone set, cooking banana
Nakitembe	AAA-EA	East African Highland Bananas – Nakitembe clone set, cooking banana
Mbwazirume	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
FHIA-17	AAAA	Hybrid, dessert banana
FHIA-25	AABB	Hybrid, cooking banana
Musa balbisiana	BB	Fertile diploid parent

The shoot-tip cultures were incubated at 26±2°C with 16 h photoperiod supplied by fluorescent tubes (94 μmol m^{-2} s^{-1}) for 4 weeks. Cultures were routinely sub-cultured on fresh semi-solid regeneration medium every 3–4 weeks. For the elongation and maturation of shoots, individual shoots were transferred to semi-solid medium supplemented with BAP (3 mg l^{-1}) and indole-3-acetic acid (IAA, 0.3 mg l^{-1}). After 3 weeks, the elongated individual shoots were transferred to a rooting medium containing indole-3-butyric acid (IBA, 1 mg l^{-1}) and incubated in the culture room under normal conditions. The rooted plantlets with 3–4 leaves were used for in vitro screening experiments.

For in vivo screening tests the rooted plantlets were transferred to sterile soil in plastic bags (20 cm× 13 cm) 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.

Preparation of bacterial suspensions for plant inoculation

A single colony of each bacterial isolate was inoculated into 25 ml of YTS medium 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 the pellet was re-suspended 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.

Optimization of inoculation procedures

The in vitro plantlets of banana cv. Pisang Awak (Kayinja) with 3–4 fully expanded leaves and developed roots were removed from the growth medium. The culture medium adhering to roots was removed with the help of sterile forceps and plantlets inoculated with *Xcm* isolate 006/wks/05. Two inoculation procedures were evaluated. (1) Root dipping method – The roots of in vitro plantlets were damaged with a cut using a scalpel and then dipped into 25 ml of bacterial suspension (10^8 cfu ml^{-1}) in a Petri dish (90 mm) for 30 min. Excess bacterial suspension was

removed by blotting the plantlets on sterile tissue paper. The inoculated plantlets were cultured in baby food jars (98.5×59 mm, Sigma-Aldrich cat no. V0633) containing 30 ml of regeneration medium. The cultures were incubated under normal laboratory conditions at 26±2°C with a 16 h photoperiod supplied by fluorescent tubes (94 μmol m^{-2} s^{-1}). Injured roots of the control plantlets were dipped in sterile water. (2) Pseudostem injecting method – 100 μl of bacterial suspension (10^8 cfu ml^{-1}) was injected into the middle part of the pseudostem of in vitro plantlets using a syringe fitted with a 28 gauge needle. Plantlets were then cultured in baby food jars (98.5×59 mm, Sigma-Aldrich cat no. V0633) with 30 ml of regeneration medium and incubated under normal laboratory conditions. Ten plantlets were used for each treatment and each treatment was repeated three times. The control plantlets were injected with sterile water. The inoculated plantlets were assessed every day for 6 weeks for appearance of BXW disease symptoms characterized by chlorosis or necrosis and complete wilting of plants.

Screening of in vitro plantlets

Eight banana cultivars were tested with 16 isolates of *Xcm* by injecting inoculum into the pseudostem of in vitro plantlets. Ten plantlets of each banana cultivar were inoculated with each bacterial isolate, whereas control plantlets were injected with sterile water. The treatments were laid out in a randomized factorial design (8 cultivars × 16 isolates) and the experiment was repeated three times. The pathogenic bacteria were re-isolated from wilted plants and identified as *Xcm* on the basis of their characteristic morphology as yellowish, mucoid and circular colonies on YTSA-CC semi-selective medium.

Screening of in vivo potted plants

Eight banana cultivars were screened for resistance to *Xcm* isolate 006/wks/05 using the pseudostem injecting method. Ten week-old tissue culture grown plants transferred into sterile soil in plastic bags (20 cm×13 cm) were used for inoculation. For the in vivo experiment, only one pathogen isolate was used on the eight banana cultivars. Again, the trial was repeated in three separate experiments. Ten plants per cultivar were used for each experiment. Ten plants of each cultivar inoculated with sterile water were used as the control.

Disease assessment due to BXW

Plants were assessed every day for 6 weeks for disease symptoms, with preliminary symptoms evaluated as chlorosis or necrosis of leaves, and developed symptoms of complete wilting of plants. Wilt incidence was measured as number of wilted plants from total number of plants inoculated. The relative resistance of cultivars to BXW was evaluated 6 weeks after inoculation based on wilt incidence and the following disease rating scale; Resistant (R) – no plants wilted; Moderately Resistant (MR) – <50% plants wilted, Susceptible (S) – >50% plants wilted, Highly Susceptible (HS) – all plants wilted.

Experimental design and statistical analysis

The homogeneity of variance of the three repeated experiments was confirmed using Levene's test. Hence, the treatment means presented were over the three replicates. Analysis of variance (ANOVA) was conducted using the GLM procedure in SAS, and separation of the treatment and interaction means (where significantly different) was by least significant difference (LSD) at $P=0.05$ (SAS 2003). The time interval between inoculation and appearance of disease symptoms, complete wilting, and wilt incidence in eight banana cultivars inoculated with 006/wks/05 isolate of *Xcm* using in vitro and in vivo methods were compared using Pearson correlation analysis in the SAS programme (2003).

Results

Optimization of inoculation procedures

The plantlets inoculated by dipping damaged roots in bacterial inoculum suspension did not show any symptoms of disease even after 30 days of inoculation. In contrast, the method of injecting inoculum into the pseudostem of in vitro plantlets produced symptoms of chlorosis and necrosis within 10–11 days after inoculation with complete wilting 22–25 days after inoculation for all plantlets. Control plantlets, inoculated with sterile water, remained healthy. Therefore, the pseudostem injection method was selected as the optimal procedure and was used for all subsequent screening experiments.

Table 3 Time interval (mean number of days) between inoculation and appearance of first disease symptoms after inoculating eight banana cultivars with isolates of *Xanthomonas campestris* pv. *musacearum* using the in vitro screening method

Isolates	Cultivars/mean[a] number of days for appearance of first disease symptoms[b]								
	Mpo	Nkt	Mbw	P. Awak	S. Ndi	FHIA-17	FHIA-25	M. balb	LSD ($P \leq 0.05$)
001/gyz/05	13.3	19.7	13.7	11.7	13.3	12.7	14.7	21.3	1.3
002/wks/05	13.7	16.0	14.7	11.3	13.7	12.3	15.3	20.3	0.8
006/wks/05	12.0	18.7	13.0	10.7	12.7	13.0	13.7	21.0	1.2
007/wks/05	12.7	16.0	13.3	11.7	13.3	13.3	15.3	22.0	1.5
036/lra/05	14.3	18.7	14.3	11.7	13.7	13.0	15.3	23.0	1.1
038/lra/05	12.3	19.7	13.7	10.7	12.7	12.3	14.3	21.7	1.0
031/Kib/05	12.7	16.7	14.0	10.3	13.3	13.3	15.3	23.0	1.3
033/Kib/05	14.3	16.0	13.7	11.3	13.0	12.7	15.3	21.7	1.7
045/kla/05	13.0	16.3	13.7	11.0	13.3	13.3	15.0	23.0	1.0
019/iga.05	14.3	16.3	12.7	10.7	13.7	12.0	14.7	20.3	0.9
023/kay/05	13.3	19.3	14.0	11.7	13.7	14.0	16.0	24.0	1.1
003/muk/05	13.3	18.7	13.7	11.7	13.3	13.0	14.3	21.7	1.3
041/nsg/05	12.0	16.0	12.7	10.3	12.7	12.7	13.7	21.7	1.7
014/luw/05	13.0	16.7	13.3	11.3	13.0	13.3	15.3	20.7	1.9
043/msd/05	13.3	16.3	13.3	11.3	13.3	12.3	15.0	24.3	1.8
034/kibo/05	13.3	16.7	13.0	11.3	13.3	13.3	14.3	22.3	1.7
LSD ($P \leq 0.05$)	1.5	1.2	1.2	1.3	0.8	1.2	2.1	2.2	

Mpo Mpologoma, *Nkt* Nakitembe, *Mbw* Mbwazirume, *P. Awak* Pisang Awak, *S. Ndi* Sukali Ndiizi, *M. balb Musa balbisiana*

[a] Mean of three replicates

[b] The disease symptoms were chlorosis or necrosis on leaves of inoculated plants

Table 4 Time interval (mean number of days) between inoculation and complete wilting of plants of eight banana cultivars inoculated with isolates of *Xanthomonas campestris* pv. *musacearum* using the in vitro screening method

Isolates	Cultivars/mean[a] number of days for complete wilting								
	Mpo	Nkt	Mbw	P. Awak	S. Ndi	FHIA-17	FHIA-25	M. balb	LSD ($P \leq 0.05$)
001/gyz/05	35.0	44.0	36.3	27.7	32.0	35.3	37.3	–	1.9
002/wks/05	33.7	44.7	36.3	27.0	31.7	33.7	43.0	–	2.5
006/wks/05	28.0	43.7	30.7	24.0	30.0	36.0	40.0	–	3.3
007/wks/05	33.3	44.0	34.0	28.7	32.7	31.3	38.7	–	3.8
036/lra/05	31.7	42.0	31.0	25.3	31.3	37.3	36.7	–	6.2
038/lra/05	32.3	44.3	34.3	27.7	31.0	28.3	36.3	–	4.5
031/kib/05	35.3	42.3	27.3	25.0	30.0	35.3	36.7	–	2.6
033/kib/05	35.3	44.3	31.3	26.3	31.7	36.3	39.7	–	2.4
045/kla/05	35.3	43.7	35.7	28.0	32.3	35.7	38.3	–	1.9
019/Iga/05	33.7	41.0	29.7	26.7	30.7	33.7	39.0	–	2.2
023/kay/05	35.0	43.0	33.7	27.7	31.7	29.7	39.0	–	2.4
033/muk/05	34.7	42.7	35.7	27.0	32.3	35.7	40.0	–	2.2
041/nsg/05	35.7	43.0	37.3	25.7	32.3	33.7	38.3	–	3.4
014/luw/05	31.3	42.0	30.3	26.3	30.7	30.0	38.3	–	2.4
043/msd/05	35.7	44.7	34.0	27.3	31.7	31.7	38.7	–	2.2
034/kibo/05	34.3	44.0	36.0	26.7	32.7	31.0	37.0	–	2.5
LSD ($P \leq 0.05$)	3.2	4.9	2.9	3.4	2.0	3.8	2.4	–	

Mpo Mpologoma, *Nkt* Nakitembe, *Mbw* Mbwazirume, *P. Awak* Pisang Awak, *S. Ndi* Sukali Ndiizi, *M. balb Musa balbisiana*

– = no wilting observed

[a] Mean of three replicates

Screening of in vitro plantlets

For the eight cultivars of banana tested, control plantlets inoculated with water did not show any disease symptoms while the plantlets inoculated with all the isolates of *Xcm* developed leaf chlorosis or necrosis. However, the incidence of disease and incubation period for the appearance of symptoms varied significantly ($P<0.0001$) between cultivars (Table 3). The cv. Pisang Awak developed symptoms, on average, 11 days after inoculation for all plants tested, while *M. balbisiana* took longer (22 days) and only 15–20% of the plants showed symptoms. The youngest leaf of *M. balbisiana* showed necrotic and chlorotic patches but recovered from these and the plants were subsequently healthy. The response of the

Fig. 1 Comparison between different banana cultivars for complete wilting, 6 weeks after inoculation with *Xanthomonas campestris* pv. *musacearum* using the in vitro screening method; **a** Mpologoma, **b** Pisang Awak, **c** Mbwazirume, **d** Sukali Ndiizi, **e** FHIA-17, **f** FHIA-25, **g** Nakitembe, **h** *Musa balbisiana* and **i** control plant inoculated with water

cvs Mbwazirume, Mpologoma, Sukali Ndiizi, FHIA-17 and FHIA 25 was similar with first symptoms appearing, on average, 12–14 days after inoculation, whereas, cv. Nakitembe had a significantly ($P<$ 0.0001) longer incubation period (average 18 days) for appearance of symptoms.

Plants of all the cultivars tested except for *M. balbisiana* eventually wilted completely. The period between inoculation and complete wilting varied significantly ($P<0.0001$) between cultivars (Table 4, Fig. 1) as did the incidence of completely wilted plants (Table 5). No plants of cv. *M. balbisiana* wilted completely and eventually these plants resembled healthy control plants. In contrast, all the plants of Pisang Awak wilted completely 24–28 days after inoculation. For cvs Mbwazirume, Mpologoma, Sukali Ndiizi and FHIA-17, complete wilting was observed in 83–90% of plants 30–35 days after inoculation, whereas for FHIA-25 only 75% of plants wilted 36–43 days after inoculation. Some of the plants of cv. Nakitembe which showed initial symptoms of necrosis recovered, resulting in only 45% wilting 42–44 days after inoculation. The bacteria were recovered from all the wilted plants and circular yellow mucoid colonies of *Xcm* appeared on semi-selective medium confirming that symptoms were due to the bacteria that was used as inoculum.

Analysis of variance showed significant variation ($P<0.0001$) in susceptibility of eight banana cultivars to BXW. There was no significant difference ($P=$ 0.92) between the 16 isolates of *Xcm* in wilt incidence within the same cultivar, whereas a significant difference ($P<0.0001$) was observed in incubation period for appearance of symptoms and complete wilting of plants.

Screening of in vivo potted plants

As a consequence of results showing no significant difference ($P=0.92$) in wilt incidence between isolates of *Xcm*, only one isolate (006/wks/05) was used to test resistance in eight banana cultivars using in vivo potted plants. For the eight cultivars tested, control plants did not show symptoms but following inoculation they varied in their response (Table 6, Fig. 2) similar to results produced by the in vitro screening

Table 5 Wilt incidence in eight banana cultivars inoculated with isolates of *Xanthomonas campestris* pv. *musacearum* using the in vitro screening method

Isolate	Cultivars/mean[a] wilt incidence							
	Mpo	Nkt	Mbw	P. awak	S. Ndi	FHIA-17	FHIA-25	M. balb
001/gyz/05	83.3	43.7	86.7	100.0	86.7	80.0	70.0	0
002/wks/05	86.7	43.7	86.7	100.0	83.3	93.3	80.0	0
006/wks/05	83.3	42.7	80.0	100.0	80.0	90.0	70.0	0
007/wks/05	90.0	43.7	83.3	100.0	83.3	86.7	76.7	0
036/lra/05	83.3	48.7	86.7	100.0	86.7	96.7	80.0	0
038/lra/05	86.7	46.3	83.3	100.0	80.0	86.7	73.3	0
031/Kib/05	86.7	47.0	83.3	100.0	86.7	90.0	73.3	0
033/Kib/05	83.3	43.7	86.7	100.0	80.0	83.3	73.3	0
045/kla/05	80.0	46.3	83.3	100.0	80.0	93.3	76.7	0
019/Iga/05	80.0	43.7	83.3	100.0	83.3	90.0	73.3	0
023/kay/05	90.0	46.3	86.7	100.0	86.7	90.0	70.0	0
033/muk/05	86.7	48.3	83.3	100.0	80.0	86.7	80.0	0
041/nsg/05	83.3	43.7	86.7	100.0	80.0	90.0	73.3	0
014/luw/05	86.7	43.7	86.7	100.0	83.3	90.0	76.7	0
043/msd/05	83.3	43.7	83.3	100.0	86.7	86.7	76.7	0
034/kibo/05	83.3	43.7	83.33	100.0	80.0	93.3	80.0	0
Mean[b]	84.8d	44.9b	84.58d	100.0f	82.9d	89.2e	75.1c	0a
Rating	S	MR	S	HS	S	S	S	R

Wilt Incidence % = [No. of plants wilted/total number of plants inoculated] × 100

Mpo Mpologoma, *Nkt* Nakitembe, *Mbw* Mbwazirume, *P. Awak* Pisang Awak, *S. Ndi* Sukali Ndiizi, *M. balb Musa balbisiana*

HS Highly susceptible, *S* Susceptible, *MR* Moderate resistant, *R* Resistant

[a] Mean of three replicates

[b] Means with the same letter are not significantly different

Table 6 Comparison of time period (mean number of days) between inoculation and appearance of disease symptoms, complete wilting of plants, and wilt incidence in eight banana cultivars inoculated with 006/wks/05 isolate of *Xanthomonas campestris* pv. *musacearum* using the in vivo and in vitro screening methods

Cultivars	Mean[a,b] number of days for appearance of disease symptoms[c]		Mean[a,b] number of days for complete wilting		Mean[a,b] wilt incidence		Rating
	In vivo	In vitro	In vivo	In vitro	In vivo	In vitro	
Mpologoma	14.0c	12.0d	33.7c	28.0c	86.7d	83.3 d	Susceptible
Nakitembe	17.0b	18.7b	50.7a	43.7a	40.3b	42.7 b	Moderately Resistant
Mbwazirume	13.3d	13.0d	32.0c	30.7c	83.3d	80.0 d	Susceptible
Pisang Awak	11.0e	10.7e	26.3e	24.0e	100.0f	100.0f	Highly Susceptible
Sukal Ndiizi	13.3d	12.7d	31.3d	30.0c	86.7d	80.0d	Susceptible
FHIA-17	13.3d	13.0d	30.0d	36.0d	93.3e	90.0 e	Susceptible
FHIA-25	15.0c	13.7c	44.7b	40.0b	66.7c	70.0c	Susceptible
M. balbisiana	22.3a	21.0a	–	–	0a	0a	Resistant
LSD ($P \leq 0.05$)	1.6	1.2	2.2	3.3	7.8	10.5	
Pearson correlation	$r=0.947$, n = 24, $P<0.0001$		$r=0.836$, n=21, $P<0.0001$		$r=0.968$, n=24, $P<0.0001$		

Wilt Incidence % = [No. of plants wilted/total number of plants inoculated] × 100

Mpo Mpologoma, *Nkt* Nakitembe, *Mbw* Mbwazirume, *P. Awak* Pisang Awak, *S. Ndi* Sukali Ndiizi, *M. balb Musa balbisiana*

[a] Mean of three replicates

[b] Means with the same letter are not significantly different within each column

[c] The disease symptoms were chlorosis or necrosis on leaves of inoculated plants

procedure. The incidence of disease, incubation period for the appearance of symptoms and complete wilting varied significantly ($P<0.0001$) between cultivars. The banana cv. Pisang Awak developed chlorotic or necrotic symptoms 11 days after inoculation and all plants wilted completely 26 days after inoculation. Cultivars Mbwazirume, Mpologoma, Sukali Ndiizi and FHIA-17 developed chlorosis or necrosis 13–14 days after inoculation with complete wilting in about 83–93% of plants 30–33 days after inoculation. Cultivar FHIA-25 first developed symptoms 15 days after inoculation and complete wilting 44 days after inoculation in about 66% of plants, whereas Nakitembe showed necrosis 17 days after inoculation and complete wilting 50 days after inoculation in about 40% of plants. About 10% of *M. balbisiana* plants showed chlorosis and necrosis in the youngest leaf only about 22 days after inoculation, but these plants recovered from disease and no wilted plants were observed. The banana cultivars tested using in vivo plants were classified into the same groups of resistance as previously using in vitro plantlets (Table 6). Pearson correlation indicated that the in vitro and in vivo methods were strongly correlated ($r=0.96$; $P<0.0001$) for wilt incidence and ($r=0.95$; $P<0.0001$) for appearance of disease symptoms.

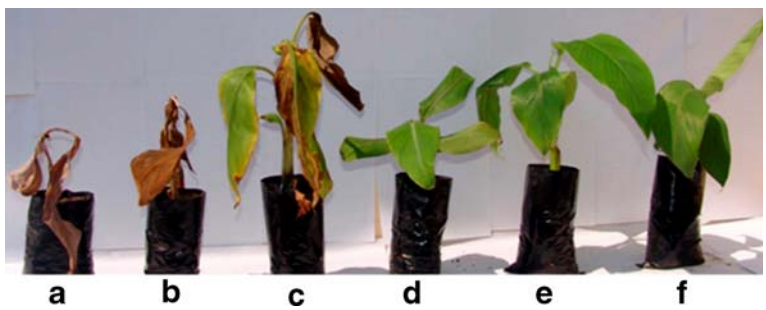

Fig. 2 Comparison between different banana cultivars for complete wilting, 6 weeks after inoculation with *Xanthomonas campestris* pv. *musacearum* using in vivo potted plants; **a** Pisang Awak, **b** Mpologoma, **c** FHIA-25, **d** Nakitembe, **e** *Musa balbisiana* and **f** control plant inoculated with water

Evaluation of banana cultivars for resistance

The response to artificial inoculation with *Xcm* varied between the cultivars of banana tested (Tables 5, 6). The relative resistance of cultivars was evaluated based on the incidence of wilted plants both in vitro and in vivo (Tables 5, 6). Cultivar Pisang Awak was found to be highly susceptible with 100% wilt incidence with the in vitro screening method, which was further confirmed with in vivo potted plants. Cultivars Mbwazirume, Mpologoma, Sukali Ndiizi, FHIA-17 and FHIA-25 showed a similar pattern of wilting and were rated as susceptible to BXW. Banana cv. Nakitembe showed wilt incidence of about 40–45% and was rated as moderately resistant. Cultivar *M. balbisiana* was rated as resistant as none of the plants completely wilted after inoculation of in vitro or in vivo plants.

Discussion

Continuous and intense evaluation of banana germplasm for disease resistance is one of the basic requirements for effective and sustained implementation of integrated disease management programmes. Identification of disease resistance depends greatly on adequate assessment and disease evaluation methods. Currently, banana germplasm is screened in the field which is labour and time-consuming and subject to variation because of the non-uniform distribution of pathogen inoculum in the soil. Artificial inoculations with uniform inoculum in the field attract regulatory concerns and can pose a risk to neighbouring farmers. The technique, described here, using in vitro plantlets is thus a valuable tool for rapid and mass screening of banana cultivar resistance to BXW. Evaluation of germplasm for resistance to several plant diseases using rapid screening methods has been previously reported for several crops (Asea et al. 2005; Denman et al. 2005; Engle et al. 2003; Kull et al. 2003; Onyeka et al. 2005; Steventon et al. 2002). Similar early evaluation methods have also been developed for screening *Musa* spp. for resistance to the fungal disease, black leaf streak (Mobambo et al. 1994; Twizeyimana et al. 2007).

Variation in isolate pathogenicity can influence the value of controlled environment resistance screening but for BXW we found no variation in pathogenicity between the 16 isolates tested on the eight cultivars of banana. The root-dip method is one of the most common methods of inoculation of plant seedlings (Steventon et al. 2002) but in this study the inoculation method of dipping damaged roots was not successful. Experiments in our laboratory showed that *Xcm* cannot grow on acidic media and the pH of tissue culture medium used (pH 5.8) was acidic. Subsequent tests showed *Xcm* cannot grow on this tissue culture medium. Because the dipped roots were in contact of this tissue culture medium, this may explain why *Xcm* could not multiply and induce disease symptoms. In contrast, the inoculation method of injecting bacterial inoculum into the pseudostem was successful in inducing disease.

The characteristic symptoms of BXW of leaf chlorosis and necrosis and plant wilting were observed after artificial inoculation. Symptoms following natural infection in young plants were similar to those we observed from artificial inoculation, with leaves progressively wilting and the youngest leaves affected first, resulting in plant death. *Musa balbisiana* resisted BXW while for the other cultivars the incubation period for appearance of symptoms, complete wilting and incidence of wilted plants varied. Wilting occurred earliest in the highly susceptible cv. Pisang Awak but was delayed in the moderately resistant cv. Nakitembe. All plants wilted in the highly susceptible cultivar while only about 40–45% plants of the moderately resistant cultivar wilted. Some yellow patches were observed in one leaf of *M. balbisiana* and symptoms did not spread, possibly due to a hypersensitive reaction (Atkinson et al. 1985) and the plants recovered and thereafter appeared healthy and no *Xcm* 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 the whole plant to the pathogen (Kiraly 1980; Klement and Goodman 1967).

Disease development in the in vitro plants and in vivo plants were well correlated and cultivars were consistently ranked using the two methods. The symptoms produced in vitro were very similar to the in vivo screening tests and represented those due to BXW under natural conditions in the field. The commonly grown beer banana cv. Pisang Awak (known locally in Uganda as Kayinja) was found to be the highly susceptible, while the cooking banana

cv. Nakitembe was moderately resistant and *M. balbisiana* identified as resistant. Pisang Awak was reported to be the highly susceptible cultivar with typical inflorescence infection in the fields (Eden-Green 2004). Nakitembe has been found to suffer less from BXW in the field. Nakitembe could be deployed in the field by recommending it to the farmers to reduce the impact of BXW, while the cv. *M. balbisiana,* identified as resistant, could be of use to future breeding programmes.

The screening procedure using in vitro plantlets offers huge potential for the rapid testing of large number of cultivars. The response to BXW can be demonstrated within 6 weeks using this novel technique and highly susceptible germplasm can be identified and eliminated from further evaluations. The various means of infection in the field are by insect transmission through the male flowers, through the roots, infected plant materials, or the use of contaminated tools (Eden-Green 2004). This method of artificial inoculation in the pseudostem is similar to natural infection through injury by contaminated tools. Disease development is also affected by the age of the host plant (Agrios 1997). Therefore, promising resistant cultivars identified by this screening method should be allowed to continue to grow into mature plants and then evaluated for resistance in the field with natural infection (through flowers or roots) as the final confirmatory test. This method using in vitro plantlets can be used for the screening of *Musa* germplasm with large numbers of cultivars for resistance to *Xanthomonas* wilt and other bacterial diseases.

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