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Banana influences on differential expression of hypersensitive response and pathogenicity gene f (*hrpf*) in *Xanthomonas campestris* pv. *musacearum*

Mudonyi, B.[1]*, Tusiime, G.[2], Kubiriba, J.[1], Tushemereirwe, W. K.[1] and Changa, C. M.[1]

[1]National Agricultural Research Laboratories PO Box 7065 Kampala, Uganda.
[2]College of Agriculture and Environmental Sciences, Makerere University PO Box 7062, Kampala, Uganda.

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Banana *Xanthomonas* wilt is a devastating disease of cultivated banana in East and Central Africa, manifesting as initial leaf wilting, premature fruit ripening and eventual death of all infected plants leading to total yield loss. In order to contribute towards development of effective disease control options, *hrpf* expression by *Xanthomonas campestris* pv *musacearum* (*Xcm)* during establishment of *Xanthomonas* wilt infection was determined. To successfully initiate infection, plant pathogenic bacteria deliver effector proteins into host cells using specialized protein transport system such as the Type III secretion system (TTSS). It is a syringe needle-like translocation apparatus essential for delivery of effector proteins into the host cells and hypersensitive response and pathogenicity gene f (*hrpf*) encodes one of the structural proteins for effector protein delivery. In this study, *hrpf* expression by *Xcm* during establishment of *Xanthomonas* wilt infection was determined in minimal medium amended with extracts from susceptible banana genotypes and banana host plants. Total RNA was isolated from *Xcm* recovered from inoculated plants and also from minimal medium amended with banana extracts; cDNA synthesised and *hrpf* amplified by PCR using gene specific primers. Findings showed that *Xcm* multiplied in susceptible host banana and minimal medium amended with their extracts but not in resistant *M. balbisiana* and its extract. *hrpf* gene was thus amplified from cDNA samples of susceptible banana genotypes and their extracts suggesting its expression and involvement in the successful establishment of *Xanthomonas* wilt disease by *Xcm*.

Key words: *Xanthomonas campestris* pv. *musacearum* (*Xcm)*, Type three secretion system (TTSS), hypersensitive response and pathogenicityf (*hrpf*) gene, banana.

INTRODUCTION

Banana *Xanthomonas* wilt (BXW) caused by *Xanthomonas campestris* pv. *musacearum* (*Xcm)* is a rapidly spreading disease that leads to total destruction of cultivated banana in East and Central Africa (Tushemereirwe et al., 2003). Typical BXW symptoms are leaf wilting, premature fruit ripening and eventual

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

death of infected plants (Karamura et al., 2008). All indigenous banana varieties that are widely grown by the farmers are susceptible to *Xcm (*Ssekiwoko et al*.,* 2006). If left uncontrolled, BXW epidemic is projected to cause economic loss of $2 to $8 billion within a period of one decade. The loss of such magnitude ultimately endangers food security and livelihood of more than 80% of the population that is dependent on banana (Tushemereirwe et al., 2003). With continued devastation of banana by *Xcm*, there has been increasing need to understand molecular mechanisms of the infection process and bacteria effector proteins involved with the aim of developing appropriate control measures.

Most plant pathogenic bacteria are equipped with injectosomes for delivery of effector proteins to the interior of host cells for example *Pseudomonas syringae*, *Erwinia amylovora*, *Pantoae* agglomerans members of injectosome family *Hrp*1 and *Burkholderia Pseudomallei*, *Ralstonia solanacearum* and *X. campestris* which belong to injectosome family *Hrp*2 (Galan and Collmer, 1999). The TTSS that is encoded by the hypersensitive response and pathogenicity (*hrp*) genes is used to secrete and translocate effector proteins from bacteria into host cells. Basically, the TTSS is a syringe needle-like translocation apparatus, made up of inner and outer membrane rings and a protruding filament called pilus which functions as a conduit to guide the translocation of effector proteins to the interior of the host cells (Jin et al*.,* 2001; Webber et al*.,* 2005). After delivery into the host, effector proteins manipulate host cell functions and break host defences, allowing bacteria to multiply to cause disease development (Alfano and Collmer, 2004; Yang and White, 2005; Grant et al., 2006; Gurlebeck et al., 2006; White et al., 2009; Kay and Bonas, 2009; Buttner and Bonas, 2010). However, resistant and non-host plants recognise specific effector proteins triggering the hypersensitive response (HR). HR is a rapid, local, programmed cell death that is induced upon recognition of the pathogen and concomitant with the inhibition of pathogen growth within the attacked resistant plants. In resistant hosts, these effector proteins show an 'avirulent' activity limiting the pathogen's host range.

A single *Xanthomonas* genome encodes 20-30 TTSS effector proteins and this varies between species and strains. The variance in TTSS effector proteins produced among diverse species and strains of bacteria is believed to be the main determinant for host range of a given pathogen (Zou et al., 2006). 26 functional TTSS effector (*hrp)* candidate genes within the NCBI data base of *Xcm* and *X. vasicola* pv *vasicolorum (Xvv*) were identified although only 15 coded for products related to the *hrp* cluster (Aritua et al., 2008; Data not published). Six *hrp* genes (*hrpA- hrpF)* were identified and isolated from *X. campestris* pv*. vesicatoria* (*Xcv)* and *X. oryzae* pv oryzae (*Xoo*) where they were responsible for pathogenicity and induction of hypersensitive response *HR* (Li et al., 2011). Of all the *hrp* genes, *hrpf* was found to be indispensible for type III secretion *in vitro* and essential for introduction of virulence factors into the plant by *Xcv* (Zou et al., 2006). It was upon this background that *in vitro* study of minimal medium amended with crude extracts from different banana genotypes was designed to establish whether chemical compounds in these extracts could influence *Xcm* growth and therefore expression of *hrpf*. If *Xcm* growth *in vitro* has a bearing on the rate of *hrpf* expression both *in vitro* and *in planta*, it would then mean that different banana genotypes have different genes/compounds that determine either susceptible or resistant reaction. The study therefore, correlates growth of *Xcm in vitro* to *hrpf* expression and compares it with wilt disease development and *hrpf* expression *in planta*. While most bananas are susceptible, *M. balbisiana*, a wild relative is resistant (Ssekiwoko et al., 2006) and yet it is not known if the different banana genotype responses to *Xcm* infection are influenced by expression of *hrpf*. In addition, the general belief that for successful host colonization *Xanthomonads* express *hrpf,* a member of the *hrp* gene cluster which encodes the TTSS had not been confirmed for *Xcm*/banana pathosystem.

MATERIALS AND METHODS

In vitro culture and recovery of *Xcm*

In vitro studies were conducted in various liquid media including minimal medium (containing 20 mM NaCl, 10 mM $(NH_4)_2PO_4$, 5 mM $MgSO_4$, 1 mM $CaCl_2$, 0.16 mM KH_2PO_4, 0.32 mM K_2HPO_4 and 10 mM sucrose in 1 L of distilled water). Also studies were extended to minimal medium amended with banana extracts from *M. balbisiana* (BB) resistant to *Xcm,* Psangawak (ABB) popularly called "Kayinja in Uganda" which is very susceptible to both tool and insect transmitted *Xcm* and 'Saba' (ABB); susceptible mainly to insect transmitted *Xcm*. Minimal medium pH was adjusted to 7.4. To prepare the banana extracts, 10 g of leaf tissues harvested from three months old tissue culture plants were crushed in a blender, mixed with 100 ml of minimal medium and filter sterilised using 0.2 micro filters. 30 ml of each amended extract was dispensed in sterile 100 ml conical flasks.

Xcm from a preserved culture at National Agricultural Research Laboratories (NARL) was used as inoculum. A single colony of pure culture grown on Yeast Peptone Glucose Agar (YPGA) medium for 48 h at 28ºC (Figure 4) was multiplied on the same medium by sub culturing. The cells were mixed with sterile water in a MacConkey bottle to form a suspension and optical density (OD) adjusted to a concentration of 0.1 at OD_{600} ($1x10^8$ CFU).

30 ml of amended and 30 ml of non-amended (control) culture media were inoculated with 0.5 ml of *Xcm* suspension, and incubated at 28°C on an orbital shaker at 100 rpm. A completely randomised design was adopted for the experiment where each treatment (media type) was replicated four times and the experiment repeated 3 times. Bacterial growth was determined at 24, 36, 48, and 60 h after inoculation by spectrophotometry. In addition, cells were recovered respectively after each time period, pelleted and immediately frozen in liquid nitrogen and stored at -80ºC awaiting RNA extraction.

Plant inoculation and recovery of *Xcm*

Three months old tissue cultured banana plantlets of Psang Awak,

Saba and *M. balbisiana* were inoculated with 0.5ml of bacterial suspension at the leaf petiole of the youngest leaf using syringe and needle. They were allowed to grow in pots in a screen house. *Xcm* was recovered after 7, 14 and 21 (for Pisang Awak and Saba) but 32 and 40 days after inoculation for *M. balbisiana* corresponding with *Xcm* incubation period in it. To recover *Xcm*, leaves were surface sterilised with 70% ethanol, sliced into small pieces and suspended in 30 ml of sterile water to allow *Xcm* to ooze out for 1 h (Angela and Rosato, 2003; Gottig et al., 2008). Suspended cells were recovered by centrifugation at 6000 rpm and 25°C and immediately frozen at -80ºC awaiting RNA extraction. Meanwhile plants were observed for 40 days for symptom development and disease progression.

RNA extraction, cDNA synthesis and PCR

Bacterial cells recovered from minimal medium with or without extracts and inoculated plants were washed twice with TE buffer (1 M Tris Hcl, pH 7.4 and 0.5 M EDTA, PH 8.0) to remove salts and *Xanthan* gum. RNA was extracted following the phenol-SDS method for bacterial total RNA extraction as described by Mahuku (2004), with slight modifications. 440 µl of phenol emulsion/phenol saturated buffer containing 200 µl of phenol, 240 µl of CTAB extraction buffer (1 M Tris HCl, pH 8.0; 0.5 M EDTA; pH 8.0; NaCl 81.76 g; β-mercaptoethanol 0.1% pH 8.0; PVP-40, 20 g) was used for RNA isolation. Cells in a falcon tube were chilled in liquid nitrogen and later ground to a powder in a mortar with a pestle. The powder was recovered in sterile 1.5 ml Eppendorff tubes and mixed with 440 µl of hot phenol saturated extraction buffer. This was followed by vortexing for 5 min and then incubated for 15 min at 65°C with mixing by inversion every after 5 min and finally left to cool at room temperature for 2 min. The suspension was then cleaned with 200 ml of 24:1 of chloroform iso amyl alcohol with mixing by inversion in order to break the cell walls. Contents were centrifuged for 10 min at 12000 rpm at room temperature and the supernatant pipetted into new sterile Eppendorff tubes and step above repeated in order to extract as much RNA as possible. The aqueous phase was collected and precipitated with $1/10^{th}$ volume of ammonium acetate (10 M) and an equal volume of ice-cold isopropanol at room temperature and allowed to rest for 15 min. The RNA pellet was recovered by centrifugation at 12000 rpm for 10 min. The pellet was washed with 500 µl of 70% ethanol by centrifugation for 3-5 min, air dried for 40 min and re-suspended in 40 µl of diethylpyrocarbonate (DEPC treated/nuclease free water). The resuspended RNA was then kept at -80°C for downstream applications.

RNA synthesised was DNase treated using the ready to use master mix kit (Bioneer, 2012) as follows; 2 µl RNA was pipetted into sterile Eppendorff tubes and mixed with 1 µl of 10× reaction buffer, 1 µl of DNase1 (1 U/µl) and 6 µl of DEPC-treated water making up a total reaction volume of 10 µl. The reaction was incubated at 37°C for 30 min and then 1 µl of 25 mM EDTA (EDTA is an exonuclease inhibitor, DNase1 is a 5' exonuclease inhibitor) or 1 µl of DNase stop solution was added in each tube. The reaction was again incubated at 65°C for 10 min to heat inactivate the DNase1 then placed on ice for 1 min. The effectiveness of DNase treatment was analyzed on 1% agarose gel electrophoresis to confirm that all residual DNA was removed before cDNA was synthesized from total RNA.

The cDNA was synthesized using the ready to use master mix kit (Bioneer, 2012) following the manufacturer's instructions. The reaction was constituted by; 2 µl of 4 µM RNA sample, 4 µl of 10X reaction buffer, 2 µl of 10 µM dNTPs, 2 µl of random oligo nucleotide primers, 0.25 µl of 40 U/µl RNasin, 1 µl of 10 U/µl reverse transcriptase. These were mixed and topped to 20 µl with sterile de-ionised water. The reaction mixture was incubated at 42°C for 60 min and terminated at 70°C for 15 min using the Mygenie 96 thermo cycler (Bioneer) to inactivate the reverse transcriptase**.** Tubes were later chilled on ice for 1 min and the reaction collected by brief centrifugation in a micro centrifuge. The cDNA synthesized was immediately used for PCR.

PCR was conducted using the constitutively expressed 16s rRNA gene primers (forward 5' TGGTAGTCCACGCCCTAAACG 3' and reverse 5'CTGGAAAGTTCCGTGGATGTC 3' (Gottig et al., 2008) and the *Xcmhrpf* gene specific primers Forward GACGAGTGGAAGGAATTTGC, and Reverse ACATGTCCCCACCTTGAATC (designed at NARL Kawanda by Aritua Valentine). For the PCR reactions, the following components were mixed on ice, 2 µl of 0.5 µM RNA sample, 2 µl of oligonucleotide primers (forward and reverse), 10 µl of a Bioneer PCR premix (containing 0.25 mM dNTPS, 1X reaction buffer, 2 mM $MgCl_2$) and topped up with 6 µl RNase-free water to make up to 20 µl total reaction mixture. PCR conditions for the constitutively expressed 16s rRNA were; initial denaturation of 94^oC for 2 min; then 30 cycles run each at cycle denaturation of 94°C for 1 min, primer annealing of 54°C for 1 min and extension of 72°C for 1 min and a final extension of 72°C for 10 min. PCR conditions for *Xcmhrpf* gene were; initial denaturation temperature of 95°C for 5 min, then 35 cycles each run at cycle denaturation of 94°C for 20 s, primer annealing of 60°C for 40 s, an extension of 72°C for 1 min and a final extension of 72°C for 10 min.

RESULTS

Effect of crude banana leaf extracts on medium colonisation by *Xcm*

The *Xcm* colonisation of minimal medium containing sucrose as carbon source was determined by measurement of turbidity or optical density using a spectrophotometer. Colonisation varied significantly with crude leaf extracts from different banana genotypes. *Xcm* colonisation followed a normal growth curve except for the medium amended with *M. balbisiana* leaf extracts that was barely detectable (Figure 1). Colonisation was more in minimal medium with leaf extracts of banana genotypes 'Kayinja' and 'Saba' than that without extracts or that with extracts of *M. balbisiana.* Colonisation of minimal medium amended with 'Kayinja' and 'Saba' leaf extracts or that without extracts increased exponentially with time to reach a peak 24 h after inoculation and thereafter declined steadily. Between 0 and 10 h there was no significant difference in colonisation of minimal medium without extracts and that with leaf extracts of 'Kayinja' and 'Saba'. Between 10 and 24 h, colonisation among all the media evaluated was significantly different. Optimum colonisation of medium without extracts and that with extracts of 'Kayinja' and 'Saba' was reached between 20 and 30 h after inoculation. Colonisation steadily declined at about 24 h after inoculation. Colonisation of those media did not decline further or increase 60 h after inoculation.

Banana bacterial wilt (BBW) symptoms on infected banana plants

BBW symptoms incited on infected banana plants varied

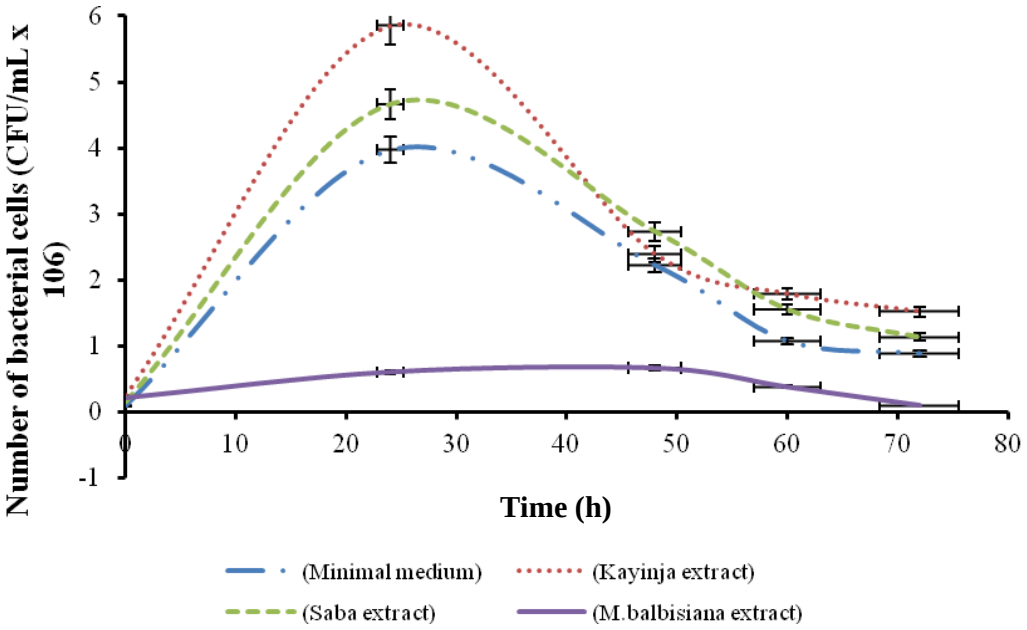

Figure 1. Growth curve of *Xcm* during colonisation of minimal medium amended with or without leaf extracts from susceptible and resistant banana genotypes. Error bars represent 95% confidence interval.

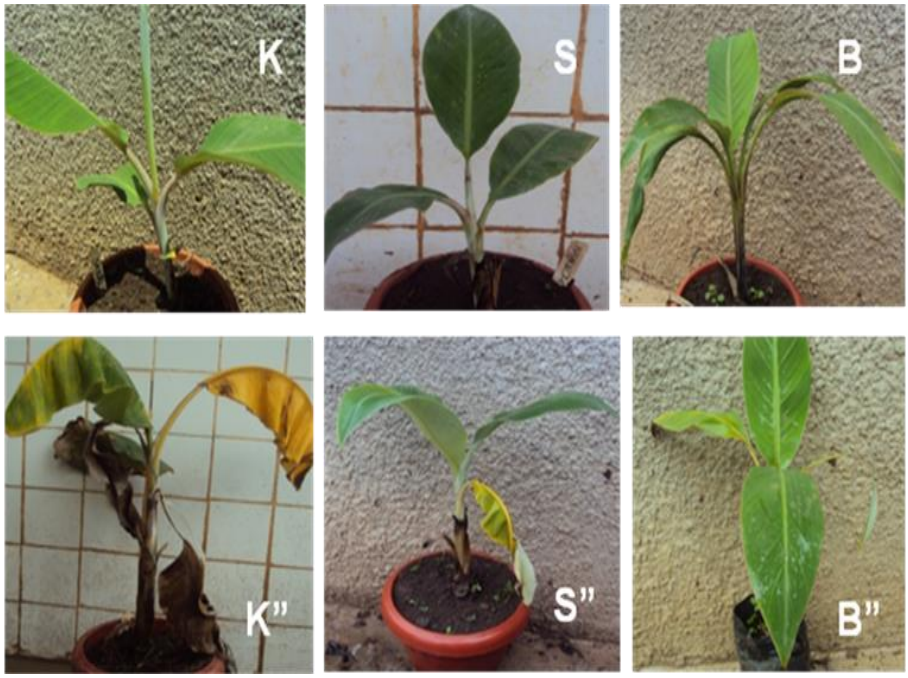

Figure 2. Banana bacterial wilt symptoms on infected plants of genotypes Kayinja (K"), Saba (S") and *M. balbisiana* (B") inoculated with *Xcm* (Bottom). Negative control reference plants of these genotypes; Kayinja (K), Saba (S) and *M. balbisiana* that were inoculated with water (Top) did not develop BBW symptoms throughout the observation period.

with the genotype inoculated (Figure 2). Typical BBW symptoms that progressed up to leaf wilting and eventual plant death after 32 days were incited on banana cv Kayinja. BBW symptom development on genotype Saba

Figure 3. Colony characteristics of 48 hr old YPGA culture of Bacteria re-isolated from inoculated plants was typical of *Xcm*.

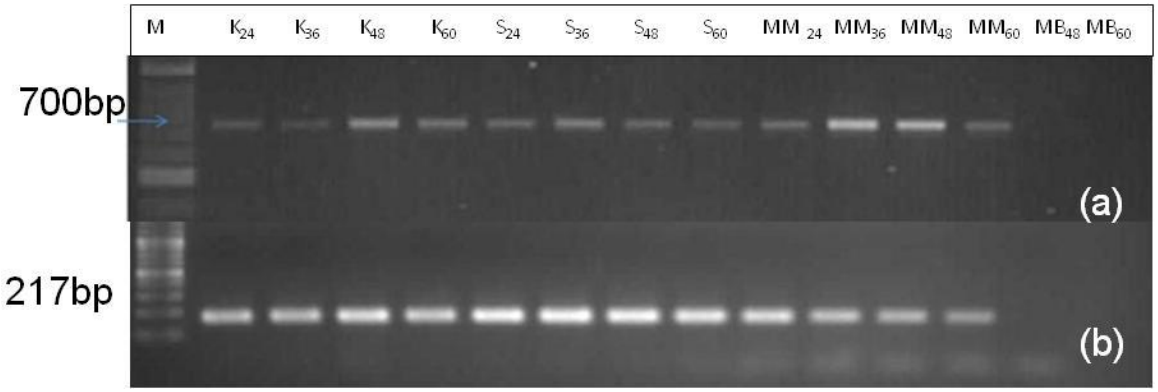

Figure 4. RT-PCR amplification products obtained with primers (a) specific for detection of 700 bp fragment of *hrpf* expressed by *Xcm* after 24, 36, 48 and 60 h of multiplication in minimal medium without (MM_{24}, MM_{36}, MM_{48}, MM_{60}) or with leaf extracts from susceptible banana cv Kayinja (K_{24}, K_{36}, K_{48}, K_{60}), moderately resistant banana genotype Saba (S_{24}, S_{36}, S_{48}, S_{60}) and resistant banana genotype *M. balbisiana* (MB_{48}, MB_{60}). Internal quality control RT-PCR was with primers (b) specific for detection of 217 bp fragment of 16S rRNA housekeeping gene. Lane M represents 100kb Molecular weight DNA marker.

was delayed but eventually infected plants also died. The BBW symptoms on genotype *M. balbisiana* took more than 35 days to develop and were restricted to the inoculated leaf only and symptoms later cleared. Also bacteria that showed yellow, mucoid, shiny, smooth colony growth characteristics on YPGA media were re-isolated from potted symptomatic plants of genotypes 'Kayinja', and 'Saba' (Figure 3). Ooze of bacterial cells was not recovered from the genotype *M. balbisiana.* Downstream h*rpf* expression studies during infection of banana performed utilizing bacterial ooze from inoculated plants were thus only carried out for banana genotpes 'Kayinja' and 'Saba'.

Detection of h*rpf* expression during infection of banana

RT-PCR internal positive control was carried out by detecting 217 bp fragment of *Xcm* 16S rRNA housekeeping gene. The fragment was detected from *Xcm* cDNA synthesized for samples that were obtained at 7, 14, and 21 h after inoculation of banana genotypes 'Kayinja' and 'Saba' (Figure 4). The intensity of 217 bp fragment band on 1% agarose gel was the same for all the samples analyzed. Intensity of 700 bp fragment of *hrpf* detected for *Xcm* samples isolated from both minimal medium and during infection of banana genotypes

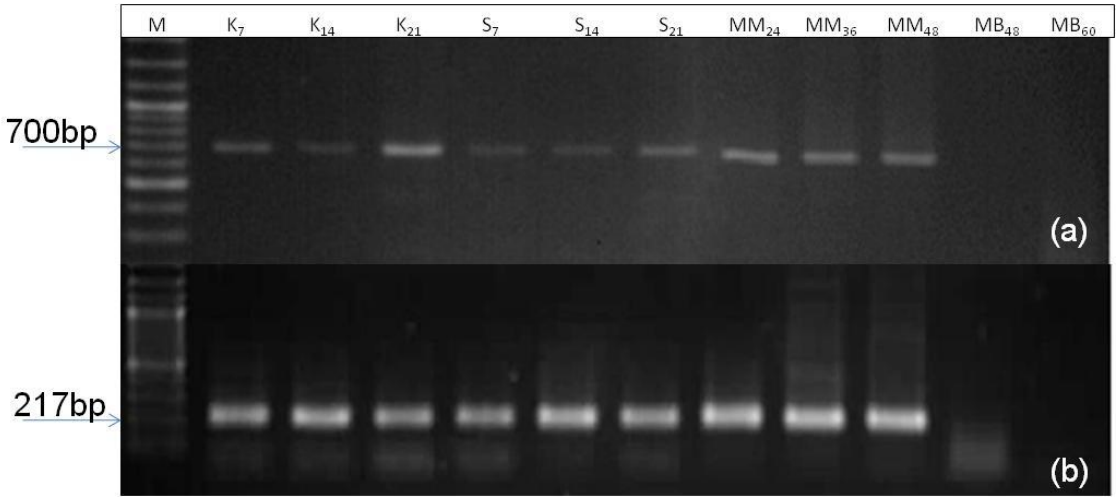

Figure 5. RT-PCR amplification products obtained with primers (a) specific for detection of 700bp fragment of *hrpf* expressed by *Xcm* during infection of susceptible banana cv Kayinja (K_7, K_{14} and K_{21}) and moderately resistant banana genotype Saba (S_7, S_{14} and S_{21}) at 7, 14, and 21 days post inoculation. Expression was not detected in resistant banana genotype *M. balbisiana* (MB_{48} and MB_{60}). Internal quality control RT-PCR was with primers (b) specific for detection of 217 bp fragment of 16s rRNA housekeeping gene. Lane M represents 100 kb Molecular weight DNA marker.

'Kayinja' and 'Saba' varied with time. Band of 700 bp fragment detected was more intense for samples of *Xcm* recovered at 36 and 48 h from minimal medium amended with leaf extracts of Kayinja and Saba (Figure 4) and 21 days after inoculation than that for the samples isolated 7 and 14 days during infection of banana (Figure 5). Meanwhile, intensity of 700 bp fragment band detected for *Xcm* samples from minimal medium without leaf extracts was uniform (Figure 5).

DISCUSSION

This study sought to determine the *Musa* genotype effects on *in vitro* multiplication of *Xcm* and expression of *hrpf* gene during infection. *Hrpf* is indispensible for translocation of virulence factors into plant host cells (Buttner and Bunas, 2010). In this study, *Xcm* colonization of minimal medium followed the normal growth curve pattern (Figure 1). Colonisation was significantly affected by banana genotype leaf extracts used for amendment of minimal medium. Leaf extracts of banana genotypes 'Kayinja' and 'Saba' that are susceptible to BBW (Figure 2) enhanced medium colonization by *Xcm* while leaf extracts *of M. balbisiana* a wild relative of banana that is resistant to BBW (Figure 2) suppressed medium colonization by *Xcm*. Recovery of *Xcm* cells from minimal medium amended with leaf extracts of the resistant genotype *M. balbisiana* was also impossible. On the other hand *Xcm* cells were recovered from medium amended with leaf extracts of susceptible banana genotype'Kayinja' and 'Saba'. Susceptible banana genotype extracts support multiplication and resistant genotypes suppress multiplication of *Xcm.* Since the leaf extracts were in form of a homogeneous solution, it suggested that *M. balbisiana* extracts most probably contained inhibitory chemical compounds and metabolites that could have significantly diminished ability of *Xcm* multiplication *in vitro*. Similar inhibitory compounds and metabolites are not found in susceptible banana genotypes like 'Kayinja' and 'Saba'. *Xcm* multiplication also depended on degree of banana genotype susceptibility because *in vitro* multiplication was more in minimal medium amended with leaf extracts of the highly susceptible genotype, 'Kayinja' than in minimal medium amended with leaf extracts of moderately tolerant banana genotype 'Saba' suggesting that the level of production of those compounds and metabolites may vary with degree of banana genotype susceptibility. Already, Yulu et al. (2013) reported, that there was no detectable expression of genes controlling ethylene production during infection of *M. balbisiana* by *Xcm* but that expression of those genes was relatively high in 'Saba' and 'Kayinja'. This study also seems to support the possibility that resistance to *Xcm* in banana is a heritable attribute since *Xcm* cells were not recovered from artificially inoculated *M. balbisiana* plants which was not the case for susceptible banana genotypes Kayinja and Saba in pot trials. The intensity on 1% agarose gel, of 700bp fragment of *hrpf* (Figures 4 and 5) varied with degree of susceptibility of banana genotype and duration of infection also suggesting there was differential expression of *hrpf* gene in *Xcm* during medium colonization and infection of banana. *Hrpf* and other

members of the *hrp* gene cluster are known to be critical in the initial infection process by bacterial pathogens because they are used to construct bacterial pili (needle/injectisome) through which virulence factors are introduced into plant (host) cells (Cornelis et al*.,* 2009). *Hrpf* gene that encodes the TTSS was previously predicted to be associated with *Xcm*/banana infection (Aritua et al*.,* 2007). The findings of this study suggest that *Xcm* expresses this gene during infection of susceptible banana plants. The *hrpf* that was detected in *Xcm* during infection of banana was consistent with similar studies involving interaction of *X. oryzae* pv. *oryzae* with rice (Zou et al*.,* 2006) and *X. campestris* pv. *vesicatoria* in pepper and tomato (Schulte and Bonas, 1992).

This finding establishes information that may be useful in the development of genetically modified banana expressing RNAi molecules for silencing of expression of *hrpf* gene in *Xcm* and thus decreasing pathogen capacity to cause infection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Alfano JR, Collmer A (2004). Type Three Secretion System effector proteins: double agent in bacterial disease and plant defence. Annu. Rev. Phytopathol. 42:385-414.

Angela M, Yoko B, Rosato (2003). A simple method for in vivo expression studies of *Xanthomonas axonopodis* pv.*citri*. Curr. Microbiol. 47(5):400-403.

Aritua V, Nanyonjo A, Kumakech F, Tushemereirwe W (2007). Rep-PCR reveals a high genetic homogeneity among Ugandan isolates of *Xanthomonas campestris* pv *musacearum*. Afr. J. Biotechnol. 6(3):179-183.

Aritua V, Parkinson N, Thwaites R (2008). Characterisation of *Xanthomonas* sp. Causing wilt on enset and banana and its proposed reclassification as strain of *X*. *Vasicola*. Plant Pathol. 57:170-177.

Bioneer Biotechnologies (2012). Ready-to-use PCR master mix for cDNA synthesis from total RNA.

Buttner D, Bonas U (2010). Regulation and secretion of *Xanthomonas virulence* factors. FEMS. Microbiol. Rev. 34:107-133.

Cornelis GR, Agrain C, Sorg I (2009). Length control of extended protein structures in bacteria and bacteriophages. Curr. Opin. Microbiol. 9:201-206.

Galan JE, Collmer A (1999). Type three secretion machines, bacterial devices for protein delivery into host cells. Science 284:1522-1528

Gottig N, Garavaglia BSN, Daurelio LD, Valentine A, Gehring C, Orellano EG, Ottado J (2008). *Xanthomonas axonopodis* pv**.** *citri* uses a plant natriuretic peptide-like protein to modify host homeostasis. PNAS 105(47):8631-18636.

Grant SR, Fisher EJ, Chang JH, Mole MB, Dangle JL (2006). Subterfuge and manipulation: Type Three effector proteins of phytopathogenic bacteria. Annu. Rev. Microbiol. 60:425-449.

Gurlebeck D, Thieme F, Bonas U (2006). Type Three effector proteins from the plant pathogen *Xanthomonas* and their role in the interaction with the host plant. J. Plant Physiol. 163:233-255.

Jin Q, Hu W, Brown I, McGhee G, Hart P, Jones AL, He SY (2001). Visualization of secreted *Hrp* and *avr*. Proteins along the *Hrp*. Pilus during type III secretion in *Erwinia amylovora* and *Pseudomonas syringe*. Mol. Microbiol. 40:1129-1139.

Karamura EB, Turyagyenda FL, Tinzara W, Blomme G, Ssekiwoko F, Eden-Green S, Molina A, Markham R (2008). *Xanthomonas* wilt of bananas in East and Central Africa. Diagnostic and Management Guide. Bioversity International, Uganda. INIBAP ISBN. pp. 978-982.

Kay S, Bonas U (2009). How *Xanthomonas* Type Three effectors manipulate the host plant. Curr. Opin. Microbiol. 12:37-43.

Mahuku GS (2004). A simple extraction method suitable for PCR - Based analysis of plant, fungal and bacterial DNA. Plant Mol. Biol. Rep. 22:71-81.

Schulte R, Bonas U (1992). Expression of *Xanthomonas campestris* pv *vesicatoria hrp* gene cluster, which determines pathogenicity and hypersensitive response on pepper and tomato. J. Bacteriol. 32:815-823.

Ssekiwoko F, Tushemereirwe W, Batte M, Ragama PE, Kumakech A (2006). Reaction of banana germplasm to inoculation with *Xanthomonas campestris* pv. *musacearum*. Afr. Crop Sci. J. 14(2):151-155.

Weber E, Ojanen-Reuhs T, Huguet E, Hause G, Romantschuk M, Korhonen TK, Bonas U, Koebnik R (2005). The type III-dependent *Hrp* pilus is required for productive interaction of *Xanthomonas campestris* pv. *vesicatoria* with pepper host plants. J. Bacteriol. 187:2458-2468.

White FF, Potnis N, Jones JB, Koebnik R (2009). The type III effectors of *Xanthomonas*. Mol. Plant Pathol. 10:749-766.

Yang B, White FF (2005). Diverse members of the *AvrBs3/Pth*, A family of type III effectors are major virulence determinants in bacterial blight disease of rice. Mol. Plant Microbe Interact. 17(11):1192-1200.

Yulu MS. (2014). Role of ethylene in Banana host defense against *Xanthomonas campestris pv. musacearum*. MSc Thesis, Makerere University, Kampala. 69 p.

Zou LF, Wang XP, Xiang Y, Zhang B, Li YR, Xiao YL, Wang JS, Walmsley AR, Chen GY (2006). Elucidation of the *hrp* Clusters of *Xanthomonas oryzae* pv. oryzicola That Control the Hypersensitive Response in Nonhost Tobacco and Pathogenicity in Susceptible Host Rice. Appl. Environ. Microbiol. 72(9):6212-6224.