

## Full Length Research Paper

## Evaluation of banana genotype resistant to *Xanthomonas* wilts disease (*Xanthomonas campestris* pv. *musacearum*) in south east of Ethiopia

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*Xanthomonas* wilt caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) is one of the most important constraints to banana production. The use of resistant banana varieties would be a long-term and cost-effective solution to control any pathogen. Therefore, identifying this pathogen resistant banana genotype is one of the basic requirements for effective management. The current study was therefore initiated to evaluate banana genotypes for resistance to banana *Xanthomonas* wilt. The experiment was conducted at Arsi University greenhouse from 2014 to 2015 GC. Banana and enset disease were collected and bacterial isolates were isolated and characterized based on different characterization tests. Twelve (12) banana genotypes were inoculated with three Xcm isolates ( $I_1$ ,  $I_2$  and  $I_3$ ) in a factorial experiment arranged in CRD with six replications. Disease assessment data was conducted and analyzed. Bacterial isolates were isolated and the identity of the isolated strains was confirmed as Xcm. The analysis of variance for incubation period, wilting incidence and disease severity revealed significant variations ( $p < 0.05$ ) among banana genotype and isolates. The results revealed that “Cadaba” genotype was found to have the lowest wilt incidence of 16.67%, severity index 15.07% and longest incubation period 5.28 and 9.33 weeks for initial and complete wilting respectively, moderately resistant to the pathogen and producers preferred for multiplication. Butuza, Grandy nani, Robusta and Willams genotype were determined as moderately susceptible having wilting incidence of 21-30%. However, “Nijuru Genotype” showed the highest wilting incidence of 66.67%, severity index 38.78% followed by “Matooke that could be used as highly susceptible checks in future screening trail. Results also revealed that among Xcm isolates, isolate-  $I_2$  is the most aggressive, while isolate  $I_1$  is the least aggressive. As the current work revealed, the potential variation among banana genotype reaction to Xcm infection, genotype that showed moderately susceptible reaction should be further evaluated against Xcm.

**Key words:** Banana genotype, incubation period, wilt incidence, *Xanthomonas campestris* pv. *musacearum*.

### INTRODUCTION

Banana is the world's fifth most important food crops after maize, rice, wheat and cassava (Tripathi, 2011). In Ethiopia, it is the second major fruit crop after citrus. It is grown in southern and western parts of the country, which

are mainly confined to low to mid altitudes where there is adequate rainfall or irrigation (Seifu, 1999). The main banana growing areas are located at Arba Minch in southern Ethiopia and south western Ethiopia (Temesgen

et al., 2004). Bananas are produced mainly in traditional agricultural systems by small-scale farmers throughout the country. Its' production is largely for the local market and home consumption. Banana (*Musa spp.*) is typically a cultivated fruit crop mainly as food and all its cultivars are eaten as dessert in Ethiopia. According to the CSA (2008), the total area under banana production is estimated to be over 29064.03 hectares with the annual production of about 1943331 quintal. The total annual banana production in Ethiopia is 66.86 qt/ha.

Despite banana's importance as a food crop, its production and productivity are threatened by various biotic and abiotic factors (Sharrock et al., 2002). Mainly biotic stresses like disease, insect (mealy bug) and nematodes are a leading cause of banana loss. Based on the distribution and the damage incurred on the banana production, *Xanthomonas* wilt caused by *Xanthomonas campestris* pv. *musacearum* is considered to be the most serious production constraint. It is known to be the most threatening and important problem to banana production (Melese et al., 2014).

This pathogen is very destructive and completely kills the plant at all growth stage and cause heavy total yield loss at many localities in Ethiopia (Dagnachew and Bradbury, 1968, 1974; Dereje, 1985; Gizachew, 2000; Quimio and Mesfin, 1996). It is widely distributed in the high, mid and lower altitude areas of the central, southern and southwestern enset growing regions of Ethiopia with different degree of severity (Dereje, 1985; Spring et al., 1996). More recently, the disease has been reported as more common on banana than enset in western Ethiopia (Temesgen et al., 2004). Today, banana yield losses due to banana xanthomonas wilt have been estimated at 30 to 52% of the annual production (Karamura et al., 2006). In addition, 70 to 80% of disease incidence and 100% yield loss were recorded for many juice bananas in Uganda (Tushemereirwe et al., 2002). Once the pathogen has initiated infection, damage limitation is extremely difficult and the disease is impossible to cure (Eden-Green, 2004).

The *Xanthomonas* wilt disease has been endemic to Ethiopia, significant constraint on enset and banana production in the Ethiopian highlands for over four decades and was first reported and described in the late 1960's (Dagnachew and Bradbury, 1968, 1974). However, in recent years, the epidemics of *Xanthomonas* wilt with significant damage have been reported on banana in Uganda in 2001 (Tushemereirwe et al., 2004). Further outbreak and establishment were also confirmed on banana in eastern Congo, in the Lake Victoria region of Tanzania, Rwanda and Kenya (Aritua et al., 2008; Biruma et al., 2007 and Ndungo et al., 2006). Banana production losses caused by this pathogen threaten the

food security of about 100 million people and the income of millions of farmers in the Great Lakes region of Central and Eastern Africa, who depend on banana fruit for food and export trade (Tripathi et al., 2009) and also threatens food security of over 15 million of Ethiopians' who utilize Enset as a staple or co-staple food (Brandt et al., 1997).

According to Ssekiwoko et al. (2006a), there is no effective control measure against the *Xanthomonas* wilt, except the use of different cultural strategies. Currently, the control of *Xanthomonas* wilt depends on the use of cultural practices that include the use of disease-free planting materials, early detection and distraction of the diseased plants, cleaning and disinfecting of farming equipment, and rotation of infected sites with non-host crops and restriction of introduction of foreign plant materials into gardens (Brandt et al., 1997). Adoption of de-budding, meaning removal of the male bud by forked stick had been able to prevent insect vector spread to cultural practices (Blomme et al., 2005). Despite this cultural work, *Xanthomonas* wilt epidemics are increasingly more difficult to control because recommended cultural practices are labor exhaustive and not timeliness being implemented by producers.

Host resistance is the most cost-effective and simplest method of controlling any disease caused by plant pathogens (Young and Danesh, 1994). Based on a screening trial of local and exotic banana genotypes for reaction to Xcm, no genotype was found to be 'immune' to infection (Temesgen et al., 2006; Awasa Agriculture Research Center Progress Report, 2000). However, *Musa balbisiana*, a wild type of banana, was identified as the most resistant to *Xanthomonas* wilt in Uganda (Ssekiwoko et al., 2006a). Therefore, further exploring resistant banana genotype or identifying the most resistant banana genotype is a base for developing cultivars with resistant to *Xanthomonas* wilt through conventional breeding or biotechnology which would be a long-term and cost-effective solution.

Since its discovery in the 1960s in Ethiopia and recently in East Africa, some studies have been conducted to control *Xanthomonas* wilt in the country. According to Tripathi et al. (2008) and Getachew et al. (2006), even though no natural banana cultivars and genome groups have complete genetic resistance to Xw, they differ in degree of susceptibility. However, research on *Xanthomonas* wilt that involves searching for resistant banana cultivars which generated under tissue culture protocol has been given very little due attention. Therefore, exploring and identifying the most pathogen resistant banana genotype which are developed through tissue culture is one of the basic requirements for effective and sustained implementation of integrated disease management program. There is limited knowledge on the

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**Table 1.** Description of the *Xanthomonas campestris* pv. *musacearum* isolates used for the pathogenicity tests.

Isolate code	Location	Altitude (mas)	Plant species sampled
I <sub>1</sub>	West/Dire Inchini/Bola	2560	Enset Sabbara clone
I <sub>2</sub>	Southern /Sidama/Yirgalem/Dale	2749	Banana Pisawak genotype
I <sub>3</sub>	Southwest/Wonchi/	2671	Enset clone Hiniba

pathogenicity and aggressiveness of *X. campestris* pv. *musacearum* (Xcm) strains to various banana genotypes. Thus, for effective control of this pathogen, the current study was designed to evaluate different banana genotypes for resistance to banana Xanthomonas wilt under artificial inoculation conditions.

## MATERIALS AND METHODS

### Collection of diseased banana and enset samples, plant species and pathogen characterization

Diseased banana and enset samples were collected from the major banana and enset growing districts Southern region, west and south-west of Ethiopia, viz., Southern region/Sidama, West/Dire Inchini and Southwest/Wonchi districts. Diseased pseudostem samples were collected from one kebele in each district in random sample. The samples were labeled properly and brought into Arsi University College of agriculture and environmental science for further studies. After isolation and detection it was labeled as I<sub>1</sub> from enset in Dire inchini, I<sub>2</sub> from banana in Sidama Yirgalem and I<sub>3</sub> from enset in Wonchi (Table 1). Identity of the isolated bacteria was confirmed following colony growth on semi selective medium (sucrose peptone agar medium: 20 g sucrose, 5 g peptone, 0.5 g K<sub>2</sub>H<sub>3</sub>PO<sub>4</sub>, 0.25 g MgSO<sub>4</sub> and 15 g agar in 1 L sterilized distilled water) (Mwangi et al., 2007) and Gram staining reaction tests (Schaad, 1988). In addition, physiological tests, that is, gelatin liquefaction and starch hydrolysis testes as well as catalase reaction were carried out (Dickey and Kelman, 1988). For pathogenicity test, different banana genotypes were collected as described in Table 2.

### Preparation of inoculum

Pathogenicity tests were carried out on the different banana genotypes using the three different Xcm isolates. The Xcm isolates were isolated from the sample of naturally infected cultivated enset and banana, and used for this purpose as mentioned above. Before inoculation of test plants, the isolates were grown on YDA and incubated at 28°C for 2 days. The concentration of each bacterial suspension was adjusted to 0.3 OD at 460 nm, which is equivalent to 10<sup>8</sup> cfu/ml bacteria cells, using spectrophotometer (Gizachew, 2000).

### Pathogenicity testing on different banana genotypes

A factorial experiment with three Xcm isolates (I<sub>1</sub>, I<sub>2</sub> and I<sub>3</sub>) as sub-factors, and 12 banana genotype as main-factors were carried out to determine the pathogenicity of the isolates on banana genotype. The experiment was laid in completely randomized design (CRD) with six replications. For this purpose, twenty four suckers of each

**Table 2.** Plant materials used for pathogenicity test and their sources.

Tested plant type	Source
Banana genotype	
Williams	MARC
Giant Cavandish	MARC
Dwarf Cavandish	MARC
Nijuru	MARC
Robusta	MARC
Cardaba	MARC
Grandy nani	MARC
Poyo	MARC
Butuza	MARC
Kitawire	MARC
Ducasse hybrid	MARC
Matoke	MARC

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genotype was used and planted into plastic bag (100 cm in diameter and 100 cm height), which were filled with sun-dried mixture of top soil : sand : manure at a ratio of 3:2:1 (Quimio, 1992) and allowed to establish for three months (Figure 1). Three months after planting, at four to seven leaf stages, each genotype was inoculated with 3 ml of a virulent Xcm isolate suspension whose cell concentration was adjusted to 1x10<sup>8</sup> cfu/ml at lower base of the newly expanded central leaf petiole using 10 ml sterile hypodermic syringe needle (Dereje, 1985; Gizachew et al., 2008a). One plant per each banana genotype was inoculated with each three Xcm isolates (I<sub>1</sub>, I<sub>2</sub> and I<sub>3</sub>) and one plant per banana genotype but the control plant was inoculated with the same volume of sterile. Then, inoculated plants were covered with a wet plastic bag for 48 h. Each treatment was replicated six times (Figure 2).

### Disease assessment

Disease assessment for the pot experiments were started one week after inoculation and proceeded at a week interval for four consecutive months after inoculation. Wilt incidence, incubation period for the first wilting symptom and time for complete wilting was recorded at time of disease assessment. In addition, disease severity was assessed using standard disease scales of 0-5 (Winstead and Kelman, 1952) where 0: no symptom; 1: only the inoculated leaf wilted; 2: 2-3 leaves wilted; 3: four leaves wilted; 4: all leaves wilted and 5: plant dead). The severity grades were converted into percentage of severity index for analysis (Cooke, 2006). The area under percent severity index progress curve



**Figure 1.** Established banana genotype for artificial inoculation of *Xanthomonas* wilt under pot culture condition for three months.



**Figure 2.** Artificial inoculation of banana genotype by *Xanthomonas* wilt pathogen.

(AUPSiPC) for each treatment was computed using the formula adopted from Jerger and Vijanen-Rollinson (2001). The severity grades were converted into percentage of severity index for analysis (Cooke, 2006).

$$PSI = \frac{SNR \times 100}{NPR \times MSS}$$

Where PSI is percent severity index; SNR is the sum of the numerical rating; NPR is number of plant rated, MSS is the maximum score of the scale. Means of severity from each scoring date was used in the data analysis. Disease incidence/wilt incidence was calculated according to the following formula:

$$WI = \frac{NPCW \times 100}{NPPT}$$

Where: WI– Wilt incidence, NPSWS– number plants completely wilted, NPPT- number of plants per treatment. The area under percent severity index progress curve (AUPSiPC) for each treatment was computed using the formula adopted from Jerger and Vijanen-Rollinson (2001).

$$AUPSiPC = \sum_{i=1}^{n-1} 1/2 (x_i + x_{i+1}) (t_{i+1} - t_i)$$

Where n is total number of assessment times,  $t_i$  is the time of the  $i^{th}$  assessment in weeks from the first assessment date,  $x_i$  is the percentage of the disease severity or disease incidence at  $i^{th}$  assessment. AUPSiC-area under percent severity index progress curve was expressed in percent-weeks because severity ( $x$ ) was expressed in percent and time ( $t$ ) in weeks.

### Data analysis

Analysis of variance was performed for data of disease parameters (wilt incidence, percent of severity index and Incubation period) using General Linear Model of SAS computer package (SAS, Institute Inc. 2003). Means were separated with LSD at 5% probability level using the t-test. The degree of resistant to Xw was determined on the basis of WI (%) as: highly resistant (HR): 0% wilting; resistant (R): 1-10% wilting; moderately resistant (MR): 11-20% wilting; moderately susceptible (MS): 21-30% wilting; susceptible (S): 31-50% wilting; highly susceptible: > 50% wilting (Bayoumi and El-Bramawy, 2007).

## RESULTS AND DISCUSSION

### Isolation, identification and morphological, biochemical and physiologically of the bacterial isolates

Out of the collected symptomatic banana and enset samples, three had detectable Xcm isolates. Spreading the bacteria ooze obtained from the symptomatic banana and enset pseudostem on the cellobiose cephalixin agar (CCA) media resulted in the growth of typical bacterial isolates with smooth mucoid colonies having light to deep yellow and creamy color after three days of incubation period at 28°C. And also after leaf and pseudostem pieces were plated, the mucoid growth of bacterial isolates was observed on the media. These observations were consistent with the finding of Kidist (2003), who indicated the Xcm colonies from banana and cultivated enset as being light to yellow and creamy.

Pink to reddish colored cells were observed after Gram staining, and isolates were classified under Gram negative bacteria. All tested bacteria isolates did not dissolve in 3% KOH solution rather caused the KOH solution to become a thin strand of slime in appearance, when the mixed bacteria culture in the solution was lifted with the inoculating loops, further confirming their identity as Gram negative. All isolates have formed gas bubbles, when dissolved with three drops of 3% H<sub>2</sub>O<sub>2</sub>, and hence were catalase positive. The reaction of the three isolates to Gram staining, catalase reaction and KOH reaction were found to be consistent with the description given by Kidist (2003) and Gizachew (2000) and are also similar to general characteristic of *X. campestris* described by Bradbury (1984).

Further, each isolate was tested for gelatin liquefaction after 3 to 7 days of incubation. When inoculated gelatin tubes were observed without tilting, there was circle formed by growth of bacteria, floating at the upper part of the inoculated test tubes as compared to the non-inoculated control. When inoculated tubes were tilted, gelatin liquefaction was observed in all inoculated test tubes and was taken as positive and the non-inoculated control remained solid. Hence, the bacterial isolates were capable of hydrolyzing gelatine. This result is in agreement with the description given by Dagnachew and

Bradbury (1968), who stated ability of some Xcm isolates to liquefy gelatin.

With regard to starch hydrolysis reaction, each isolate showed 1 to 2 cm clear zone around their growth, which is an indication of starch hydrolysis but un-inoculated plate remain unchanged. The result of morphological, physiological and biochemical tests indicated that all isolates from symptomatic samples, fit the characteristics of *X. campestris* pv. *musacearum*. These results are in line with description of cultural characteristics of the Xanthomonas wilt pathogen by Kidist (2003) and Dereje (1985).

### Evaluation of banana genotype for resistance to Xcm pathogen

#### *Incubation period for symptom expression and wilt incidence, disease severity and area under disease progress curve*

Disease assessment started a week after inoculation, and the earliest external typical disease symptoms were observed 3 to 5 weeks post inoculation on the infected banana genotype. These include collapse of plantlets and folding down of the leaf blade along the midribs followed by scalding and dull green appearance of the central inoculated leaf. This was followed by yellowing starting at the apex, stepwise wilting of leaves, drying and wilting of the whole plant and finally death and rotting (Figure 3). Yellowish bacterial ooze was observed, when pseudostem and leaf petiole were cut. Such typical symptoms were described under field and experiment condition by Gizachew (2000), Dereje (1985) and Alemayehu et al. (2016).

The analysis of variance for incubation period in relation to the appearance of both initially wilting and complete wilting of plants, wilt incidence and disease severity index revealed significant differences ( $p < 0.05$ ) among genotypes and isolates. However, the interaction effect was not significant.

The mean of incubation period ranged from 3 to 5 weeks for initial wilting, while the period varied between 6 and 11 weeks for complete wilting (Table 3). The result showed that banana cultivars vary with regards to earliness and intensity of symptom expression. In this experiment, among the tested genotypes, Matooke was found to have the shortest incubation period (3 weeks) followed by Nijuru, (3.11 weeks), Gaint Cavendish (3.11) and Dwarf cavendish (3.39 weeks), while Butuza and Carduba had the longest incubation period (5.11 and 5.28 weeks respectively) for appearance of initial wilting. Matooke, Nijuru, Dwarf cavendish and Gaint cavendish were significantly differently from other genotypes for early showing disease symptom (Table 3). So the current finding showed that Xcm can survive in banana tissue for periods of over three weeks without showing any external





**Figure 3.** The appearance of disease symptoms three weeks after inoculation on cultivated different banana genotype.

symptoms or latently found in the side of banana tissue. Ibrahim (2013) found that the period of symptom expression for Xcm isolate inoculated banana leaves was within five weeks. Mwangi et al. (2006) also found that banana cultivars vary with regards to earliness and intensity of symptom expression.

The number of weeks required to complete wilting varied between six and 10 weeks among banana genotypes and six to nine among isolates (Table 3). This result was in conformity with Temesgen et al. (2006), who reported complete wilting between six and 16 weeks after inoculation. Among banana genotypes, Cardaba took

longer (9.33 weeks) to show complete wilting as compared to other tested banana genotype, while Nijur had the shortest incubation period (5.8 weeks) for complete wilting. There was significant difference between Cardaba and Nijuru for incubation period for complete wilting, while there were no statistically significant differences among Robusta, Kitawire, Poyo, Willams, Ducasse hybrid, Grandy nani and Butuza and also among Matoke, Gaint cavandish and Dwarf cavandish (Table 3). Such kind of variation in symptom expression for either initial or final completely wilting of entire plant could indicate the degree of susceptibility or tolerance

**Table 3.** Incubation period for initial (IP) and complete wilting (CWP) (weeks), percent of wilt incidence (%DI) and percent severity index progress (PSI) on 12 banana genotype inoculated with three *Xcm* isolates under greenhouse condition.

Banana genotype	IP (week)	CWP (week)	%DI	%PSI
Matooke	3.06 <sup>b</sup>	6.4 <sup>fg</sup>	66.67 <sup>a</sup>	38.78 <sup>a</sup>
Nijuru	3.11 <sup>b</sup>	5.8 <sup>g</sup>	55.56 <sup>ab</sup>	30.05 <sup>ab</sup>
Giant cavandish	3.11 <sup>b</sup>	6.75 <sup>defg</sup>	44.44 <sup>abc</sup>	28.45 <sup>ab</sup>
Dwarf cavandish	3.39 <sup>b</sup>	6.5 <sup>efg</sup>	38.89 <sup>bcd</sup>	25.71 <sup>ab</sup>
Robusta	3.89 <sup>ab</sup>	6.8 <sup>bcdef</sup>	38.89 <sup>bcd</sup>	28.45 <sup>ab</sup>
Kitawire	3.78 <sup>ab</sup>	7.43 <sup>bcdef</sup>	33.33 <sup>bcd</sup>	21.11 <sup>ab</sup>
Poyo	3.5 <sup>b</sup>	7.5 <sup>bcde</sup>	33.33 <sup>bcd</sup>	18.71 <sup>ab</sup>
Williams	3.94 <sup>ab</sup>	7.86 <sup>bc</sup>	27.78 <sup>cd</sup>	23.60 <sup>ab</sup>
Ducasse hybrid	4.33 <sup>ab</sup>	7.83 <sup>bc</sup>	27.78 <sup>cd</sup>	24.49 <sup>ab</sup>
Grandy nani	4.06 <sup>ab</sup>	8.0 <sup>b</sup>	22.78 <sup>cd</sup>	21.61 <sup>ab</sup>
Butuza	5.11 <sup>a</sup>	7.75 <sup>bcd</sup>	22.22 <sup>cd</sup>	29.74 <sup>ab</sup>
Cardaba	5.28 <sup>a</sup>	9.33 <sup>a</sup>	16.67 <sup>d</sup>	15.87 <sup>b</sup>
LSD	1.97	1.128	21.58	14.32
<b>Isolates</b>				
I <sub>1</sub>	4.49 <sup>a</sup>	8.83 <sup>a</sup>	83.33 <sup>a</sup>	41.22 <sup>a</sup>
I <sub>2</sub>	3.39 <sup>b</sup>	7.08 <sup>b</sup>	41.11 <sup>b</sup>	19.60 <sup>b</sup>
I <sub>3</sub>	3.76 <sup>b</sup>	6.88 <sup>b</sup>	28.33 <sup>b</sup>	16.15 <sup>b</sup>
LSD	0.72	0.75	10.79	5.839
%CV	23.803	10.94	23.01	21.20

Means followed by the same letter in the column are not significantly different at 5% level of significance, I<sub>1</sub>- Isolate from infected enset West Showa, I<sub>2</sub>- Isolate from infected banana southern region, I<sub>3</sub>- isolate from infected Enset South western region, data represent mean of six replications.

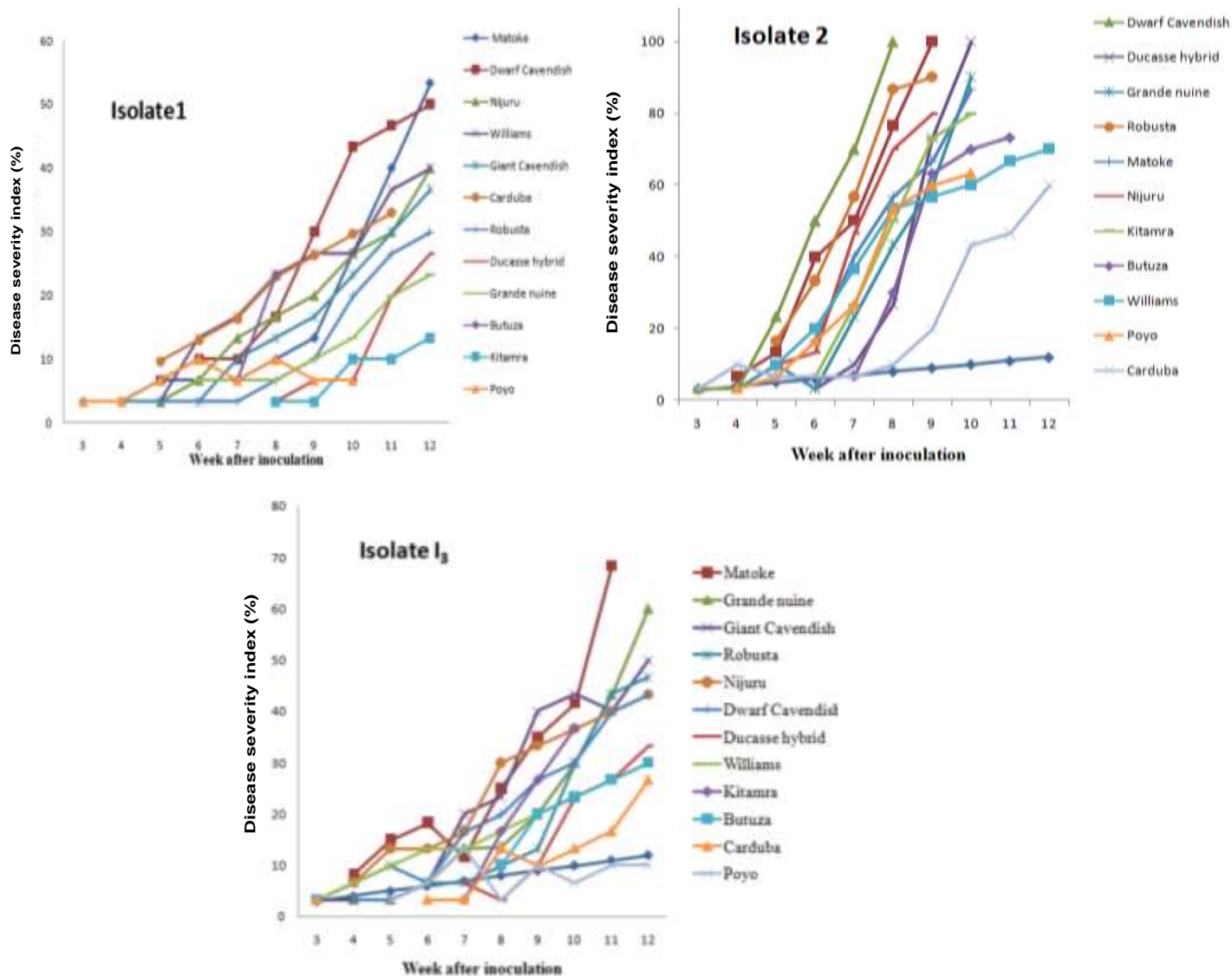
of the genotypes. Thus, Carduba delaying of initial and complete wilting symptom expression showed tolerance to *Xcm* infection, while Matooke showed the earliest initial and complete wilting found susceptible for this pathogen.

Comparisons were made across the isolates for initial and complete wilting, isolate I<sub>2</sub> and I<sub>1</sub> caused initial wilting the earliest (3.39 and 3.76 weeks after inoculation, respectively), while Isolates I<sub>3</sub> took about 4.46 weeks for first wilting of the host. Most plantlets inoculated with Isolates I<sub>2</sub> and I<sub>3</sub> completely wilted but most of the banana genotype plantlet inoculated with isolate I<sub>1</sub> was not completely wilted. Among the three isolates of *Xcm* used in this study, *Xcm* isolate I<sub>2</sub> and I<sub>1</sub> had shorter incubation periods (7.08 and 8.83 weeks, respectively) for causing the complete wilting as compared to isolates I<sub>3</sub> (9.21 weeks). As a result, among the three isolates of *Xcm* used in this study, I<sub>2</sub> isolated from banana genotype from highland area resulted in the earliest causing initial and complete wilting of most tested banana genotype. This suggested the most virulence and aggressive nature of isolates I<sub>2</sub>. However, isolate I<sub>1</sub> was found to be a weaker pathogen as compared to other isolates. The result of the present study is in accordance with the finding of Alemayehu et al. (2016), who reported that

significance differences among isolates were recorded to induce initial wilting three to four weeks after inoculation under greenhouse condition. Similarly, Ibrahim (2013) reported that there was difference among *Xcm* isolates for inducing disease symptom on inoculated genotype.

Disease severity was high for most banana genotypes after inoculation with isolates I<sub>2</sub> and I<sub>3</sub>. 80-100% of disease severity indexes were recorded for banana genotype Nijuru, Giant cavandish, Matoke, Dwarf cavandish and Ducasse hybrid at 8 to 9 weeks after being inoculated with isolate I<sub>2</sub>. Isolate I<sub>3</sub> caused 30 to 70% severity at 9 to 12 weeks after inoculation, while isolate I<sub>1</sub> resulted in 6.67 to 53.33% severity at 8 to 12 weeks after inoculation (Figure 4). Averagely, 83.33% of disease incidence and 41.22% of disease severity index were caused by isolate I<sub>2</sub>. Isolate I<sub>3</sub> caused average wilt incidence of 43.11%, and 19.6% disease severity index. Isolate I<sub>1</sub> had significantly lower disease incidence (28.33%) and severity index (16.15%) (Table 3). This further confirmed the most aggressive nature of isolate I<sub>2</sub> as compared to the remaining two isolates.

Complete disease severity index (100%) on Giant cavandish, Dwarf cavandish and Ducasse hybrid, 90% on Grande nani and Robusta, and 86.7% on Matooke were



**Figure 4.** *Xanthomonas* wilt disease development expressed as percent of severity index (%) on twelve banana genotype under greenhouse condition over time after inoculation (week) Note: Xcm Isolates I<sub>1</sub> and I<sub>3</sub> from onset and Isolate I<sub>2</sub> from banana.

recorded at 8 to 9 weeks after inoculated with isolate I<sub>2</sub>. Similarly, 68.33% of disease severity on Matoke and 60% severity index on Grande nani were recorded at 11 and 12 weeks, respectively after inoculation with isolates I<sub>3</sub>. Less than 50% of disease severity index were recorded on the other banana genotype being inoculated with Isolates I<sub>1</sub> (Figure 4).

Based on the evaluation of their reaction, none of the twelve banana genotype types had complete resistance to Xcm isolates used in this study. Among banana genotypes type tested in the current experiment, Cardaba was significantly different from the others having lowest wilting incidence (16.67%) and disease severity (15.07%) and categorized as moderately resistant. Other banana genotypes include Butuza, Grandy nani, Robusta and

Williams were determined as moderately susceptible having wilting incidence of 21 to 30%. On the other hand, Nijuru and Matoke were relatively highly susceptible with 66.67 and 55.6% average wilting incidence, 38.78 and 32.05% of severity index, respectively. 31 to 50% wilting incidence were recorded on Ducasse hybrid, Poyo, Gaint Cavandish, Dwarf cavandish and Kitawire were determined as susceptible (Table 3). The results suggest higher susceptibility of Nijuru and Matoke, and moderately resistance of Cardaba to Xcm infection. Hence, this banana genotype showed moderate resistance to *Xanthomonas* wilt making pathogen to be multiplied and used by producers and help as one disease management option in addition to cultural practices.



## Conclusion

Banana is one of the major food crops in the low to mid lands of the East of Africa. However, its production is threatened by a number of abiotic and biotic factors. Among biotic factor, Xanthomonas wilt caused by *X. campestris* pv. *musacearum* is one of the most important constraints to banana production. The only recommended control measures for Xanthomonas wilt are cultural practices. So, in addition to cultural practices, the use of resistant banana varieties would be a long-term and cost-effective solution. Therefore, exploring tolerance banana genotype is one of the basic requirements for control of this pathogen. Thus, the current study was designed with the objective to evaluate different banana genotypes for resistance to banana Xanthomonas wilt under artificial inoculation conditions.

Diseased samples of banana and enset were collected and bacterial isolates were isolated from collected symptomatic samples and characterized based on pathogenicity, morphological, physiological and biochemical tests. All the tests confirmed the identity of the isolated strains as *X. campestris* pv. *musacearum*. 12 banana genotypes were inoculated with three isolates in a factorial experiment arranged in CRD with six replications. Disease assessment was carried out every week for four months and all the disease parameters data were collected, measured and analyzed.

Pathogenicity tests involving inoculation of different banana genotype with three isolates of *Xcm* revealed significant variations ( $p < 0.05$ ) among the isolates and genotype in terms of incubation period, wilting incidence and disease severity. Among *Xcm* isolate,  $I_2$  was found to be the most aggressive, while  $I_1$  was the least aggressive. In the study of evaluation of 12 banana genotype tolerance to *Xcm*, among all, "Cardaba" genotype exhibited moderately tolerant against Xanthomonas wilt. Therefore, "this banana genotype could be considered as tolerant genotype to the pathogen and it can be used as a Xanthomonas wilt management component. Butuza, Grandy nani, Robusta and Willams genotype were determined as moderately susceptible having wilting incidence of 21 to 30%. On the other hand, genotype "Nijuru" showed the highest wilting incidence, severity index and shortest incubation period followed by "Matoke" and both genotype could be used as highly susceptible checks in future screening trail. The results suggest the higher susceptibility of Nijuru and Matoke and moderate resistance of Cardaba to *Xcm* infection.

In general, the current study showed that banana genotype varies in reaction to Xanthomonas wilt pathogen. In these regards, use of tolerant genotype along with cultural practices and sanitary control measure is viewed to be the most feasible of the Xanthomonas wilt management. In the future, producers or farmers should prefer to multiply Cardaba genotype due to its moderate resistance to Xw. Additionally, the banana genotype that

showed a moderately susceptible reaction to the wilt pathogen should be further evaluated against *Xcm* isolate under field conditions. More research is needed considering the various banana genotype from the different banana growing regions and research center to explore resistant gene in banana genotype.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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