

## Full Length Research Paper

# Natural occurrence and pathogenicity of *Xanthomonas* bacteria on selected plants

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The bacterial genus *Xanthomonas* consists of several species of economic importance, among which *Xanthomonas campestris* pv. *musacearum* (*Xcm*), the cause of enset and banana wilt is the most important in tropical Africa. However, the natural occurrence and host range of this species is yet to be clarified. The objectives of this study were to verify the presence of *Xanthomonas* bacteria on plants growing in and around enset gardens in South and Southwest Ethiopia, and to elucidate the pathogenicity of *Xcm* strains to cultivated and wild plants. Several economical and ornamental plants were assessed for wilting in South and Southwest Ethiopia. Wilting was visible on *Canna* spp. with 9.8% incidence and 30% prevalence, while reddish streak symptoms (typical of *Xanthomonas* bacteria) were observed on the leaves of sugarcane, sorghum and wild sorghum with disease incidence ranging from 20 to 80%, and prevalence varying from 30 to 100%. The pathogenicity of three *Xcm* isolates to five plant species was tested in a factorial experiment arranged in CRD with five replications. All the tested *Xcm* isolates were found to be pathogenic to banana, cultivated and wild enset, *Canna indica*, *Canna orchoides*, maize, sorghum and finger millet. The analysis of variance for incubation period and disease incidence revealed significant differences ( $p < 0.05$ ) among test plants and isolates. Results suggest marked variations among test plants' ability to resist the bacterium. Variations were also evident in the aggressiveness of the bacterial isolates. On the other hand, enset and banana did not show any symptom after being inoculated with four *Xanthomonas* isolates from other crops.

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

## INTRODUCTION

The genus *Xanthomonas* is composed of several species of economic importance as they affect the production of different crops all over the world. A member of this genus, *Xanthomonas campestris* pv. *musacearum* (*Xcm*), has been implicated in threatening the crop enset

(*Ensete ventricosum* (Welw.) Cheesman) in Ethiopia since the 1960s (Yirgou and Bradbury, 1968; Dereje, 1985; Weldemichael, 2000).

The disease has also been implicated as causing a serious threat on banana production thereby the

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livelihood of thousands of people throughout the Great Lake Region of Africa (Wasukira et al., 2012). Previous work based primarily on DNA sequences and fatty acid data has shown that strains of *X. campestris* pv. *musacearum* have very close homology to strains of *Xanthomonas vasicola* and most likely belong to this species. Accordingly, the name *X. vasicola* has been proposed for *X. campestris* pv. *musacearum* (Aritua et al., 2007). However, we will use the previous name *Xcm* (currently still the official name) throughout this paper.

The initial symptoms by *Xcm* on enset and banana occur on the central leaf and spread to all parts. The earliest symptoms are usually loss of turgor and wilting in the spear (youngest emerging leaf) or one or more of the young leaves, sometimes preceded by yellowing and distortion, especially in young plants. Bacterial ooze exudes when a non-dry part of the plant is cut. A cut made through the petioles of newly infected enset plants reveals browning of the vascular bundles and yellowish or grayish masses of bacterial ooze come out from the vascular bundles (Tripathi et al., 2009). Cross sections at the base of the pseudostem and corm show discoloration of the vascular bundles with large bacterial pockets and grayish or yellowish exudates with brownish to black spots, respectively (Wondimagagne, 1981; Ashagari, 1985).

The main known natural host plants to *X. campestris* pv. *musacearum* are Banana (*Musa* spp.) and cultivated enset (*Ensete ventricosum*) both of which belong to the *Musaceae* family and order zingiberales (Yirgou and Bradbury, 1968, 1974). However, the host range of this pathogen appears rather controversial. While Ssekiwoko et al. (2006) reported *Xcm* as being able to only infect monocots that belong to the families *Musaceae* and *Cannaceae*, Mwangi et al. (2006) ruled out grasses like maize, sorghum and napier grass along with such crops as common beans, cassava, taro, sweet potato and tobacco as hosts to the pathogen. On the other hand, *Xanthomonas* species have been reported in sweet potato, sugar cane, maize, common beans and sorghum (Hernandez and Trujillo, 1990; Destefano et al., 2003; Mkandawire et al., 2004; De Cleene, 2008; Todorović et al., 2008). *Xcm* was also found to be pathogenic to maize and sugarcane (Aritua et al., 2008; Karamura, 2012). Wild *Musa zebrina*, *Musa ornata* and *Canna indica* were also reported as potential alternative hosts for this pathogen (Ssekiwoko et al., 2006).

Enset bacterial wilt caused by *X. campestris* pv. *musacearum* was first reported in Ethiopia by Yirgou and Bradbury (1968) and has since spread to all the enset growing regions in Ethiopia (Brandt et al., 1997). However, most of the studies conducted in Ethiopia thus far focus on surveying the disease in some areas and characterizing the pathogen based on biochemical tests (Wondimagagne, 1981; Ashagari, 1985; Spring et al., 1996; Bobosha, 2003; Addis et al., 2004).

Screening some cultivated enset clones for wilt

resistance and studying the survival and dispersal of the pathogen have also been investigated although not thoroughly (Weldemichael, 2000; Addis et al., 2006; Weldemichael et al., 2008a and b). Studies on the occurrence of the disease on plants other than enset and banana are lacking under Ethiopian conditions, and are very limited even throughout Africa. Besides, the pathogenicity of *Xcm* isolates to plants growing in and around enset gardens has not been well established.

Therefore, this study was designed to: 1) verify the presence of *Xanthomonas* bacteria on plants growing in and around enset gardens in South and Southwest Ethiopia, and 2) elucidate the pathogenicity of *Xcm* strains to various plants.

## MATERIALS AND METHODS

### Assessing plants around enset gardens for *Xanthomonas* spp. infection

A field survey was carried out to assess some crops that is, *Canna* spp., sugar cane (*Sacharum officinarum*), cultivated sorghum (*Sorghum bicolor*), and wild sorghum (*Sorghum halepense*). The survey was carried out by visiting enset and banana producing areas in South and Southwest Ethiopia. During the survey, data were collected on the type of plants growing in and around each field; incidence of disease on each of the above plants as proportion of plants with visible symptoms. Besides, specimens were collected from each plant and brought to the laboratory for verification. 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 1l sterilized distilled water) (Mwebaze, 2007; Mwangi et al., 2007), catalase (Dickey and Kelman, 1988) 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.

### Pathogenicity tests

Pathogenicity tests were carried out to determine the possible host range of the pathogenic *Xcm* and the reaction of various plant species. The experiment had a factorial design with isolates as sub-factors and test plants as main factors. It was arranged in a completely randomized design with five replications.

Three *Xcm* isolates (*I*<sub>1</sub>, *I*<sub>2</sub> and *I*<sub>3</sub>) were isolated from naturally infected cultivated enset, wild enset and banana, respectively (Table 1), and used for the pathogenicity test on cultivated enset, wild enset, banana, *Canna* species and cereal crops (maize, sorghum and finger millet) collected from different areas (Table 2). Each isolate was collected by taking bacterial ooze in the field using a toothpick and then suspending the ooze in a test tube half filled with sterilized distilled water according to Weldemichael (2000). Before inoculation of test plants, the concentration of each bacterial suspension was adjusted using a spectrophotometer to 0.3OD at 460 nm, which is equivalent to 10<sup>9</sup>cfu/ml bacteria cells.

Seedlings of banana, enset and *Canna* spp. were transplanted into pots (22x22 cm), filled with a sun-dried mixture of soil, sand and manure at a ratio of 3:1:1 (Quimio, 1992), then allowed to establish for three months (four to seven leaf stage). Inoculation of test plants with each bacterial isolate was done by injecting an aliquot of 3 ml of the bacterial suspension into the petiole base of the newly expanding central leaf using a 10 ml sterile hypodermic

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

Isolate code	Location	Altitude (masl)	Plant species sampled
I <sub>1</sub>	Keffa/Gimbo/Ufundo	1.692	Wild enset (Epoo)
I <sub>2</sub>	Keffa/Gimbo/Ufundo	1.702	Banana (Pisang awak)
I <sub>3</sub>	Sidama/Hagerselam/Calbessa	2.752	Cultivated enset (Gullumo)

**Table 2.** Plant materials used for the pathogenicity tests, their designation and source.

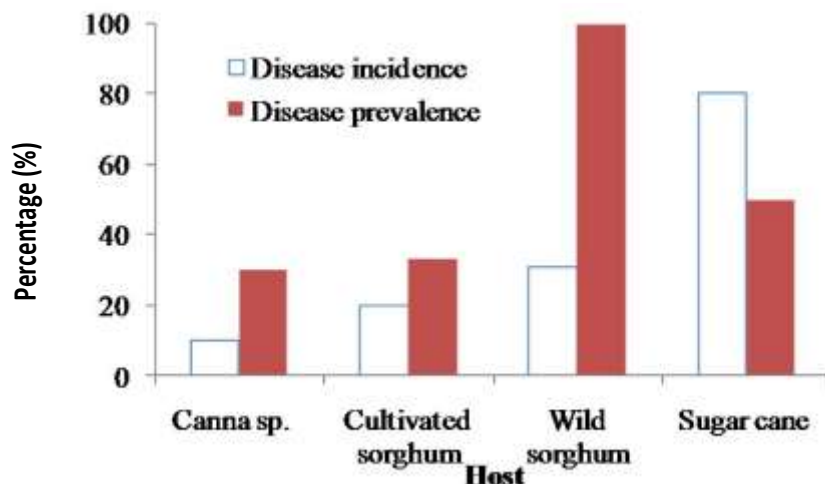
Tested plant species	Cultivar/clone	Designation	Source
Banana	Pisang awak	Pa	Yirgalem
Cultivated enset	Meziya	Mz	Areka
Cultivated enset	Mandaluka	Ml	Areka
Cultivated enset	Hiniwa	Hn	Areka
Cultivated enset	Ashure	As	Areka
Cultivated enset	Digmaz	Dg	Areka
Cultivated enset	Zerate	Zr	Areka
Cultivated enset	Sorpie	Sp	Areka
Cultivated enset	Hiniba	Hb	Areka
Cultivated enset	Buffare	Bf	Areka
Cultivated enset	Geziwet	Gz	Areka
Wild enset	Erpa	Erp13	Areka
Wild enset	Epoo	Epo1	Kaffa collection
Wild enset	Epoo	Epo2	Kaffa collection
Wild enset	Epoo	Epo3	Kaffa collection
Wild enset	Epoo	Epo4	Kaffa collection
Wild enset	Epoo	Epo5	Kaffa collection
Wild enset	Epoo	Epo6	Areka
Wild enset	Erpa	Erp18	Areka
Wild enset	Erpa	Erp19	Areka
<i>Canna</i> spp.	<i>Canna indica</i>	CI	Kaffa collection
<i>Canna</i> spp.	<i>Canna orchoides</i>	Co	HCA
Maize	Melkessa-1	Mk	HCA
Maize	ACV6	AC	HCA
Sorghum	Gambella 1107	Gb	HCA
Sorghum	IS-18758	IS	HCA
Sorghum	RTxTAM	RT	HCA
Sorghum	BTx623	BT	HCA
Wild sorghum	<i>Sorghum halepense</i>	Sh	MARC
Finger millet	Tadess	Td	ANARC
Finger millet	Pandet	Pd	ANARC

HCA-Hawassa College of Agriculture; MARC-Melkassa Agriculture Research Center; ANARC-Arsi Negella Agriculture Research Center, Erpa and Epoo are vernacular names of wild ensets.

syringe (Ashagari, 1985). Inoculated plants were then covered with a wet plastic bag for 48 h.

For the cereals, seeds of each species were planted in plastic pots (18x18 cm) filled with a sun-dried sterile mixture of soil, sand and manure (3:1:1) and 324 mg of urea per pot; this amount of urea was re-applied six weeks after planting. About 10 seeds were

planted in drills and thinned to five plants/pot two weeks after planting. The cereals were inoculated at one month old (three to four leaf stage) by wounding and spraying techniques, that is, their leaves were physically struck with very fine sterile sand paper, sprayed with 3 ml of each bacterial isolate suspension and covered with a transparent plastic bag for 48 h (Hussien, 2001). Negative



**Figure 1.** Mean disease incidence and prevalence of *Xanthomonas* spp. on *Canna*, sorghum, sugar cane and wild sorghum.

controls of each plant species were inoculated with the same quantity of sterile distilled water.

#### Disease assessment

Data were collected on incubation period (period between inoculation and first wilting symptom) and the number of plants showing disease symptoms was recorded weekly starting from one week after inoculation for four consecutive months. Disease incidence was calculated according to the following formula:

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

Where, DI: disease incidence, NPCW: number of plants completely wilted, NPPT: number of plants assessed.

In addition, disease severity was assessed using a standard disease scale of 0 to 5 (Winstead and Kelman, 1952) where 0: no symptom; 1: only the inoculated leaf wilted; 2: 2 to 3 leaves wilted; 3: four leaves wilted; 4: all leaves wilted and 5: plant dead). The severity grades were converted into percentage 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. Severity from each scoring date was converted to area under percent severity index progress curve (AUPSiPC) using the formula by Jerger and Vujanen-Rollinson (2001) as follows:

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

Where, n is total number of assessments,  $t_i$  is the time of the  $i^{\text{th}}$

assessment in weeks from the first assessment date,  $x_i$  is the percentage of the disease severity or disease incidence at  $i^{\text{th}}$  assessment. AUPSiPC is the area under percent severity index progress curve was expressed in percent-weeks.

#### Data analysis

Analysis of variance was performed for data on disease parameters (wilt incidence and incubation period) using the General Linear Model of SAS computer package (SAS, Institute Inc., 2003). Means were separated by least significant difference (LSD) at 5% probability level.

## RESULTS

### Disease incidence on plants around enset garden and bacterial isolates characterization

Visible disease symptoms (yellowing of the leaf at margin side and tip, wilted leaf and blade folded upward and inward and also dry leaf) were evident on diseased plants. Reddish-brown streaks were also recorded on the grasses that is, cultivated and wild sorghum, and sugar cane. Disease incidence (proportion of infected plants in a field) varied from 10% on *Canna* sp. to 80% on sugar cane (Figure 1). Disease prevalence (proportion of fields with at least one diseased plant) ranges between 30% on *Canna* sp. and 100% on wild sorghum.

When diseased (wilted) plants were plated on sucrose peptone agar medium (a semi selective medium for *Xanthomonas*), deep yellow colonies grew out from sugarcane and sorghum, and yellow colored colonies were observed growing from *Canna*. All of these isolates were found to possess negative reaction to Gram staining, and positive reaction to catalase reaction. Inoculation of the isolates to enset and banana did not



**Figure 2.** (A left) Death of enset plant ('Astara' clone) after inoculation with an *Xcm* isolate collected from cultivated enset and (A right) healthy looking control plant of same clone; (B) wilting of the enset clone 'Sorpie' after inoculation with an *Xcm* isolate obtained from wild enset.

induce any symptom and hence the isolates were considered non-pathogenic to enset and banana.

#### Pathogenicity of *Xcm* isolates to various plants

The pathogenicity of *Xcm* isolates to various plants was tested in two experiments. Although, the results of both experiments were consistent; the average of the two experiments was presented in the current report.

#### Banana and cultivated enset

Disease assessment started a week after inoculation and the earliest typical external disease symptoms were observed two to four weeks post inoculation on 'Pisang awak' and enset clones. These included folding down of the leaf blade along the midrib, followed by scalding and dull green appearance of the central inoculated leaf. This was followed by yellowing, starting at the apex, sequential wilting of leaves, drying and wilting of the whole plant and finally plant rotting and death (Figure 2). Yellowish bacterial ooze was observed when pseudostem and leaf petiole were cut. In the current experiment, there were significant variations among clones and isolates in terms of incubation period, disease incidence and area under disease severity index progress curve (Tables 3 to 5). However, the interaction was not significant (data not shown).

Among the tested plants, the banana cultivar 'Pisang awak' was found to have the shortest incubation period followed by the enset clone 'Mandaluka'. Enset clone 'Mezya' had the longest incubation period. This clone

**Table 3.** Incubation period (IP) (weeks), percent disease incidence (%DI) and area under percent severity index progress curve (AUDSPC) for 10 cultivated enset clones and a banana cultivar, 'Pisang awak', inoculated with three *Xcm* isolates under greenhouse conditions.

Clone	IP	%DI	AUDSPC
Pisang awak	2.8 <sup>c</sup>	73.3 <sup>a</sup>	1039 <sup>a</sup>
Mezya	4.5 <sup>a</sup>	26.7 <sup>g</sup>	534 <sup>f</sup>
Mandaluka	2.9 <sup>c</sup>	60.0 <sup>b</sup>	891 <sup>abc</sup>
Hiniwa	3.3 <sup>bc</sup>	33.3 <sup>f</sup>	649 <sup>ef</sup>
Ashure	3.3 <sup>bc</sup>	53.3 <sup>c</sup>	751 <sup>bcd</sup>
Digmaz	3.7 <sup>b</sup>	33.3 <sup>f</sup>	611 <sup>ef</sup>
Zerate	3.7 <sup>b</sup>	33.3 <sup>f</sup>	693 <sup>def</sup>
Sorpie	3.2 <sup>bc</sup>	46.7 <sup>d</sup>	833 <sup>bcd</sup>
Hiniba	3.1 <sup>bc</sup>	53.3 <sup>c</sup>	728 <sup>cde</sup>
Geziwet	3.1 <sup>bc</sup>	73.3 <sup>a</sup>	919 <sup>ab</sup>
Buffare	3.1 <sup>bc</sup>	40.0 <sup>e</sup>	649 <sup>e</sup>
LSD	0.7011	5.288	184
<b>Isolates</b>			
I <sub>1</sub>	3.95 <sup>a</sup>	5.5 <sup>c</sup>	438 <sup>c</sup>
I <sub>2</sub>	3.4 <sup>b</sup>	56.4 <sup>b</sup>	857 <sup>b</sup>
I <sub>3</sub>	2.7 <sup>c</sup>	81.8 <sup>a</sup>	981 <sup>a</sup>
LSD	0.366	2.76	95.88
% CV	29.06	15.29	23

Means in a column followed by the same letter are not significantly different at 5% level of significance. I<sub>1</sub>: Isolate from wild enset, I<sub>2</sub>: Isolate from banana ('Pisang awak'), I<sub>3</sub>: Isolate from cultivated enset clone ('Gullumo'). Data represent mean of five replications.

also had the lowest average wilt incidence (27%) across

**Table 4.** Incubation period (IP) (weeks), percent disease incidence (%DI) and area under percent severity index progress curve (AUDSPC) for nine wild enset types inoculated with three *Xcm* isolate under greenhouse condition.

Wild enset	IP	%DI	AUDSPC
Epoo1	3.3 <sup>bc</sup>	26.7 <sup>de</sup>	621
Epoo2	3.0 <sup>c</sup>	55.6 <sup>a</sup>	805
Epoo3	3.6 <sup>bc</sup>	40.0 <sup>bc</sup>	803
Epoo4	4.6 <sup>a</sup>	15.3 <sup>f</sup>	460
Epoo5	3.0 <sup>c</sup>	33.3 <sup>dc</sup>	537
Epoo6	3.5 <sup>bc</sup>	33.3 <sup>dc</sup>	786
Erpa13	3.4 <sup>bc</sup>	46.7 <sup>b</sup>	775
Erpa18	3.1 <sup>bc</sup>	60.00 <sup>a</sup>	747
Erpa 19	4.1 <sup>ab</sup>	25.00 <sup>e</sup>	630
LSD	0.95	7.45	357.5
<b>Isolates</b>			
I <sub>1</sub>	3.90 <sup>a</sup>	3.45 <sup>c</sup>	537 <sup>b</sup>
I <sub>2</sub>	3.59 <sup>a</sup>	48.28 <sup>b</sup>	815 <sup>a</sup>
I <sub>3</sub>	2.83 <sup>b</sup>	70.00 <sup>a</sup>	831 <sup>a</sup>
LSD	0.445	3.48	165.16
CV	24.83	16.28	24

Means followed by the same letter in the column are not significantly different at 5% level of significance. I<sub>1</sub>: Isolate from wild enset, I<sub>2</sub>: Isolate from banana ('Pisang awak'), I<sub>3</sub>: Isolate from cultivated enset clone ('Gullumo'). Data represent mean of five replications.

**Table 5.** Incubation period (IP) (weeks), percent disease incidence (%DI) and area under percent severity index progress curve (AUDSPC) for *Canna* spp. and various cereals inoculated with three *Xcm* isolates under greenhouse conditions.

Plant	Species/cultivar	IP	%DI	AUDSPC
<i>Canna</i> spp.	<i>Canna indica</i>	3.8 <sup>abc</sup>	77.8 <sup>a</sup>	845 <sup>a</sup>
	<i>Canna orchoides</i>	3.8 <sup>a</sup>	73.3 <sup>a</sup>	701 <sup>ab</sup>
Maize	Melkassa1	2.8 <sup>efg</sup>	40 <sup>d</sup>	436 <sup>de</sup>
	ACV6	3.0 <sup>defg</sup>	66.7 <sup>b</sup>	700 <sup>b</sup>
Sorghum	Gambella 1107	2.5 <sup>fg</sup>	33.3 <sup>d</sup>	519 <sup>c</sup>
	BTx623	3.1 <sup>def</sup>	33.3 <sup>d</sup>	519 <sup>c</sup>
	IS-18758	3.2 <sup>cde</sup>	50 <sup>c</sup>	589 <sup>bc</sup>
	RTxTAM	2.4 <sup>g</sup>	25 <sup>ef</sup>	525 <sup>c</sup>
Wild sorghum	<i>Sorghum halepense</i>	2.8 <sup>efg</sup>	12.8 <sup>f</sup>	513 <sup>c</sup>
Millet	Tadess	3.2 <sup>bcdde</sup>	16.7 <sup>f</sup>	327 <sup>de</sup>
	Pandet	3.8 <sup>a</sup>	8.4 <sup>f</sup>	292 <sup>e</sup>
	LSD	0.6253	12.2981	144.35
Isolates	I <sub>1</sub>	3.6 <sup>a</sup>	19.6 <sup>c</sup>	495 <sup>b</sup>
	I <sub>2</sub>	3.1 <sup>b</sup>	38.9 <sup>b</sup>	572 <sup>a</sup>
	I <sub>3</sub>	2.7 <sup>c</sup>	55.3 <sup>a</sup>	593 <sup>a</sup>
	LSD	0.28	2.3174	74.639
	%CV	23.49	14.15	33

Means in a column followed by the same letter are not significantly different at 5% level of significance. I<sub>1</sub>: Isolate from wild enset, I<sub>2</sub>: Isolate from banana ('Pisang awak'), I<sub>3</sub>: Isolate from cultivated enset clone ('Gullumo'). Data represent mean of five replications.

the three isolates, while 'Pisang awak' and 'Geziwot' had the highest wilt incidence (73%), followed by 'Mandaluka' (60%). Furthermore, the highest AUPSPC (1039) was recorded on 'Pisang awak' followed by 'Geziwet' and 'Mandaluka' in that order, while the lowest AUPSPC (534) was recorded on 'Mezya'. Thus, the banana cultivar 'Pisang awak' and enset clone 'Geziwot' were suggested to be highly susceptible to *Xcm* as compared to the other clones tested in the current experiment.

When comparisons were made across isolates, isolate  $I_3$  caused wilting the earliest (2.7 weeks after inoculation) while the other two isolates,  $I_1$  and  $I_2$ , took about four weeks and three weeks, respectively, to induce symptoms (Table 3). Most plantlets inoculated with isolates  $I_2$  and  $I_3$  completely wilted but most of the enset clones and some 'Pisang awak' plantlets inoculated with isolate  $I_1$  did not wilt completely. Moreover, wilt incidence and area under the disease severity index progress curve were significantly the lowest for isolate  $I_1$ . On the other hand, isolate  $I_3$  caused the earliest wilting and disease parameters after inoculation with this isolate were significantly greater than for the others. As a result, among the three isolates of *Xcm* used in this study, the wild *Xcm* isolate  $I_1$  was found to be a weaker pathogen as compared to isolates  $I_3$  and  $I_2$ . In contrast, isolate  $I_3$ , which was obtained from cultivated enset in Sidama zone of southern Ethiopia, was the most virulent and aggressive.

### Wild enset

The first disease symptoms on wild enset plants were recorded a week after inoculation as yellowing from the apex to the edge of the inoculated leaf and water-soaked lesions along the inoculated leaf's midrib. Two to five weeks after inoculation leaf wilting and yellowing symptoms were observed on most plantlets (Figure 2). Yellowish bacterial ooze was observed when pseudostem and leaf petiole were cut. Such symptoms are similar to typical *Xanthomonads* bacterial wilt symptoms described on the banana cultivars and cultivated enset under field and experimental conditions.

Like the cultivated enset clones, the wild enset types also reacted differentially to the isolates of *Xcm*. Significant variations were observed among the wild enset types and *Xcm* isolates in terms of incubation period, disease incidence and area under disease severity index progress curve (Table 4).

The mean number of weeks required for the appearance of initial symptoms on wild enset clones varied between three and five. The incubation period was shorter on wild ensets clones such as 'Epoo5', 'Epoo2', 'Erpha18' and 'Erpha13', while 'Epoo4' had the longest incubation period among the tested wild enset clones. None of the nine wild enset types tested in the current experiment showed complete resistance to *Xcm* isolates

used in this study. Among wild enset type tested in the current experiment, wilt incidence was the highest (60%) on 'Erpa18' followed by 'Epoo2', which had the highest AUPSPC (805). Thus these two wild enset types were found to be highly susceptible to *Xcm*. On the other hand, the wild enset 'Epoo4' had significantly the lowest wilt incidence and AUDSPC, making it relatively more tolerant to the pathogen.

In this experiment too, incubation period was the longest for isolate  $I_1$ , while isolate  $I_3$  had the shortest incubation period. Symptom appearance after inoculation with  $I_1$  was delayed by one to two weeks compared to the other two isolates. Most of the plantlets inoculated with isolates  $I_2$  and  $I_3$  were completely wilted 10 weeks after inoculation. On the other hand, only one plantlet of 'Epoo3' inoculated with isolate  $I_1$  completely wilted at the same time of assessment. This difference between isolates in inducing symptoms on tested plants indicates variations in aggressiveness among the isolates. Disease incidence and severity were also high for most wild enset after inoculation with isolates  $I_2$  and  $I_3$ . One hundred percent disease severity indexes were recorded at 5 to 9 weeks after inoculation on wild enset with isolate  $I_3$  (data not shown). Isolate  $I_2$  caused 60 to 100% severity at 7 to 11 weeks after inoculation, while isolate  $I_1$  resulted in 40 to 60% severity at 8 to 14 weeks after inoculation. On average, 70% disease incidence and AUDSPC value of 831 were caused by isolate  $I_3$ . In contrast, isolate  $I_1$  had significantly lower disease incidence and AUDSPC. This further confirmed the most aggressive nature of isolate  $I_3$  as compared to the remaining two isolates.

### *Canna* spp. and cereals

Among the suspected alternative host plants, *Canna* spp., maize, sorghum and finger millet varieties were tested for the reaction to three *Xcm* isolates. Two to three weeks after inoculation, typical external disease symptoms were observed on some plantlets of these suspected plants. On *Canna* plantlets, water soaked lesions developed along the inoculated leaf's midrib within two weeks after inoculation and after three to four weeks some inoculated leaves wilted and leaf blade folded upward and inward, turned yellow, dried and died. However, new suckers that emerged from the corm after the inoculated plantlet kept growing. This may be related to inability of the bacteria to colonize the corm of the *Canna* plants.

In maize, the first symptom observed on the inoculated leaf was necrosis and discoloration or yellowing of the leaf, starting from the tip to the bottom of the leaf, three to four weeks after inoculation. Gradual wilting along the midrib to the edge of the inoculated leaf was also observed. In sorghum varieties, lesions or discoloration initially developed at the tip of the inoculated leaf two weeks after inoculation. Thereafter, the lesions at the tip

of the leaf gradually elongated to the midrib and then to the leaf blade. Eventually, a yellowing symptom appeared on the leaf blade and, in severe cases, a burned appearance at the margin of the leaf. In addition, leaves withered and turned brown, wilted, dried and dropped off. The observed symptoms on finger millet varieties were discoloration starting from the tip to bottom of the leaf and finally turning yellow and dried.

The analysis of variance for incubation period, disease incidence and AUDSPC revealed significant differences among varieties and isolates (Table 5). The number of weeks to the appearance of first disease symptoms varied between two and half, and four among cereal cultivars. Among the tested plants, initial symptoms appeared the earliest on the sorghum cultivar 'RTxTAM' and the latest on the finger millet cultivar 'Pandet'. Each of the inoculated plant species reacted differently to the three isolates of *Xcm*. Disease incidence was in excess of 70% on *C. indica* and *C. orchoides* and reached 67% on the maize variety 'ACV6' (Table 5). Disease incidence was negligible on the finger miller variety 'Pandet'. This variety showed initial symptoms but then the disease progressed quite slowly. The second longest incubation period and lowest AUDSPC were recorded from the other finger millet variety, 'Tadess'. The current results may suggest the more resistant nature of finger millets as compared to the other cereals.

Isolates of *Xcm* differed in their ability to cause the disease on *Canna* spp. and the various cereals. Disease symptoms were induced the earliest by isolate I<sub>3</sub> followed by isolate I<sub>2</sub>. The highest disease incidence was induced by isolate I<sub>3</sub> on *Canna indica* and *Canna orchoides*. The same isolate caused up to 60% disease incidence on maize, cultivated and wild sorghum and finger millets. On average, the highest AUDSPC value of 593 was recorded when plants were inoculated by isolates I<sub>3</sub> (Table 5). This was significantly higher than the AUDPSC from isolate I<sub>1</sub>. In a trend similar to that from cultivated and wild enset, and banana, isolate I<sub>3</sub> was found to be the most aggressive on *Canna* spp. and cereals.

## DISCUSSION

Bacterial wilt caused by *X. campestris* pv. *musacearum* is considered as one of the major biotic stresses threatening enset and banana (Thwaites et al., 2000). The enset-*Xcm* pathosystem remains one of the least studied pathosystems to date. The current study objectives were to determine enset bacterial wilt occurrence on various plants commonly grown in and around enset farms in South and Southwest Ethiopia and elucidate the pathogenicity of *Xcm* isolated from different group of plants.

During the field survey, different plants that is, *Canna* sp., cultivated and wild sorghum, and sugarcane were assessed for symptoms associated with the

*Xanthomonas* bacteria. Results reveal the prevalence of symptoms associated with the *Xanthomonas* bacteria ranging from 30 to 100% with disease incidence varying from 10 to 80%. Thus, these plants were considered as possible alternate hosts to the *Xcm* bacteria. Ssekiwoko et al. (2006) also reported 80 to 100% disease incidence on *C. indica* in a pot experiment, while Ashagari (1985) identified *C. orchoides* as a host for the *Xcm* pathogen. In the present study, *Xanthomonas* from any of these plants did not induce observable symptoms on enset and banana, and hence the isolates were considered as non-pathogenic to enset and banana. On the other hand, the *Canna* spp. and all the aforementioned cereals crops were found to be susceptible to *Xcm* from enset and banana.

The current study reveals the pathogenicity of *Xcm* to cultivated and wild enset, banana, *Canna* spp., and several grasses. In contrast, Ssekiwoko et al. (2006) reported that *Xcm* infects only monocots belonging to the two families Musaceae and Cannaceae. Mwangi et al. (2006) has also excluded maize and sorghum from possible hosts of *Xcm*. On the other hand, Aritua et al. (2008) and Karamura (2012) have reported maize and sugarcane developing disease after being artificially inoculated with *Xcm*. Aritua et al. (2008) even reported genetic similarities between *Xcm* isolates on one hand, and isolates of *Xanthomonas vasicola* pv *holcicola* from sorghum and *Xanthomonas vasicola* pv *vasculorum* from sugarcane on the other. Inoculation of maize with *Xcm* resulted in the development of full blown yellow-brown streaks (Karamura, 2012), a result that coincides with this study findings.

Significant variations ( $p < 0.05$ ) existed among the isolates in terms of incubation period, wilting incidence and severity. In general, isolate I<sub>3</sub> from cultivated enset in South Ethiopia was found to be the most aggressive, while I<sub>1</sub> from wild enset plant was the least aggressive. Variability in terms of pathogenicity among *Xcm* isolates was also reported by Weldemichael (2000). This was contrary to a report by Aritua et al. (2007) that revealed low level of genetic variation among the pathogen isolates collected from different African countries.

However, the current pathogenicity test findings contradict those of Tripathi et al. (2009), who reported no significant differences in pathogenicity among *Xcm* isolates. Our findings thus call for more research in diversity of the pathogen populations. Besides, the results confirm the need to consider isolate variation in breeding for bacterial wilt resistance. We recommend this isolate be used in future resistance screening trials. We also suggest that molecular studies including sequencing be carried out to understand the genetic basis of variation in pathogenicity of the isolates.

The test plants also differed significantly in their degree of susceptibility to *Xcm*. The banana cultivar Pisang awak, *C. indica*, and enset clones Geziwot, Mandaluka, wild enset Epoo2 and Erpa18 showed high disease



incidence and severity, and short incubation period, and hence were considered as most susceptible. Enset clones Mezya, wild enset Epool4, and finger millet cultivar Pandet had lower disease severity and longer incubation period, and hence were considered relatively tolerant to the pathogen. While not much work has been done to assess the susceptibility of wild enset to *Xcm*, the enset clone 'Mezya' was also found to be more tolerant to *Xcm* infection by Ashagari (1985) and Weldemichael (2000).

The current work reveals the potential various plants including wild enset may play in harboring *Xcm* pathogenic to both cultivated enset and banana. Hence, care must be taken to minimize the risk of the pathogen being spread from the wild to agricultural fields. Further characterization of the *X. campestris* pv. *musacearum* strains from wild enset, cultivated enset and banana should be carried out by using the existing available detection methods. In addition, the genetic diversity among both the host and the pathogen should be investigated further. Additional tests on the *Xcm* isolates to different plant species should be carried out to elucidate the potential of wild and cultivated plants in harboring and disseminating the pathogen.

### Conflict of interests

The authors have not declared any conflict of interests.

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