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Evaluation of Enset Clones for Their Reaction to Bacterial Wilt of Enset (*Xanthomonas campestris* pv. *musacearum*) in Gurage Zone, Southern Ethiopia

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Abstract

Bacterial wilt of enset is one of the major threats for the enset production in Gurage Zone, Southern Ethiopia. Even though the use of resistant clones has been an effective management strategy for the disease, such clones are not well identified. Hence, twenty five enset clones, collected from Gurage zone, were evaluated for their reaction to *Xanthomonas campestris* pv. *musacearum* (Xcm) in potted experiment under greenhouse conditions. Twelve suckers were grown for all the 25 clones and ten were inoculated with Xcm pathogen at a concentration of 10^8 cfu/ml and the other suckers were kept untreated as a control. All the 25 enset clones showed wilt symptom but at varying levels of the disease severity. Only one clone (Gezwet) was resistant, 7 clones moderately resistance, 6 clones susceptible and the other 11 clones were highly susceptible to the disease based on wilt incidence, incubation time and Area Under the Disease Progress Curve (AUDPC). The mean incubation period ranges from 16.2 (on Yeregye clone) to 42.2 days (on Gezwet clone). The complete wilting period was also longer for the resistant clone and shorter for susceptible clones. Therefore, Gezwet clone and the other moderately resistant clones were recommended for farmers to incorporate in their farming.

Keywords: *Xanthomonas campestris*, Bacterial wilt, Enset clones, Evaluation, Incidence.

1. Introduction

About 20% of the human population in Ethiopia depends on enset as a food source (Brandt *et al*., 1997). Enset (*Ensete ventricosum*, Ethiopian banana) is a multipurpose crop used as a source food for humans and animals, as medicine (Africa RISING, 2014), and in construction as well as in many cultural practices. This shows that cultivation of enset can significantly improve food security at household and at a national level. Enset is a staple food crop for over 20 million people in the southern part of Ethiopia (Dereje, 2012). The plant has a high nutritive value and is highly productive (Mohammed *et al*., 2013).

However, Bacterial Wilt of Enset (BWE), caused by *Xanthomonas campestris* pv. *musacearum* (Xcm), is the major enset production constraint for all enset producing regions. Bacterial wilt attacks enset at any developmental stage of the plant, including full maturity (Brandt *et al*., 1997). Fikre *et al*. (2012) reported cultural practices and sanitation (e.g., removal of infected plant and plant parts) control measures are the most principal control measures for BWE. On the other hand, curative mechanisms, the use of disease free sucker for planting material, crop rotation, and the use of resistant clones can serve as viable management options for BWE. The identification of infected plants and their early removal are seen as a key part of the control system.

Bacterial wilt of enset disease usually destroys enset plants resulting in total yield loss and threatening the livelihood of millions of people who depend on enset as a staple food source (Brandt *et al*., 1997). The disease attacks almost all varieties of commonly grown banana cultivars (Tripathi *et al*., 2007) and enset clones (Gizachew *et al*., 2008), but to varying extents. There is a high genetic diversity in cultivated enset populations in Ethiopia (Almaz *et al*., 2002; Birmeta, 2004; Yemane and Fassil, 2006; Bizuayehu, 2008). Farmers often cultivate various numbers of enset clones in mixture in their farms. They give vernacular names for each clone. There are more than 66 enset clones in Gurage Zone (Haile, 2009), which is one of the potential enset producing areas in Ethiopia.

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The presence of resistance/tolerance in enset clones to Xcm has been reported despite the fact that no clone was found to be completely resistant (Dereje, 1985; Gizachew *et al*., 2008, Tariku *et al*., 2015). However, further research is needed to consider the vast wealth of enset genetic resources in different enset-growing regions. Continuous and intense evaluation of enset clones for disease resistance is one of the basic requirements for effective and sustained implementation of integrated disease management programs.

Variable levels of clonal response to the Xcm disease were observed under farmers' field conditions and while using artificial inoculation (Anita *et al*., 1996). A number of studies have been conducted for the evaluation of some enset clones for Xcm pathogen (Dereje, 1985; Gizachew *et al*., 2008; Tariku *et al*., 2015); however, compared to the rich source of enset land races and the variability of the pathogen (Fikre and Gizachew, 2007; Befekadu *et al*., 2014), a continuous evaluation of the clones is important and recommended. Enset farmers commonly grow combinations of clones in their enset fields and each clone is basically grown for its specific use. Hence, this study was proposed to evaluate the enset clones for their reaction to Xcm at Gurage Zone, Southern Ethiopia.

2. Materials and Methods

Hypersensitivity Test

In order to separate the pathogenic and non-pathogenic bacterial isolates, the hypersensitivity test was conducted on tobacco (*Nicotiana tabacum* L.) plants. The inoculum was prepared by suspending bacterial cells from 48-hrs-old cultures into Sterilized Distilled Water (SDW) at a density of 10^8 cfu/ml. Then two milliliters of the bacterial cell suspension was injected into the leaves of two-month-old tobacco seedlings using hypodermic syringe and needle. The control plants were inoculated with distilled water. Isolates, showing complete collapse of tissues around the injection point, were considered as positive for the test and identified as pathogenic isolates (Quimio, 1992).

Pathogenicity Test

Bacterial isolates that induced hypersensitivity on the tobacco plants were subjected to pathogenicity test on susceptible enset clone, particularly on Astara clone (Gizachew *et al*., 2008). The enset suckers were grown in a greenhouse condition for two months. Bacterial colonies were grown on yeast extract dextrose calcium carbonate agar (YDC) medium for two days and suspended into SDW. One-year-old (two months after transplant) enset suckers were inoculated with 3 ml of the bacterial cell suspension of 460 nm (10^7-10^8 cfu/ml bacterial cell concentrations) by using a spectrophotometer. After development of the symptom, re-isolation of the pathogen was undertaken from infected leaf petiole at the point of inoculation and re-cultured and used for further work.

Enset Clones

A total of 25 enset clones (Table 1) was evaluated for reaction to Xcm pathogen at Plant Protection Site - Hawasa University, Hawasa, Ethiopia. Twelve one-year-old suckers of each of the 25 clones were collected from enset growing areas of Gurage Zone and grown in pots at Hawasa University in a greenhouse condition. The average temperature and relative humidity of the greenhouse during the experimental period was 22^{o}C and 70%, respectively. About 9 kg of well-mixed clay soil was placed into each pot. The enset clones were collected from areas with the same environmental conditions from farmer fields. The suckers were developed from a single corm for each clone. The clones were evaluated for their reaction to the pathogen under artificial inoculation. Lemat and Nechewe clones were included as tolerant/resistant checks, while Astra was used as a susceptible check (Gizachew *et al*., 2008).

Inoculum Preparation and Inoculation

Bacterial ooze was collected from the inoculated plants used in the pathogenicity test. The exudates were aseptically collected at the cut end of petioles and leaf sheaths with the help of tooth pick and suspended in SDW. A loopful of the suspension was streaked on YDC plate for multiplication of inoculum. The plates were incubated at 28^{o}C for 24 hrs. Pure bacterial colonies, showing light yellow mucoid growth typical of Xcm from the plate, were re-cultured on YDC agar and incubated at 28^{o}C for two days to produce enough bacterial culture for inoculation.

Two months after transplantation (at 4-7 leaf stages), the enset clones were inoculated by using hypodermic syringe and needle with 3 ml of 2-day-old bacterial suspension at the base of young leaf petiole. The concentration of bacteria was adjusted to 10^8 cfu/ml using spectrophotometer. Similarly, the control plants were inoculated with the same amount bacteria free of SDW. Ten suckers as replicates were inoculated with the pathogen and two suckers were inoculated with SDW as a control for each clone. Re-isolation of the pathogen from the inoculated plant was done at the end of the experiment which lasted for two months after inoculatin.

Disease Assessment

Disease data were taken 10 days after inoculation, then at a 7-day-interval for one month. The number of suckers showing wilt symptom, the time of the initial symptom (incubation period) and the complete wilting date were recorded. The percentage of the wilted plants (wilt incidence) at each assessment period was calculated according to the following formula:

$$\text{Incidence} = (NW/NT) \times 100$$

where, NT = the number of total tested plants and NW = the number of wilted plants.

The reaction of each clone was categorized into four resistance levels based on average wilt incidences at 35 DAI (days after inoculation) (Tripathi *et al*., 2007) as follows: Highly Susceptible (HS): 70-100% plants wilted, Susceptible (S): 40-69% plants wilted, Moderately Resistant (MR): less than 40% plants wilted, and Resistant (R): none of the plants wilted. Furthermore, the date of complete wilting, incubation time and average wilt incidence were used for the evaluation of clones. Similarly, percentage of wilted plants at each assessment period was used to calculate the Area Under Disease

Progress Curve (AUDPC) using the following formula (Shaner and Finney, 1977):

$$\text{AUDPC} = \sum_{i=1}^{n}[(Di_{i+1} + Di_i)/2] \times [t_{i+1} - t_i]$$

where Di_i = percentage of wilted plants at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations.

Data Analysis

Statistical software, SAS version 9.2 (SAS, 2002), was used with two-way ANOVA for analysis. Data on percent of disease incidence were arcsine transformed before the analysis. Significant difference among treatment means was tested using Duncan's Multiple Range Test (DMRT) at 5% probability level for significance. The experiment was arranged using Completely Randomized Design (CRD).

3. Results

Out of the 25 enset clones inoculated with Xcm pathogen, all of the clones showed symptoms of BWE at different assessment periods, while all the control plants inoculated with water did not show any wilt symptoms in all clones and at all assessment periods. Also, none of the evaluated enset clones was immune to the pathogen.

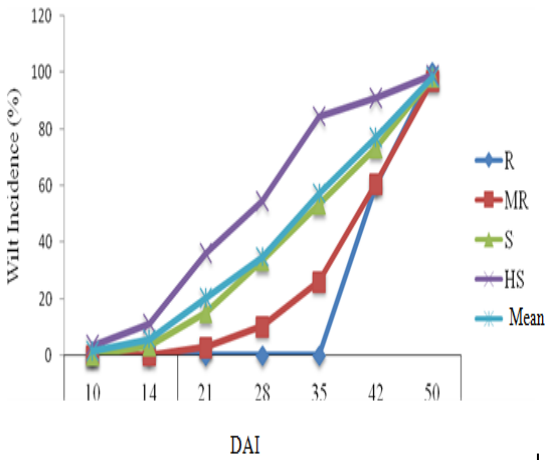
All inoculated clones' symptoms (yellowing and chlorosis) development started in the inoculated leaves. Symptom development after the artificial inoculation was similar to those observed in young plants following natural infection in the field. Significant differences ($p \leq 0.0001$) were observed in the incubation period, wilt incidence at the 35th day, complete wilting period, average incidence and AUDPC among the 25 enset clones evaluated for their resistance to Xcm pathogen. Symptom development started at the 10th day after inoculation and the mean incubation period of the clones varied from 16.2 (Yeregye) to 42.2 (Gezwet) days.

In this experiment the tested enset clones were categorized into four disease-rating groups based on their wilt incidence at 35 DAI. Accordingly, lower wilt incidence, longer incubation period, longer complete wilting period, lower mean incidence, lower AUDPC value and slow disease progression rate were associated with resistant clones, while the reverse held true for the susceptible clones (Figure 1).

The various enset clones showed significant differences in susceptibility to Xcm. The wilt incidence at the 35th DAI ranged from 0 to 100% for the evaluated enset clones. Gezwet was the only resistant clone to Xcm with no wilt incidence at 35 DAI (Figures 1 and 2), and with mean incubation period of 42.2 days and complete wilting of 71 days. Seven enset clones, namely Gimbwe, Terye, Agade, Yeshrakinke, Kechere, Badedat and Ferezye, were moderately resistant to Xcm. These clones showed wilt incidence of less than 40% at 35 DAI and an incubation period of 37.9-40.9 days. On the other hand, a complete wilting for these clones ranged from 63-70 DAI and there were no significant differences among them at 5% probability level for incubation period, complete wilting, mean incidence and AUDPC value (Tables 1 and 2).

Six enset clones, namely Kibinar, Yegendeye, Astara, Ewane, Wenadeye and Zober, were susceptible to the pathogen with an incidence at 35 DAI of 40-69%, incubation period of 31.2-38.5 days and a complete wilting from 56-70 DAI. These clones did not vary significantly from each other in disease parameters. However, Yegendiye clone performed well, with the exception of the incidence at 35 DAI all the parameters categorized it with resistant clones. The other eleven enset clones were found to be highly susceptible to Xcm pathogen with wilt incidence of 70-100% at 35 DAI, incubation period of 16.2-35.7 days and complete wilting period of 47.8-64.0 days (Table 1).

The average wilt incidence over the assessment periods ranged from 20.11 to 70.30% (Table 2). The maximum average wilt incidence was recorded on Yeregye (HS, 70.3%) and Lemat (HS, 64.29%). On the other hand, the average BWE incidence was the lowest on Gezwet (R, 20.11%) and Gimbwe (MR, 22.09%).



Disease progress was rapid on highly susceptible and susceptible clones, whereas a relatively slow progress was recorded on resistant and moderately resistant enset clones (Figure 1). Similarly, the disease progress curve was steeper initially for resistant and moderately resistant clones, while it increased faster for the susceptible and highly susceptible enset clones. The AUDPC value varied significantly ($p < 0.0001$) between the clones. The highest AUDPC (86.19%-day) was recorded on the clone Yeregye (HS), but the resistant clone Gezwet had the lowest AUDPC (21.25%-day) but not significantly different from the clones that are grouped moderately resistant, clones of numbers 2-8 (Table 2) at ($p \leq 0.05$).

Figure 1. Mean disease progress curve for resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS) clones as compared to the average (Mean) progress curve.

Table 1. Mean incubation period, complete wilting, wilt incidence at 35 DAI and disease rating for the 25 enset clones

No.	Clone name	Number of clone	Incidence at 35 DAI	Mean incubation	Complete wilting	Clone reaction rating*
1.	Gezwet	10	00.0 ^{h}	42.2 ^{a}	71.0 ^{a}	R
2.	Gimbwe	10	10.0 ^{hg}	40.9 ^{ba}	66.0 ^{bac}	MR
3.	Terye	10	20.0 ^{fhg}	38.5 ^{bac}	67.0 ^{bac}	MR
4.	Agade	10	30.0 ^{fheg}	39.5 ^{bac}	63.0 ^{bdac}	MR
5.	Yeshrakinke	10	30.0 ^{fheg}	39.8 ^{bac}	70.0 ^{ba}	MR
6.	Kechere	10	30.0 ^{egdf}	37.9 ^{ebdac}	64.0 ^{bdac}	MR
7.	Badedat	10	30.0 ^{egdf}	38.6 ^{bac}	68.0 ^{bac}	MR
8.	Ferezye	09	33.3 ^{feg}	38.0 ^{bdac}	67.8 ^{bac}	MR
9.	Kibinar	10	40.0 ^{fdeg}	32.6 ^{ebdgcf}	63.0 ^{bdac}	S
10	Yegendeye	10	50.0 ^{fdec}	38.5 ^{bac}	70.0 ^{ba}	S
11.	Zober	10	50.0 ^{fdec}	34.8 ^{ebdagcf}	62.2 ^{bdec}	S
12.	Ewane	10	60.0 ^{bdec}	34.3 ^{ebdagcf}	56.0 ^{fdeg}	S
13.	Wenadeye	10	60.0 ^{bdec}	31.6 ^{edhgcf}	60.0 ^{fdec}	S
14.	Astara	10	60.0 ^{bdec}	31.2 ^{eidhgcf}	66.0 ^{bac}	S
15.	Beresye	10	70.0 ^{bdac}	29.5 ^{eidhgf}	53.6 ^{fhg}	HS
16.	Shebrat	10	70.0 ^{bdac}	29.5 ^{eidhgf}	52.4 ^{fhg}	HS
17.	Teguaner	10	70.0 ^{bdac}	35.7 ^{ebdacf}	53.2 ^{fhg}	HS
18.	Demolejat	10	70.0 ^{bdac}	29.2 ^{eihgf}	62.0 ^{bdec}	HS
19.	Nechwe	10	80.0 ^{bac}	22.8 ^{ij}	51.4 ^{hg}	HS
20.	Kanchwe	10	80.0 ^{bac}	28.1 $^{ihg\ f}$	55.0 ^{fheg}	HS
21.	Yekeswe	10	90.0 ^{ba}	27. 8 ^{ihgf}	54.0 ^{fhg}	HS
22.	Bushrat	10	100.0 ^{a}	26.7 ^{ihg}	63.0 ^{bdac}	HS
23.	Oret	10	100.0 ^{a}	23.8 ^{ihj}	64.0 ^{bdac}	HS
24.	Lemat	10	100.0 ^{a}	18.4 ^{j}	57.4 ^{fdeg}	HS
25.	Yeregye	09	100.0 ^{a}	16.2 ^{j}	47.8 ^{h}	HS
CV (%)			12.38	15.70	12.50	
R^2value			0.91	0.63	0.64	

*This rating is based on average wilt incidences at 35 DAI (days after inoculation): Highly Susceptible (HS): 70-100% plants wilted, Susceptible (S): 40-69% plants wilted, Moderately Resistant (MR): less than 40% plants wilted and Resistant (R): none of the plants completely wilted. Means with different superscripts within the same column and class are statistically different at 5% level of significance according to DMRT.

Table 2. Arcsine transformed wilt incidence of the 25 enset clones at different disease assessment periods and their standardized AUDPC (%-day) values

		Wilt Incidence (%)								
		DAI[a]								
No.	**Clone**	**10**	**14**	**21**	**28**	**35**	**42**	**50**	**Mean**	**S AUDPC** (%-day)[b]
1.	Gezwet	0	0	0	0	0	50.77	90	20.11^{i}	21.25^{l}
2.	Gimbewe	0	0	0	0	13.29	51.33	90	22.09^{i}	23.00^{kl}
3.	Terye	0	0	0	13.29	26.57	57.1	76.72	24.81^{hi}	27.38^{kjl}
4.	Agade	0	0	13.29	13.29	32.9	50.77	90	28.61^{ghefi}	30.00^{kijl}
5.	Yeshrakinke	0	0	0	26.57	32.9	57.1	76.72	27.62^{ghfi}	30.88 ^{kijl}
6.	Kechere	0	0	0	13.29	32.9	51.33	90	26.79^{ghi}	28.25^{kjl}
7.	Badedat	0	0	0	0	32.9	50.77	90	24.81^{hi}	26.50^{kjl}
8.	Ferezye	0	0	15	30	34.62	42.12	90	30.25^{ghefi}	30.25^{kijl}
9.	Kibinar	0	26.57	26.57	32.9	39.23	57.1	90	38.91^{gcefd}	41.63^{gifh}
10.	Yegendye	0	0	13.29	26.57	45	50.77	76.72	30.34^{ghefi}	34.25^{kij}

11.	Zober	0	0	26.57	26.57	45	63.43	90	35.94^{ghefd}	40.75^{gih}
12.	Ewane	0	0	13.29	32.9	50.77	57.1	90	34.87^{ghefd}	40.63^{gih}
13.	Wenadye	0	0	26.57	51.33	51.33	57.1	90	39.48^{cefd}	47.63^{gefh}
14.	Astara	0	0	13.29	38.67	50.77	76.72	90	38.49^{gcefd}	46.13^{gefh}
15.	Beresye	0	0	32.9	39.23	57.1	63.43	90	40.38^{cebd}	49.50^{gefd}
16.	Shebrat	0	13.29	26.57	25.69	57.1	63.43	90	39.44^{cefd}	52.63^{cefd}
17.	Teguaner	0	0	0	13.29	57.1	57.1	90	31.07^{ghefi}	37.13^{ijh}
18.	Demolejat	0	0	32.9	39.23	57.1	76.72	76.72	40.38^{cebd}	50.38^{gefd}
19.	Nechwe	13.3	26.57	45	57.1	70.39	45.45	90	49.69^{cb}	66.06^{b}
20.	Kanchwe	0	0	32.9	45	63.43	76.72	90	44.01^{cbd}	54.88^{cebd}
21.	Yekeswe	0	0	19.62	45	76.72	90	90	45.91^{cbd}	56.75^{cebd}
22.	Bushrat	0	0	39.23	39.23	90	90	90	49.78^{cb}	60.25^{cbd}
23.	Oret	0	13.29	32.9	50.77	90	90	90	52.42^{b}	63.38^{cb}
24.	Lemat	26.6	39.23	50.77	63.43	90	90	90	64.29^{a}	77.25^{a}
25.	Yeregye	13.3	42.12	76.72	90	90	90	90	70.30^{a}	86.19^{a}
CV(%)									13.64	11.09
R^2									0.92	0.96

[a] days after inoculation; [b] Standard AUDPC (Area Under the Disease Progress Curve). Means with different superscripts within the same column and class are statistically different at 5% level of significance according to DMRT.

4. Discussion

In the present study, 25 enset clones from Gurage zone were evaluated for their reaction to Xcm pathogen under artificial inoculation and produced varying reactions. Some of the varieties were more tolerant to the disease while the others were susceptible. Generally, all the inoculated enset clones developed bacterial wilt symptoms to various intensities. A study by Tariku *et al*. (2015) and Gizachew *et al*. (2008) revealed that all the inoculated clones developed disease symptoms. Based on the evaluation of their reaction, none of the enset clones had a complete resistance to Xcm pathogen. Many reports indicate that there was no completely resistant enset clone to Xcm pathogen (Dereje, 1985; Gizachew *et al*., 2008), except for Mezya, which had a high resistance to the pathogen (Fikre and Gizachew, 2007). Similarly, no banana cultivar was found to be completely resistant to Xcm (Ssekiwoko *et al*., 2006; Biruma *et al*., 2007; Tripathi *et al*., 2007; Smith *et al*., 2008). None of the inoculated enset clones were recovered from Xcm infection.

This result partially agrees with the previous findings of Gizachew *et al.* (2008), who reported that Gezwet clone was susceptible, while in the current experiment it was found to be resistant but Astara was found to be a susceptible clone in both cases. Lemat and Nechwe showed a relative tolerance to Xcm (Gizachew *et al*., 2008). Conversely, both clones were found to be susceptible to the pathogen in this finding. Dereje (1985) reported that Agade was more susceptible to Xcm than the other clones tested, but here it was a moderately resistant clone. Similar findings were reported from Tariku *et al*. (2015) for Badedat clone which was found to be high resistant for BWE disease. In this experiment, Yeshrakinke was found to be a moderately resistant clone, which is in agreement with Anita *et al.* (1996), who reported that it was a tolerant clone to Xcm pathogen. Farmers in the study area also considered this clone as more tolerant to the disease.

In contrast, Gizachew *et al.* (2008) reported that Yeshrakinke was a susceptible clone. This variation might be due to the variations of isolates of Xcm pathogen (Fikre and Gizachew, 2007; Befekadu *et al*., 2014) though, this experiment was conducted in only one pathogenic isolate or it might be related to the genetic variations within the clones (a single clone may contain several sub clones). Fikre and Gizachew (2007) reported enset clones are not consistent for their resistance/tolerance across locations and time.

Both the susceptible (Astara) and the tolerant (Lemat and Nechewe) checks, used in the present study, were all found to be susceptible to the pathogen. Tariku *et al*. (2015) also reported that Astara was a susceptible clone. Although Daniel and Getaneh (2015) reported that some botanicals are effective to Xcm pathogen, no chemical is recommended to the pathogen. Hence, the use of resistant enset clones should be the most effective management approach. In the present experiment, eight tolerant clones were identified and most of them are preferred by the farmers for their agronomic trait.

5. Conclusion and Recommendations

In the present study, 25 enset clones were evaluated for their reaction to Xcm pathogen from Gurage zone in artificial inoculation. All the enset clones showed symptoms of chlorosis and/or necrosis on leaves of the inoculated plants in varying periods, whereas the control plants (inoculated with water only) did not show any kind of symptoms. However, the clones varied in their reaction to the pathogen, including incubation period, wilt incidence, complete wilting day and AUDPC value.

Among the 25 enset clones evaluated from Gurage zone, only Gezwet was the resistant clone, even clones, namely Gimbwe, Terye, Agade, Yeshrakinke, Kechere, Badedat and Ferezye, were moderately resistant, while six enset clones, namely Astara, Yegendeye, Zober, Ewane, Wenadeye and Kibinar, were categorized as susceptible enset clones. The other 11 enset clones were found to be highly susceptible to Xcm pathogen.

Considering the rich diversity of enset plants, it was anticipated that screening and evaluation of enset clones might provide a good source for effective management strategies of the disease.

The present study identified one resistant and seven moderately tolerant enset clones to the pathogen. Therefore, farmers should be encouraged to incorporate these clones in combination with other effective control measures into their farming systems. On the other hand, this study considered only 25 enset clones from Gurage zone. However, enset plant is genetically diverse in different locations and zones. Therefore, it is recommended that all enset clones be collected and evaluated for their reaction to the pathogen at the farm level of the country. These clones should also be further evaluated against a large number of Xcm isolates after being well-characterized into races or biotypes. The tolerant clones should also be further evaluated for their agronomic performances.

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