Original Research Article||

Assessment and evaluation of enset landraces to bacterial wilt (*Xanthamonas campestris* pv. musacearum) disease at Gedio Zone, Southern Ethiopia

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Abstract

Enset is an important food crop produced in Ethiopia with great role in food security especially for millions of people living in the southern and south western parts of the country. However, its production has been threatened by a devastating bacterial disease caused by *Xanthomonas campestris* pv. musacearum Field surveys in Gedio Zone and a pot experiment at Dilla University were conducted during the 2018/2019 cropping season. A total of 90 enset farms were observed at about 5 km apart. Observations of disease symptoms on farms were performed using a simple random sampling technique in a diagonal fashion within a sampling area of 10 m × 10 m. Numbers of infected and disease free enset plants in each sample were recorded. The results showed that 65% of enset farms were infected with the disease, with a mean incidence of 34.96%. Twenty Enset landraces collected from Gedeo zone were evaluated for their reaction to Enset bacterial wilt through artificial inoculation. The experiment was laid out in a Randomized Complete Design with twenty treatments assigned to experimental units in three replications. Except Maziya, all enset landraces showed wilt symptom but at varying levels of the disease incidence during the first 35 days after inoculation. Maziya was resistant enset landrace while Ganticho, Torame, Filila, Ado, Werabesa, Mindame, and Gakira were moderately resistant. Therefore, the resistant and tolerant landraces should be multiplied, demonstrated, and incorporated into farming practices. However, these should be further evaluated for a large number of Xcm isolates under both pot experiments and field conditions.

Key words: Enset, inoculums, resistance, tolerance

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INTRODUCTION

Enset (*Ensete ventricosum*) supports more than 20% of Ethiopia's population as stable and co-stable food (Tadesse, 2002; Spring et al., 1997). According to CSA (2016), enset is grown in south-western part of the country and covers considerable land area within the private holdings. The number of estimated enset trees harvested, in that agricultural year, from all over the country is 112,522,152. Thus, the total produce in the form of Amicho, Kocho, and Bula is 23,821,849.47 quintals, 28,329,103.94 quintals and 950,414.35 quintals respectively. From these next to Sidama Zone, Gedeo Zone has the second lion share accounting for 7,776,231 enset trees. Its product in the form of Amicho, Kocho, and Bula in quintals were 3,003,975.22, 3,421,855.40 and 61,909.36 quintals, respectively in 2015/2016.

Farmers use different clones for different uses like for kocho, bulla and fiber productivity, medicinal values, and rate clones based on their reaction to bacterial wilt diseases. Farmers conserve a number of enset clones ranging from 3 to 28, on average 9 clones per family farm in southern Ethiopia. The highest number of enset clones (66) was recorded in Dauro zone and the lowest number (26) in Gedeo zone. Enset contributes 23.36 % of the total gross farm income in these areas (McKnight Foundation Collaborative Crop Research Program Project., 2013). The majority of enset production is consumed by the producers as a main dish in their daily lives.

Gedeo is one of the major enset (*Ensete ventricosum*) producing zones of the Southern Nations, nationalities and People's region. Coffee and enset are the dominant perennials in the Gedeo Zone, with enset is growing over all altitudes (Gebrehiwot and Maryo, 2015). In addition, Gedeo zone is characterized by its enset based agro-forestry system and such system is

also found in Sidama, Gurage, Hadiya and Kembata zones of the country (Tadesse, 2002).

The production and productivity of enset is threatened by different biotic and abiotic factors. Among the biotic constraints, diseases caused by bacteria, fungi, nematodes, viruses as well as mammalian and insect pests have been identified as serious problems. Of all the biotic constraints, the bacterial wilt disease is the major impediment for enset production. Enset Bacterial Wilt (EBW) particularly *Xathomonas vasicola pv. musacearum* is known to cause severe damage, as it attacks and kills the plants at any growth stages, including fully matured (ready for harvest). Both the areas and the productivity of enset is declining continuously due to this disease (Endale et al., 2003; Tsegaye et al., 2007; Anita et al., 1996). The losses due to this disease can reach up to 100% under favorable condition. The disease is also the major constraint for enset production in Gedeo zone.

Host plant resistance is believed to be the most effective and economical control measures for the disease. Yet, there is no bactericide control agent recommended for the disease, hence use of EBW resistant cultivars remain to be the only practical and effective method of controlling the disease. However, the development of resistant enset clone has remained difficult, available reports related to clonal screening against bacterial wilt have indicated the possibilities of using host plant resistance. The reactions of enset clones to EBW disease was evaluated at different regions of Ethiopia and variable levels of reactions were observed (Mekuria et al., 2016, Mengistu et al., 2014; Welde-Michael et al., 2008). Gedeo zone is one of the potential areas for enset production and high diversity of enset landraces. Despite the importance of enset crops in Gedeo and the significant diversity of enset clones in the zone, the identification of enset clones' resistance to EBW disease is essential. Therefore, the objectives of this study were to determine the distribution and incidence of enset bacterial wilt disease in the study area and to evaluate the resistance of enset clones to EBW disease.

MATERIALS AND METHODS

Description of the Study Area

This study was conducted in the Gedeo zone during the 2018/2019 cropping season. The area is located about 365 km south of Addis Ababa along the main highway that connects Ethiopia and Kenya, and about 90 km south of Hawassa, the capital city of SNNPRS. The zone shares boundaries in the East, West and South with Oromia regional state, and in the North with Sidama Region. The total area of the zone is 134,708 hectares. The study area lies at elevation ranging between 1501 and 3000 masl. The major crops grown in the area are enset, maize, soya bean, cabbage, fruit trees (avocado, mango), root and tuber crops, coffee and onion. Enset -Coffee based agroforestry is the leading farming system in the area. Enset is the major crop grown in the zone; it is the most frequently and widely grown crop used for household consumption, and it is becoming one of the most valuable cash crops and a source of livelihood support for the rural community.

Field Survey and Sampling Procedures

The status of EBW at each field was assessed and recorded through direct field observations. Three districts of the zone namely: Dilla Zuriya, Bule and Gedeb were purposefully selected for their high enset production potentials. A total of 9 kebeles (three in each district) were selected based on road access and importance of the disease. A total of 90 enset farms were observed (10 in each kebele) at about 3-5 km apart. Observation for disease symptom in farms was performed with a simple random sampling technique in a diagonal fashion in a sampling area of 100 m (10 m × 10 m). The number of samples observed from each farm ranged from three to five, depending on the size of the farm. Numbers of infected and disease free enset plants in each sample were recorded. Disease incidence was calculated as the number of plants showing wilting symptoms divided by the total number of plants assessed, multiplied by 100.

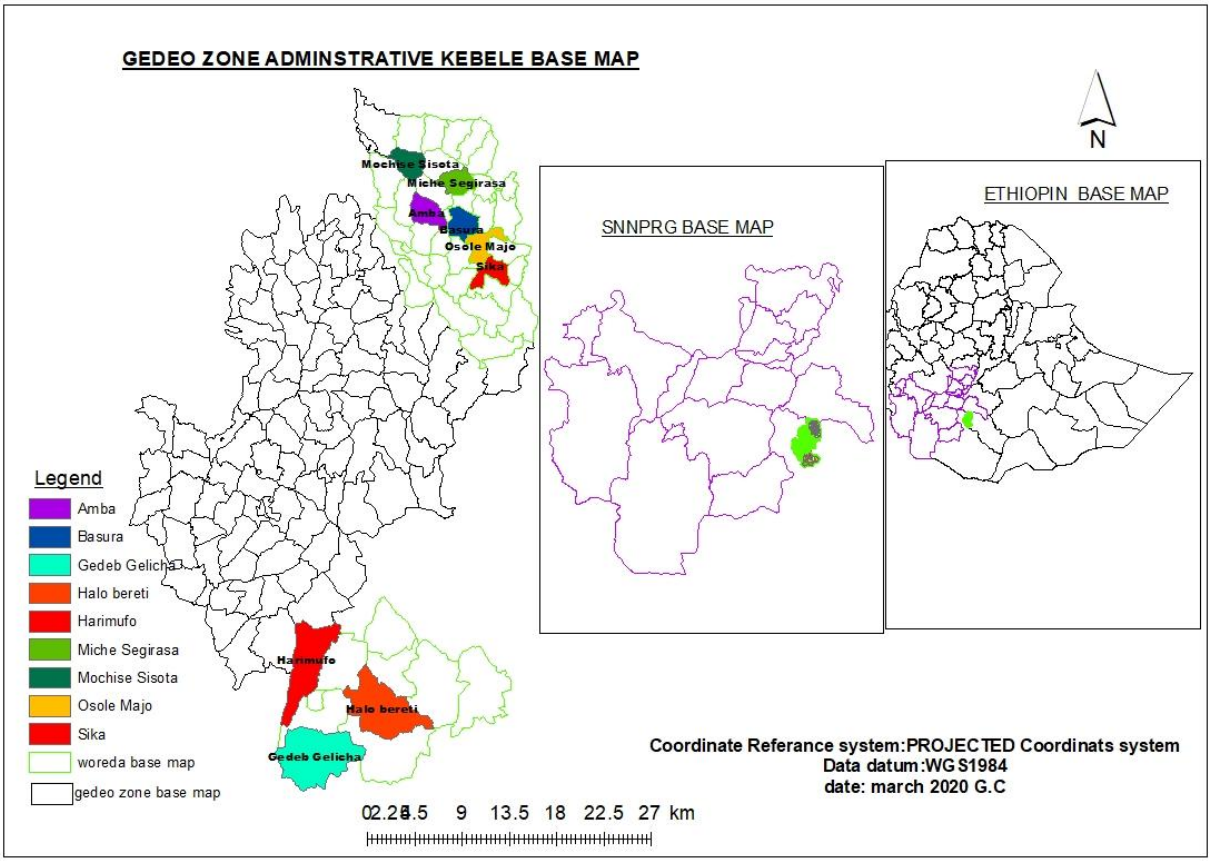

Figure 1. Maps of study sites surveyed for EBW disease

Average wilt incidence for the field was obtained. Prevalence of the disease was calculated using percentage of fields encountered with bacterial wilt disease. Disease severity was calculated based on percentage of damage observed and followed by the procedure of Horsfall and Barratt, 1945.

$$\text{Prevalence} = \frac{NWF}{NTF} X100$$

Where, NWF = the number of fields with bacterial wilt symptom and; NTF = the total number of surveyed fields.

$$\text{Wilt Incidence} = \frac{NWP}{TNP} X100$$

Where, NWP = the number of plants infected by bacterial wilt symptom and; TNP = the total number of plants assessed.

Figure 2. Identification of infected plants during field survey at Amba kebele

Isolation and Preparation of Bacterial Suspension for Inoculation

Xanthomonas campestris (Xcm) samples were collected from a disease hot spot in the Gedeb area, Gedeo Zone, southern Ethiopia. Xcm bacterial ooze from young leaves and/or pseudo stem of diseased enset plants were collected into sterile distilled water and preserved at 4℃ until use. The samples were cut into smaller pieces using sterile scalpel. Then after, the cut pieces were placed in a test tube containing 5 mL of distilled water and allowed to steam for 5 minutes until the bacterial population diffuses out of the cut tissue into the distilled water. Serial dilutions of the bacterial suspension were prepared, and a loopful of the 10^{-3} dilution was streaked onto a sterilized semi-selective growth medium (Tripathi et al., 2007).

Pathogenicity and Hypersensitivity Test

A pathogenicity test was conducted by using a susceptible enset landrace. Xcm suspension (10 mL) of 24 hrs old were tested for hypersensitivity and pathogenicity on two-month-old tobacco (*Nicotiana tabacum*) or the susceptible control enset landrace. In hypersensitivity test conducted on *N. tabacum* (Bobosha, 2003) using cultured and uncultured inoculum types independently, 2 mL of a bacterial suspension containing ~10^8 colony forming units (CFU) per mL (approximately OD600 = 0.5) was used for inoculation. A positive hypersensitive response was scored if tissue exhibited yellow clearing chlorosis limited to around the point of injection. For the initial assessment of pathogenicity tests, disease-free enset suckers of the susceptible landrace 'Astara' were infected, as described by Bobosha (2003) for 'Arkia,' which is a susceptible landrace of enset.

Inoculum Preparation

Bacterial ooze was collected from the inoculated plants used in pathogenicity test. The exudates were collected aseptically from the cut ends of petioles and leaf sheaths of freshly infected plants with the help of a toothpick and suspended in sterilized distilled water.

Inoculum of EBW and Disease Assessment

The pot experiment was organized in three replicates of 20 landraces, each landrace containing 10 plants and landraces within each replication were randomized. Thus, the entire experiment comprised a total of 600 plants. This included a total of 30 individuals of the 20 enset landraces inoculated with uncultured Xcm suspension and the remaining 10 individuals of the 20 plants comprising negative controls inoculated with sterile distilled water. Suckers of three-month-old of the landraces generated from corms of 2 years old were collected from major enset growing areas of Gedeo Zone and Areka enset clones' maintenance and multiplication site. Enset clones of Maziya were used as a resistant check. Uncultured Xcm suspensions preserved at 4℃ were used as inoculum for landrace evaluation. Suckers of enset landraces (two months after transplanting in potted soil mix) were inoculated with a 4 mL aliquot of the bacterial suspension, adjusted to ~10^8 $CFUmL^{-1}$ as described above, by infiltration with a hypodermic sterile syringe into the youngest innermost leaf petiole (Figure 3). A new sterile hypodermic syringe was used for inoculating each sucker of every landrace. The control plants were infiltrated with the same volume of sterile distilled water.

Disease evaluation was conducted at seven days interval for one and half month as suggested by Welde-Michael et al. (2008) for number of diseased plants and severity after artificial infection. Disease assessment will be done at 7, 14, 21, 28 and 35 days after inoculation. The number of infected plants per clone at each disease assessment period was recorded**.** Disease severity was calculated based on the percentage of damage observed, following the procedure of Horsfall and Barratt, 1945.

Data Analysis

The disease prevalence and incidence were analyzed from the collected data by descriptive statistics. The experimental data was subjected to analysis of variance using SAS version 9.2. Means were compared by using least significant difference (LSD) at 1% level of significance.

Figure 3. Inoculation of landraces with uncultured *Xanthomonas campustris* pv. musacearum (Xcm) suspension

RESULTS AND DISCUSSIONS

Prevalence and Incidence

Bacterial wilt disease symptoms were observed in the majority (65% and 34.96%) of the observed enset farms with disease prevalence and incidence percentage, respectively (Table 1). Disease prevalence and incidence varied among the three districts and also among kebeles within a district. The obtained disease incidence in the surveyed farms ranges from 10-66.7% with mean of 34.96%.

Table 1. Prevalence and incidence of EBW in different production locations of Gedeo Zone

District	Keble	Number of farms			Prev. (%)	Incidence%	Severity%
			*NF	*IF			
Dilla Zuria	Anba	10	4	6	60	18.8	100
	Girsa	10	6	4	40	16	100
	M/ Sisota	10	8	2	20	10	100
Bule	Basura	10	3	7	70	30	100
	Oselemajo	10	1	9	90	53.4	100
	Sicka	10	6	4	40	24.2	100
Gedebe	Gubeta	10	0	10	100	66.7	100
	Harmufo	10	1	9	90	58	100
	Hallo Berte	10	2	8	80	37.5	100
Total		90	31	59			
Average					65.6	34.96	

*NF=Non-infected; *IF =Infected; Prev =prevalence

Wilt Incidence for Landraces

The disease was mostly observed on plantations of older than four years. The farmers in the survey area were growing many different clones of enset, with variations among cultivars in their reaction to the disease. However, there was no completely resistant enset landrace. Most of the farmers were aware about the disease. Inter cropping with cactus, rouging out of the diseased plant was the common management practice followed by farmers in controlling the dispersal of the disease. But the assessed farmer's practices on the management of EBW disease were mainly related to sanitary measurements, where most farmers also believed that disease transmission is by farming tools and browsing animals. that are the most important factor, play major role in dissemination of the pathogen in their fields. Generally, the

phytosanitary measures minimize the EBW disease severity.

Table 2. Wilt incidence at various days after inoculation (DAI) and disease rating for the 20 enset landraces

No.	Clone name	EL	Disease incidence (%), DAI				DI
			7	14	21	28	
1.	Maziya	10	0	0	0	0	0.00^{h}
2.	Ganticha	10	0	0	0	1 0	10.00^{gh}
3.	Torame	10	0	0	0	1 0	10.00^{gh}
4.	Filila	10	0	0	1 0	2 0	40.00^{ef}
5.	Ado	10	0	0	2 0	2 0	40.00^{ef}
6.	Werabesa	10	0	0	1 0	2 0	30.00^{fg}
7.	Mundame	10	0	0	2 0	3 0	40.00^{ef}
8.	Gakira	10	0	0	1 0	2 0	26.67^{fg}
9.	Korkoro	10	0	1 0	2 0	3 0	56.67^{de}
10	Shagna	10	0	2 0	3 0	5 0	60.00^{cde}
11.	Kake	10	0	1 0	3 0	4 0	70.00^{bcd}
12.	Harame	10	0	1 0	2 0	4 0	60.00^{cde}
13.	Bufe	10	0	2 0	4 0	6 0	70.00^{bcd}
14.	Miqe	10	0	2 0	3 0	3 0	60.00^{cde}
15.	Dinke	10	0	3 0	5 0	6 0	80.00^{abc}
16.	Karase	10	0	2 0	4 0	6 0	80.00^{abc}
17.	Dimoye	10	1 0	3 0	5 0	7 0	80.00^{abc}
18.	Dambale	10	0	3 0	4 0	8 0	90.00^{ab}
19.	Astara	10	1 0	3 0	7 0	8 0	100.00^{a}
20.	Nifo	10	1 0	4 0	6 0	9 0	100.00^{a}
LSD							20.08
CV%							22.02

DI = disease incidence, DAI = days after inoculation; EL =

Symptom development after the artificial inoculation was similar to those observed in young plants affected by natural infection in the field. A range of symptoms was observed during the course of infection and subsequent disease development on Xcm-challenged enset landraces. At early stages of infection, up to 28 DAI, landraces showed a varying range of symptoms (Table 2), which included twisting with slight leaf curling, and drooping of the blade and tip of the inoculated leaf. The leaf blade around the Xcm inoculated area often became deformed, twisted or curved inwards. These symptoms were replaced by severe curling of the leaf edge, drooping and folding back of leaf blade from 28 DAI, were consistently observed in all landraces. Gradually, drooping from the leaf apex and folding back or collapsing of leaves became the most prominent symptom as the disease developed. All tested enset landraces showed one or more of the symptoms.

Significant differences ($p < 0.0001$) were observed in the incubation period, wilt incidence, and complete wilting period among the 20 enset landraces evaluated for their resistance to Xcm pathogen (Table 3). The various enset plants showed significant differences in susceptibility to Xcm with wilt incidence at the 35th DAI ranging from 0 to 100%. Maziya was resistant to Xcm with no wilt incidence at 35 DAI, and with mean incubation period of 50 days and complete wilting of 67.67 days. Seven enset landraces, were moderately resistant to Xcm. These ensest landraces showed wilt incidence of less than 40% at 35 DAI and an incubation period of 56-30 days. On the other hand, a complete wilting for these landraces ranged from 64-40 DAI (Table 2). Six enset landraces, were susceptible to the pathogen with an incidence at 35 DAI of 56.67-60%, incubation period of 26-25 days and a complete wilting from 38-36 DAI. The other eleven landraces were found to be highly susceptible to Xcm pathogen with wilt incidence of 70-100% at 35 DAI, incubation period of 16-36 days and complete wilting period of 48-64 days (Table 2).

Resistance Rating

Many reports indicate that there was no completely resistant enset clone to Xcm pathogen (Handora and

Michael, 2007; Gizachew et al., 2008), except for Mezya, which had a high resistance to the pathogen (Dereje, 1985).

Table 3. Mean incubation period, complete wilting and disease rating for the 20 enset landraces

No.	Clone name	EL	MIP	CW	CR*
1.	Maziya	10	50.00^{b}	67.67^{a}	R
2.	Ganticha	10	56.00^{a}	64.00^{b}	MR
3.	Torame	10	44.00^{c}	62.00^{b}	MR
4.	Filila	10	38.00^{d}	52.00^{c}	MR
5.	Ado	10	39.00^{d}	54.00^{c}	MR
6.	Werabesa	10	36.00^{de}	50.00^{d}	MR
7.	Mundame	10	33.33^{ef}	52.67^{cd}	MR
8.	Gakira	10	30.00^{fg}	40.00^{e}	MR
9.	Korkoro	10	26.00^{ghi}	38.00^{efg}	S
10	Shagna	10	25.00^{hi}	38.00^{efg}	S
11.	Kake	10	27.00^{gh}	$38.00e^{fg}$	S
12.	Harame	10	26.67^{ghi}	36.00^{fg}	S
13.	Bufe	10	26.00^{ghi}	39.33^{fg}	S
14.	Miqe	10	25.00^{hi}	36.00^{fg}	S
15.	Dinke	10	24.00^{hij}	35.00^{fgh}	HS
16.	Karase	10	23.00^{hij}	35.00^{fgh}	HS
17.	Dimoye	10	23.00^{hij}	34.00^{gh}	HS
18.	Dambale	10	22.00^{ij}	32.00^{h}	HS
19.	Astara	10	20.00^{j}	25.00^{i}	HS
20.	Nifo	10	20.00^{j}	25.00^{i}	HS
LSD			4.23	3.09	
CV%			8.33	4.39	

*MIP = Mean incubation period; CW = Complete wilting; R = resistant; MR = moderate resistant; S = susceptible; HS = highly susceptible.

The resistance rating was based on average wilt incidences at 35 DAI (days after inoculation): as 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 1% level of significance.

Similarly, no banana cultivar was found to be completely resistant to Xcm (Tariku et al., 2015; Smith et al., 2008; Tripathi et al., 2007). None of the inoculated enset clones were recovered from Xcm infection. 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 (Nifo) 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. This study shows that enset landraces vary in their reaction to enset bacterial wilt. Landraces like Ganticha, Torame (Toricho) and Filila were recovered after initial disease symptom development. In the present experiment, tolerant land races were identified.

CONCLUSIONS

Generally, all the enset landraces except maziya 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 landraces varied in their reaction to the pathogen, including incubation period, wilt incidence, and complete wilting days. Among the evaluated 20 enset landraces, only Maziya was resistant and that of Ganticha was moderately resistant, while six enset landraces, were categorized as susceptible ones. 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 20 enset landraces. However, enset plant is genetically diverse in different locations and zones. Therefore, it is recommended that all enset landraces be collected and evaluated for their reaction to the pathogen at the farm level across the country. It is also recommended that all genetic resources are further evaluated against a large number of Xcm isolates after being well-characterized into races or biotypes. The tolerant landraces should also be further evaluated for their agronomic performance.

CONFLICTS OF INTEREST

Authors declare that there is no conflict of interest regarding the publication of this article.

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