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Assessment of Disease Intensity and Evaluation of Enset Clones Against Bacterial Wilt (*Xanthomonas campestris pv. musacearum*) in Tikur Inchini and Jibat Districts of West Shewa, Ethiopia

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Abstract

Enset (***Ensete ventricosum*** Welw. Cheesman) is an important staple food crops in Ethiopia, which is widely cultivated in Southern and South western parts of the country. However, its production has been threatened by one of the devastating bacterial disease caused by ***Xanthomonas campestris pv. musacearum (Xcm)*** in enset cultivated areas of the country. The management of **Enset Bacterial Wilt (EBW)** is important to maximize the crop's yield. Therefore, the present investigation is to determine the disease intensity (prevalence and incidence) of EBW in enset growing areas of Tikur Inchini and Jibat districts of West Shewa, Ethiopia and to evaluate the field grown enset clones for resistance/tolerance to EBW using pot culture condition. Field survey of EBW disease was carried out in the main growing season during September-November, 2012. The disease assessment was made in the enset cultivated fields of selected localities in both districts. The EBW infection was recorded in different levels in both districts. A total of 75 enset cultivated fields were assessed from Tikur Inchini district, of which, 67 fields were affected with different levels of EBW disease prevalence (89.3%). The highest incidence (27%) of EBW was recorded in Waldo Hindhe locality and the lowest incidence was recorded in Homi Hane locality (14 %). From Jibat district, a total of 75 enset cultivated fields were assessed, of which, 65 fields were affected with different levels of disease prevalence (86.7%). The incidence of EBW was recorded highest in Munyo Abayi locality (25%) and the lowest incidence was recorded in Munyo Witate locality (14%). This data indicated that the disease was widely distributed with a very destructive incidence in survey areas of Tikur Inchini and Jibat districts. To evaluate the field grown enset clones for resistance/tolerance to *Xcm*, twenty number of enset clones collected from both the districts were assessed, using artificial inoculation under pot culture condition. The relative susceptibility of the cultivars to EBW was evaluated three months after inoculation based on wilt incidence. All *Xcm* inoculated enset clones expanded disease symptoms to different intensity levels after 15 to 30 days inoculation. The varieties of the disease frequency were variable ranging from 19.3 to 100%. Out of the 20 enset clones, only 6 enset clones confirmed a mean infection incidence less than 50 percent. The present investigation displays that the enset genetic copies fluctuate enset bacterial wilt by their reaction. After 30 days of introduction of inoculation, the enset clones 'Warke Bidu', 'Awenyi', and 'Kekar' showed 100 % disease symptoms. The disease symptom was detected from 'Meziya', 'Hiniba', 'Bedadet' and Warke Dima between 21 and 30 days successive inoculation. The remaining enset clones were relatively resistant/tolerant after inoculation of Xcm. Among all, 'Meziya' was found to have the lowest percentage of disease incidence (19.31%) followed by 'Hiniba' (30.18%) and 'Bedadet' (34.26%). Based on the results, none of the enset clones had resistance to Xcm. The results indicated that the enset clones, 'Meziya', 'Hiniba', 'Bedadet' and 'Warke Dima' have exhibited better resistant/tolerant clones to the bacterial wilt, under artificial inoculation conditions and these enset clones should be considered as most tolerant/resistant clones to the pathogen which could be used as a bacterial wilt management component. The results of the study indicated that the use of resistant/tolerant enset clones is one of the best approaches in the management of EBW, cheaper to the farmers and safer to environments. Hence, a resistant and tolerant outcome of the enset clones confirmed by the wilt pathogen should be further assessed in contrast to a great number of ***Xanthomonas campestris pv. musacearum***, isolates in field conditions.

Keywords: EBW, *Ensete ventricosum*, Incidence, Resistance, Susceptibility, *Xanthomonas campestris pv. musacearum*.

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1. Introduction

Enset is a perennial, herbaceous and a monocarpic, crop, belonging to the order Scitaminae with family Musaceae and the genus *Ensete* [38]. About 25 species of Ensete are equally distributed in Asia and Africa [22]. Over 20 million in Southern part of Ethiopia [17] used *E. ventricosum* as a traditional staple food crop. It is estimated that about 146 thousand hectares in Southern Nations, Nationalities and Peoples Regional State (SNNPRS) and 79 thousand hectares in Oromia are covered with enset [9]. Enset is a huge perennial herbaceous plant that grows 4-11m in height, commonly known as "**false banana**". The Enset Farming System (EFS) is among the backbone of Ethiopian agricultural economy and the most important staple food crops in Ethiopia. All plant parts of enset are utilized for different purposes. Enset is used as food in three forms: amicho, kocho and bulla. Enset gives fiber as a by-product. Enset fiber supports additional 30% of the Ethiopian fiber formation and its vigor is comparable with the fiber of Abaca. Enset is rich in carbohydrate and mineral substances like calcium and iron and the enset plantations restrict soil erosion and preserve soil, therefore, adding more nourishment to the soil farming method [37] and which also attracts the farmers because its ability to produce more food than other cultural crops on a small piece of land with minimum inputs [29]

Enset can grow in a wide range of altitude, however the best elevation for its cultivation is between 2000 and 2750 m. a. s. l. with an average annual rainfall of 1100 to 1500 mm. The crop can withstand relatively long period of drought (about 5 months). The average temperature of enset growing areas is between 10 and 21°C and a relative humidity of 63 to 80 percent. The pH of the ideal soils for enset farming is 5.6 to 7.3 relatively acidic to alkaline. Enset is a drought tolerant and multi-purpose crop. Due to its drought tolerance, enset is regarded as one of the priority crops in Ethiopia, as it makes major contribution to the food security scheme of the country [8].

Among the biotic constraints, diseases initiated by bacteria, fungi, nematodes, viruses and mammalian and insect pests have been identified as serious problems. Of all the biotic constraints, the bacterial wilt disease, is originated by *Xcm* which is the mainly significant disease influence on enset yield [25],[35],[40],[41]. Bacterial wilt caused by *Xcm*, typically characterized by wilting and death of plants is currently considered the most serious disease in Eastern and Central Africa [6]. Enset Bacterial Wilt (EBW) is known to cause severe damage, as it attacks and kills the plants at any growth stages, including full maturity (ready for harvest). Once the plants are attacked by the disease, especially at late maturity stage, it affects whole systems, and usually causing a maximum yield loss. A serious outbreak of the disease was reported by Ashagari [5] with losses up to 70 %. The results obtained from bacterial wilt disease assessment made in some enset fields of the SNNPR, showed losses up to 100% under severe damage [2]. Many researchers [1], [11], [32] reported that both the areas and the productivity of enset is declining continuously due to this disease.

In Ethiopia, disease symptoms similar to bacterial infection of banana, known as Moko disease or bacterial wilt were observed on enset for the first time in the 1930's [15]. However, a bacterium causing a wilt disease of enset was reported in Ethiopia for the first time in the 1960's [41] and named *Xanthomonas musacearum*. The same bacterium was later confirmed as causing a similar disease on cultivated banana and other *Musa* spp. [41] and was subsequently reclassified as *X. campestris pv. Musacearum* [43]. It was reported that in some areas in Western Ethiopia, it is more common on enset and banana [30]. Research on enset conducted so far is mainly limited to mapping the distribution of the disease. Determining the losses due to this disease can reach up to 100% under severe damage of plant by the pathogen under favourable condition for disease development [18], [21]. The most important factors responsible for spreading disease of bacterial wilt include disease-infected planting material, contaminated farming and processing tools, human and animal vectors. Once it appears in a field, it is easily transmitted from infected enset plant to healthy plants through different mechanisms and in some areas where the severity of the disease and loss is high. Hence the farmers are obliged to abandon the whole field and replace it with another crop [21].

Brandt et al. [8] expressed that the common recommended control method for the bacterial wilt of enset are cultural efforts such as cleaning of equipment, the use of healthy, disease-free suckers for planting material, destruction and restricted movement of diseased plants and rotation of crops. Although, the current phyto-sanitory approaches being suggested are work demanding and not simply agreed by farmers. They are at present the only known process of checking further increases of the epidemic, many managerial choices are obtainable. The use of resistant enset clones proffers a superior method to reduce the EBW (Quimio 1992). Variations on disease resistance have been observed on enset clones which are tested under field condition at Awasa Agricultural Research Center, Ethiopia [14]. The clear understanding of the taxonomy and its variability and/

Table 1. List of enset clones, districts, collection sites and an altitude for evaluation of enset clones resistance to *Xcm*

C/N	Clones Name	District	Collection site	Altitude m. a. s. l.
1	Warqee Ija	Tikur Inchini	Bola Germama	2577
2	Hadha Bishan	Tikur Inchini	Bola Germama	2572
3	Hadha Bala	Tikur Inchini	Bola Roge	2582
4	Garda Gababa	Jibat	Bilo Malima	2102
5	Bedadet	Tikur Inchini	Bola Roge	2102
6	Sabbara	Tikur Inchini	Bola Roge	2582
7	Warke Bidu	Tikur Inchini	Bola Geramama	2577
8	Hiniba	Tikur Inchini	Bola Demake	2569
9	Ferasiye	Jibat	Munyo Witate	2102
10	Kekar	Tikur Inchini	Bola Roge	2102
11	Astera	Tikur Inchini	Bola Demake	2569
12	Abba Jobir	Jibat	Tutu Jibat	2569
13	Warke Adi	Tikur Inchini	Bola Roge	2102
14	Awegene	Tikur Inchini	Bola Germama	2564
15	Warke Dima	Tikur Inchini	Bola Germama	2577
16	Garda Dhera	Tikur Inchini	Bola Roge	2102
17	Shartiye	Tikur Inchini	Bola Roge	2582
18	Awenyi	Tikur Inchini	Bola Germama	2564
19	Suite		Areka	
20	Meziya		Areka	

or similarity would help in screening the local enset clones for developing resistant/tolerant clones, as one management option. Although, it has been claimed that huge enset vernaculars are known in the country, no sufficient work has been conducted on its genetic diversity.

Enset bacterial wilt can effectively be controlled by growing of resistant varieties. Since bactericides are not readily available to small scale farmers in developing countries like Ethiopia or due to the fact that their use in low input systems is not economically justifiable, hence use of EBW resistance cultivars remain to be the only practical and effective method of controlling the disease. Host plant resistance is believed to be the most effective and economical control measures for this disease. Although the development of resistance enset clone(s) has remained difficult, available reports related to clonal screening against bacterial wilt have indicated the possibilities of using host plant resistance[1], [10]. To come up with resistant and high yielding enset clones, clone screening is very critical. In addition, despite the importance of the enset crops in Tikur Inchini and Jibat districts of West Shewa Zone, so far, no disease assessment has been made to measure the disease intensity and their by recommend a possible control method. Thus, this study was initiated to provide such elite information in Tikur Inchini and Jibat districts of West Shewa, Ethiopia. Therefore, the present study was carried out to determine the prevalence and the disease intensity of EBW in enset growing areas of Tikur Inchini and Jibat districts of West Shewa, Ethiopia and also to evaluate the resistance/tolerance of enset clones to *Xcm*.

2. Materials and Methods

2.1 Description of the Study Areas

Tikur inchini and Jibat is one of the districts in West Shewa zone of Oromiya Regional State of Ethiopia, which is located at 08°48”N latitude 037°39”E longitude and an average elevation of 2,200-2,483 and 1600-3000 m. a. s. l. respectively (Figure 1A & B). The total area of Tikur and Jibat districts is about 38,687 and 50,950 hectares respectively, out of which the cultivated land is 18,198 and 34,849 hectares and the area of enset production is 2,772 and 680

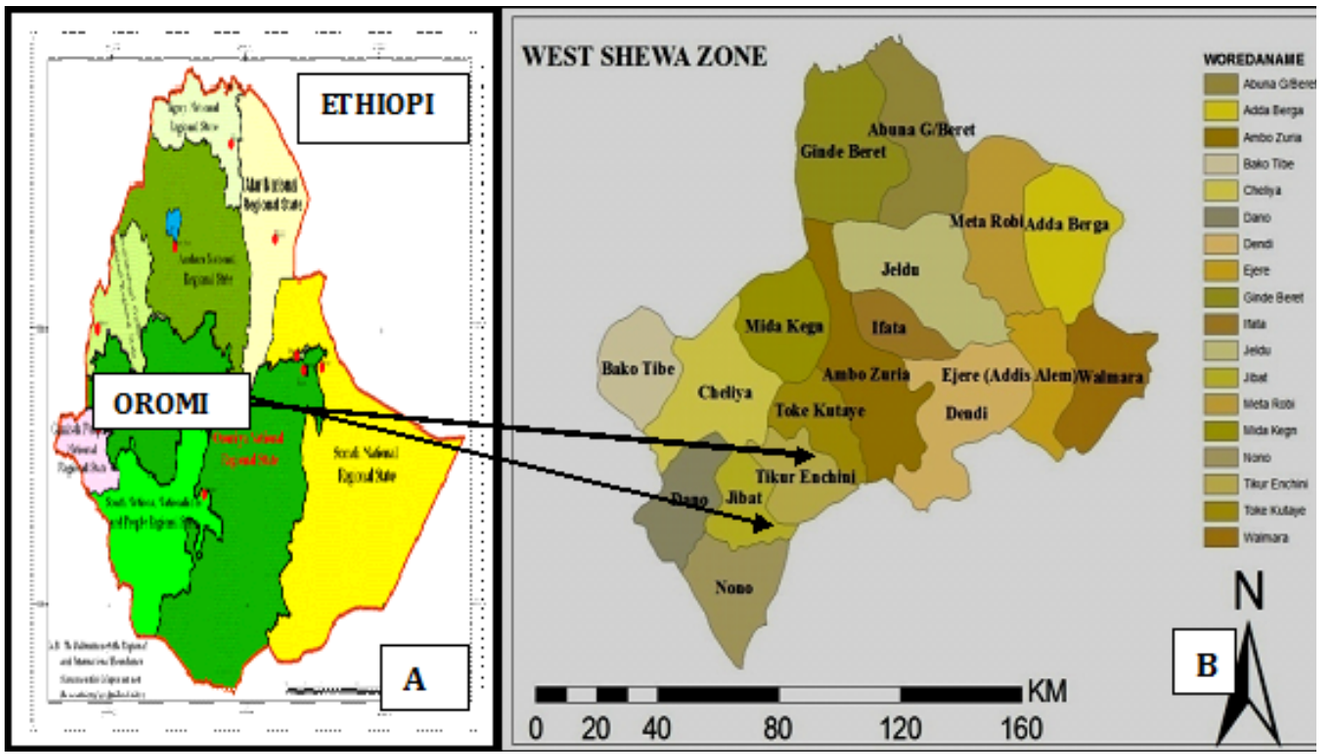

Figure 1. Map of the study areas in Oromia Regional State of Ethiopia: Tikur Inchini and Jibat districts.

hectares, respectively. The heavy rainfall was observed in both districts from onset of June to end of August. The annual rainfall in Tikur and Jibat districts ranged from 1000-1800 and 1000-1900 mm and the temperature ranged from 6 to 24°C and 18 to 24°C, respectively. The soil is classified into two main types' in Tikur Inchini district viz. brown soil (79%) and red soil (21%) and the soil is classified into three main types' in Jibat district viz. mixed soil (77%), black soil (18%) and red soil (5%).

2.2 Survey of EBW in Enset Growing Areas of Tikur Inchini and Jibat Districts

Field survey of EBW disease was carried out in the main growing season during September-November, 2012 at two major enset growing areas of Tikur Inchini and Jibat districts of West Shewa, Ethiopia From each district, five major enset growing localities (Bola Germama, Bola Demeke, Bola Roge, Homi Hane and Waldo Hindhe from Tikur Inchini district and Bilo Malima, Abayi Jibat, Maru Kombolcha, Munyo Jibat and Maru Gombo from Jibat district) were selected purposively and assessed. The selection was made based on major enset growing areas of both the districts and localities. The survey was conducted and the samples were collected through a random field inspection at every 3 km and supplemented with interviewing farmers. From each locality, fifteen enset fields were inspected randomly and the incidence and prevalence of the disease was recorded. A total of 150 enset cultivated fields in these two districts were surveyed. In addition, for each surveyed enset field, supplementary information's (like clone type, date of collection, elevation, latitude and longitude, variety plant growth stages, etc.) was collected using the survey report format. The disease assessment was made in the selected enset fields as mentioned above were recorded. The assessment was made along the two diagonal (in an "X" fashion) of the field at three points using 10 m by 10 m area. In each field, plants within the area were counted and recorded as disease and healthy by variety and the incidence of EBW was calculated as follows:

Disease incidence was calculated using the number of infected plants and expressed as percentage of total number of plants assessed.

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \times 100\%$$

Prevalence of the disease was calculated using the number of fields affected divided by the total number of fields assessed and expressed in percentage.

$$\text{Prevalence \%} = \frac{\text{Number of fields affected}}{\text{Total number of plant units assessed}} \times 100$$

The prevalence and the incidence data were analyzed by using the descriptive statistical analysis (means) over districts and altitudinal range.

Table 2. Incidence and prevalence of EBW disease in enset cultivated fields at Tikur Inchini and Jibat districts

Locality	Number of fields inspected	Altitude m. a. s. l.	Mean Prevalence (%)	Mean incidence (%)
Tikur Inchini district				
Waldo Hindhe	15	2478-2494	93	27
Bola Germama	15	2449-2584	93	26
Bola Roge	15	2521-2613	86.7	20
Bola Demeke	15	2520-2600	93	19
Homi Hane	15	2462-2490	80	14
Jibat district				
Munyo Abayi	15	2498-2571	93	25
Maru Gombo	15	2409-2444	86.6	16
Bilo Malima	15	2460-2795	93	22
Tutu Jibat	15	2526-2585	86.6	15
Munyo Witate	15	2455-2575	80	14

2.3 Collection of Data and Techniques

Both primary and secondary data were used in this study. Primary data collection was done with the help of structured questionnaire, personal interview, direct observation and key informant interview. Secondary data were collected from the main sources of Tikur Inchini and Jibat Agricultural Offices, Ambo Plant Protection Research Center Library, Ambo University Library and Internet.

2.3.1 Diseased Sample Collection

Diseased samples of enset plant parts (petiole, corm and midrib leaves) were collected from the five selected localities of the Tikur Inchini district. The altitude in which the disease collection made ranged from 2419-2613 m. a. s. l. Surface sterilized knife with 70% ethanol was used to cut samples from symptomatic enset plants[4]. The samples were brought into Ambo Plant Protection Research Center laboratory, Ambo, Ethiopia for further study. Diseased plant samples were wrapped with plastic bags and put into the ice box at about 4^0C. The samples were labelled with date of collection, locality, altitude, enset clone name, age and colour of bacterial ooze out. In the laboratory, the infected plant parts were opened (petiole and corm) and bacterial oozes were collected carefully from each collections.

2.3.2 Isolation of Xcm

The pathogen, *Xanthomonas campestris* pv. *musacearum*, was isolated from enset plant parts (petioles and midrib leaves) by growing on the selective media, Yeast Dextrose Calcium Carbonate (YDC) agar media which contains yeast 10g, dextrose 20g, calcium carbonate 20g and agar 15-20g per 1000 ml of sterile distilled water, when targeted to prepared one litre was used. Calcium carbonate was added after cooling of the other ingredients. After pouring of the prepared media onto sterile Petri plates, sample of bacterial ooze was collected from the cut ends of infected petiole and leaf sheath by tooth pick and placed into a 10 ml screw capped tube, half filled with sterile distilled water. The tubes with bacterial suspension was gently shaked and then spread 0.1 micro litre of suspension on the solid surface of the plate by using sterile L-shaped glass rod and incubated at 280C for about one to three days [7]. After 48 to 72 hours of incubation, colonies showed light yellow mucoid growth, typical of *Xcm* was transferred to individual YDC slants with 20% glycerol and maintained at 4^0C in refrigerator for further studies [28].

2.3.3 Pathogenecity Test

For the pathogenecity test, enset plants were allowed to grow on plastic pots using a mixture of soil, manure and sand in 3:2:1 ratio. The accuracy of identified suspected of Xcm on YDC agar medium isolated from enset plant species was found positive in hypersensitive reaction which was subjected to pathogenecity test. Similarly, when the plant reached 3-4 leaves stage, the injection was made on pseudo stem on 5 cm above from the base of a clean and sterile syringe [24]. Control plants were inoculated with the equal amount of liquid media. Inoculated plants were observed 3-5 weeks for symptom development [12]. Kochs's postulate was performed randomly from infected

Figure 2. Screening of Enset clones against enset bacterial wilt by artificial inoculation. Rep denotes: Replication 1, 2, & 3.

enset plants taking a piece of cut leaves using sterilized scalpel from symptomatic enset plants with yellowing and wilting symptoms. The plants were disinfected with 70% ethanol for 2-5 minutes and were rinsed in sterile distilled water repeatedly to remove all traces of disinfectants [19]. Each piece of leaves were dried on filter paper and then transferred into YDC agar medium using alcohol flamed forceps and incubated at 28^{0}C for 48h. Bacteria with mucoid growth was taken and streaked in YDC agar media and then transferred on to YDC slants, which were subjected to biochemical test for identification of the bacteria.

2.3.4 Gram Staining Reaction and Biochemical Tests

The Gram-reaction of bacterial isolate was determined by the staining method of Schaad and Stall [28] and the isolates were that appeared pink, Gram negative bacteria was subjected for further tests. The biochemical test viz. Oxidase, Catalase, Tween 80 hydrolysis [27], Starch hydrolysis [16], and KOH test[13] were made to biochemical characterization of the EBW pathogen. Based on the Bergey's Manual of Systematic Bacteriology, the bacterial isolate, *Xcm* was confirmed and identified.

2.3.5 Screening of Enset Clones for Resistance to Xcm

Screening of enset clones for resistance to Xcm, the one year old young clones of each of the twenty genotypes (Table 1) were used and planted on PVC pots (30 cm in diameter and 30 cm height) packed with 15 kg of mixture of top red soil, manure and sand of 3:2:1 ratio (Figure 2). Single clone was planted per pot (three clones per genotype represent for a replication) and each treatment was repeated thrice in a Randomized Complete Block Design. Totally 180 clones were planted. Enset suckers of Meziya and Suite of nearly having one year old was used as a resistant/tolerant and susceptible check, respectively. Bacterial suspension was prepared from pure culture of *Xcm* for artificial inoculation. The cells of the suspension were harvested into sterile distilled water and adjusted to 1x10^7 and 1x10^8 cfu/ml by dilution to a visibly cloudy suspension. Bacterial suspension of 3ml (2 days old) was used for inoculation of the clones. A sterile hypodermic syringe with metal needle of 5 ml capacity was used to inject the bacterial suspension into the petiole of the youngest open leaf. The same quantity of liquid media was injected into control plants [10], [12]. Plants incubated under pot conditions and were monitored for 8 weeks. The observation for symptom development was made at five days interval for eight weeks after inoculation. The presence of bacterial ooze and discolored vessels was checked by cutting the inoculated leaf petiole close to pseudo stem. Re-isolation of the pathogen was made from infected leaf petiole and sheaths of inoculated plants. Disease assessment was done at 8, 13, 18, 23, 28, 33, 38 to 90 days (five days intervals) after inoculation. The data was recorded on disease incubation on each plant of each clone. The number of contaminated plants per clone at each disease evaluation time was noted. Disease severity was assessed on whole plant basis of number of wilted leaves using the following scale developed by Winstead and Kelman [39]: 0: no symptoms; 1: 1 inoculated leaf wilted; 2: 2-3 leaves wilted; 3: 4 leaves wilted; 4: all leaves wilted and 5: plant dead. Since plants under control treatment did not wilt, the disease severity means for the various genotypes were analysed on one way ANOVA using SAS programme [26].

2.3.6 Data Analysis

The incidence and prevalence data were analyzed by using the descriptive statistical analysis (mean) and were presented in tables and graphs. The statistical differences for resistance among enset clones; the disease severity means for the various genotypes were analysed on one way ANOVA using SAS version 9.1[26] by using Duncan's Multiple Range Test.

3. Results and Discussion

3.1 Survey of EBW in Tikur Inchini and Jibat districts of West Shewa

3.1.1 Prevalence and Incidence of the Disease

Enset bacterial wilt disease survey was conducted at two districts viz. Tikur Inchini and Jibat of West Shewa Zone

Figure 3. A&B. Enset plants caused by EBW in Tikur Inchini Localities. C&D. Collapse of entire enset plants caused by EBW in Jibat district.

(Figures 3 A, B, C & D). EBW is frequently occurred in this part of the region in varying intensity levels in each year. In view of this, the surveys of bacterial wilt in enset fields were carried out from September to November 2012. During this period bacterial wilt disease was at its maximum incidence level. The results of the survey indicated that the EBW was not only widely distributed but was also a serious problem in both districts enset growing kebeles. The enset bacterial wilt infection was recorded in different levels at both districts. A total of 75 enset cultivated fields were assessed from Tikur Inchini district, of which, 67 fields were affected with different levels of disease prevalence (89.3%). The incidence of EBW was recorded highest in Waldo Hindhe kebele (27%) and the lowest incidence was recorded in Homi Hane kebele (14%). From Jibat district, a total of 75 enset cultivated fields were assessed, of which, 65 fields were affected with different levels of disease prevalence (86.7%). The incidence of enset bacterial wilt was recorded highest in Munyo Abayi kebele (25%) and the lowest incidence was recorded in Munyo Witate kebele (14%) (Table 2). This data indicated that the disease was widely distributed with a very destructive incidence in survey areas and it was greatly concern and economically important disease to the community of Tikur Inchini and Jibat districts. This result was in agreement with Dereje [10], who surveyed the enset bacterial wilt in enset cultivated fields at South

Table 3. Prevalence and incidence of EBW disease in different altitude ranges of Tikur Inchini and Jibat districts enset cultivated fields

Altitude Range (m. a. s. l.)	No of fields inspected	Prevalence %	Mean Incidence %
2300-2500	69	89	32
2500-2700	77	88	31
2700-2900	4	75	18
Total	150		

and South Eastern regions of Ethiopia. Recently, Tsehay [33] also reported that the presence of high severity and incidence of EBW disease in Gurage and West Showa zone. The symptoms of EBW was observed in both districts enset cultivated fields showed the yellowing of the leaves, wilting (related with failure of turgor and petiole wrinkling) and also yellowish secretion of a bacterial ooze from cut tissues of the plants, which is the characteristic of EBW. This results in agreement with the earlier reports on enset bacterial wilt assessment in enset cultivated fields by Thwaites et al. [31] and Tushemereirwe et al [34]. Totally, 150 enset fields were assessed for bacterial wilt incidence varies from 0-100% which was seen with bare enset fields and with completely dried and dead left over of enset plants scattered in many places, which confirmed with Tsehay[33]. In addition, the results of the disease survey in enset fields showed various level of EBW infection ranging from 0-100% crop loss on some sampled enset fields since 2012 as described by the interviewed farmers. Moreover, in some areas enset fields were completely destroyed due to this disease and farmers were forced to replace the field with other crops (Figure 4 A & B) in line with this, in some study areas, where the severity of the disease and loss is high, farmers is obliged to abandon the whole field and replace it with another crop such as barley, maize, wheat and as well as developing enset corm again to replace the damage of enset due to high incidence of the disease[3].

3.1.2 Intensity of Enset Bacterial Wilt Based on Different Altitude Ranges

The survey was conducted in all enset growing areas of Tikur Inchini and Jibat districts, which include altitude that ranges between 2300-2900 m. a. s. l. Of the 150 enset fields inspected, 46% of the fields surveyed were found at altitude below 2500 m. a. s. l. while 51.3% was found between 2,500-2,700 m. a. s. l. and the remaining 2.7% was located above 2,700 m. a. s. l. Out of 69 enset fields inspected in the altitude that ranges between 2,300-2500 m. a. s. l. The enset wilt was observed on 32%, with mean incidence. The highest prevalence 89% of enset bacterial wilt was recorded at lower elevation of 2300 to 2500 m. a. s. l. followed by 88% prevalence at 2,500-2,700 m. a. s. l. (Table 3). Similarly, Anita et al. [1] also reported that the disease was widely distributed in high, mid and lower altitude areas of the Central, the Southern and Southwestern enset growing regions of the country.

3.1.3 Farmer's Information About EBW in the Enset Cultivated Fields

Results of the survey indicated that EBW was not only widely distributed but also a very serious problem in all enset growing localities in both districts at different levels. All the peoples of the two districts have agreed the enset-based agricultural system, and use the crop to feed their families as well as their livestock during dry season (when the scarcity of grass occurs). The sustainability of enset agriculture is, on the other hand, threatened by EBW disease, since enset shows more significant in everyday life. According to some enset farmer's information, the regular harvesting of leaves for animals feed and sale as source of income especially for women, which is common in sampled localities of Tikur Inchini district, can increase the severity of the disease through infected tools. In general, the spread and incidence of the disease was more pronounced during the rainy than the dry seasonl10. In both survey districts, all interviewed farmers answered the major constraints for enset production was EBW disease, which is their main threat and cannot get any solution from any concerned body and all enset farmers of the two districts noticed EBW for a long years and a big impact on their production and productivity (Figure 5 A & B). According to all enset farmers' interviewed, they could be noticed variable level of clonel response against the Xcm diseases was observed in their enset fields. Farmers also used certain relatively resistant enset clones known by them to replace infected enset field. In the above mentioned districts, all interviewed farmers' enset clone Bedadet (local name for enset clones given by Western Shewa Zone farmers) are reported by the farmers to have relative resistant (tolerant) to wilt than others, but this was contrary to Tsehay [33], The susceptibility of the EBW disease of

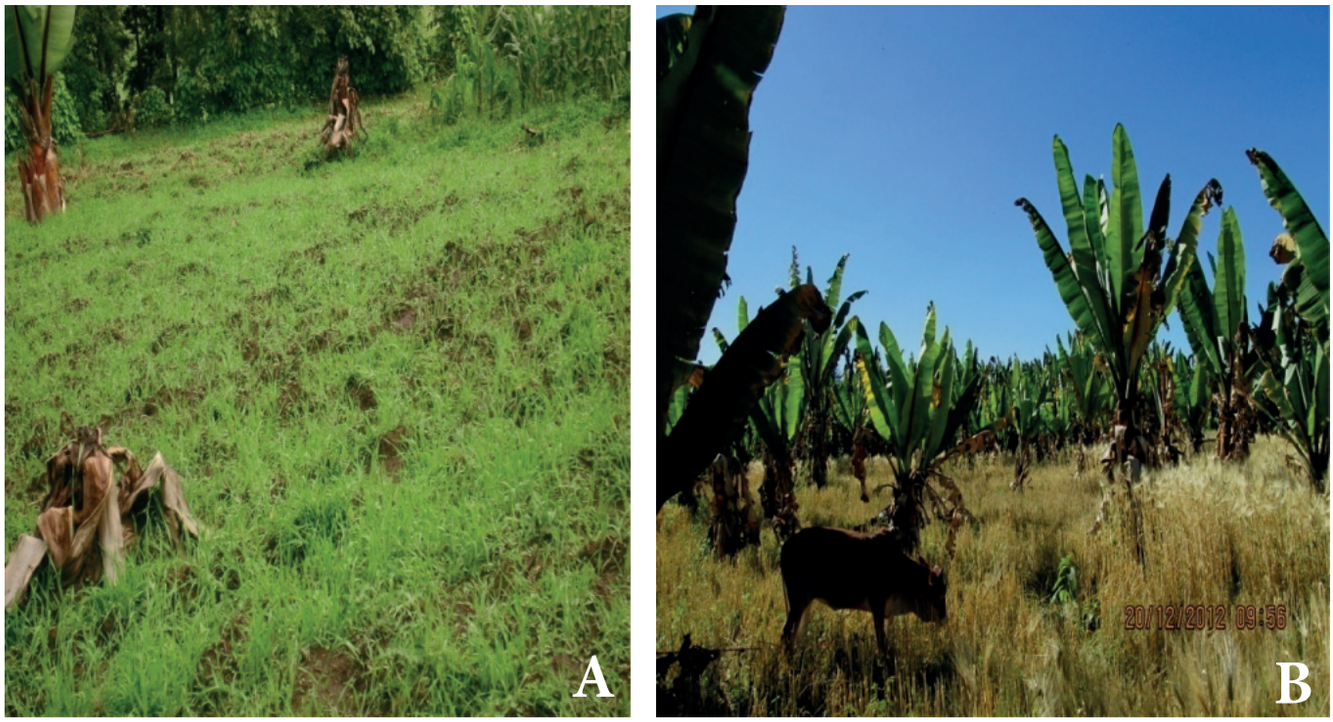

Figure 4. A & B. Enset field replaced by other crops due to EBW disease.

Sabara clone was reported in the current investigation. EBW was transmitted from infected enset plant to healthy plants through routine field activities (pruning, weeding and digging), which done for management practices once in a year especially between September-November 2012 which create conducive environment for the spread of the disease locally within the same farm and between farms, which confirmed with the reports of Karamura et al. [20] and Million, et al [23].

3.1.4 Isolation and Identification of Xcm

Enset bacterial wilt disease sample was taken from infected plant parts of leaf petioles, corm and pseudo stem showing discoloration of plant tissue with large air pocket filled with creamy or yellowish exudates (ooze) (Figure 6 A & B). The bacterial ooze sign sample was collected from Waldo Hindhe locality, Tikur Inchini district. Dereje [10] also reported that the similar yellow bacterial ooze exudates come out from the cut pseudo stem and leaf petioles of enset plants. Under laboratory condition, the YDC culture Petri plates were observed after 72 hours of incubation, bacterial colonies were developed a light yellow, circular, high convex, dome shaped and shiny appearance of mucoid colonies (Figure 7 A & B) (Table 4). The similar results were also reported by Tsehay [33], that bacterial isolates of enset grown on YDC agar culture plates produced creamy, yellow, and light yellow mucoid circular colonies with dome shaped and shiny appearance.

Table 4. Morphological and biochemical characterization of enset bacterial isolate

Isolate*	Colony appearance and shape	Colony color	Gram reaction	catalase	Oxidase	Starch hydrolysis	Tween hydrolysis	KOH solubility	Identification of bacteria
TIWH01	High convex, dome shaped and shiny appearance, mucoid circular colonies	Light yellow	-	+	-	+	-	+	*X. campestris pv. musacearum*

*The bacterial isolate was coded TIWH01= Tikur Inchini district Waldo Hindhe

Table 5. Percentage of plants for the different enset clones developing disease symptoms after artificial inoculation with *Xcm*

No	Treatment or enset clones	Number of leaves per clone before inoculation	Number of leaves wilted		
			DWISD %*	NWLIS %*	TLW %*
1	Warke Ija	7	15.333^{d}	24.41^{ab}	54.762^{abc}
2	Hadha Bishan	3	20.889^{bcd}	37.96^{ab}	57.408^{abc}
3	Hadha Bala	4	28.667^{ab}	26.85^{ab}	43.518^{bcde}
4	Garda Gababa	3	29.222^{ab}	39.82 ^{ab}	53.704^{abcd}
5	Bedadet	4	23.667^{abcd}	18.52^{b}	34.260^{def}
6	Sabara	4	20.889^{bcd}	38.89 ^{ab}	58.333^{abc}
7	Warke Bidu	4	15.889^{d}	44.26^{a}	66.667^{a}
8	Hiniba	4	30.3334^{a}	27.59^{ab}	30.186^{ef}
9	Feresiye	3	19.222^{cd}	42.59 ^{ab}	53.703^{abcd}
10	Kekar	3	17.556^{cd}	31.48 ^{ab}	66.667^{a}
11	Astera	4	26.444^{abc}	31.85 ^{ab}	57.963^{abc}
12	Aba Jobir	3	23.111^{abcd}	38.89 ^{ab}	46.297^{abcde}
13	Warke Adi	4	25.889^{abc}	30.55 ^{ab}	51.851^{abcd}
14	Awegene	3	21.444^{abcd}	34.26^{ab}	61.111^{ab}
15	Warke Dima	5	29.222^{ab}	38.33^{ab}	40.000^{cde}
16	Garda Dhera	3	18.111^{cd}	33.33 ^{ab}	57.407^{abc}
17	Shartiye	3	15.333^{d}	34.26 ^{ab}	63.889^{ab}
18	Awenyi	4	15.889^{d}	32.41 ^{ab}	66.667^{a}
19	Suite	5	19.222^{cd}	29.07 ^{ab}	57.037^{abc}
20	Meziya	7	29.778^{ab}	19.31^{b}	19.311^{f}

* DWISD=Date of wilted initial symptoms development.*NWLIS=Number of wilted leaves initial symptoms* TLW=Total leaves wilted. Mean with the same letters are not significantly different

3.1.5 Cultural Characterization

The gram negative, the catalase test, starch hydrolysis and KOH solubility were positive and the oxidase test and Tween 80 hydrolysis were negative. Now the isolate TIWH01 was confirmed and identified as *Xanthomonas campestris pv musacearum (Xcm)* (Table 4).

3.1.6 Evaluation of Enset Clones for Resistance to Xcm

Eighteen number of enset clones collected from Tikur Inchini (15) and Jibat (3) districts of West Shewa Zone and two enset clone suckers of Meziya and Suite of nearly having one year old was used as a resistant/tolerant and susceptible check, respectively, which were obtained from APPRC and assessed the resistance/tolerance of enset bacterial wilt, *Xcm* using artificial inoculation under pot culture condition at Ambo Plant Protection Research Center, Ambo, Ethiopia (Figure 8). The relative susceptibility of cultivars to enset bacterial wilt was evaluated three months after inoculation based on wilt incidence. Disease evaluation data was conducted at 5 days interval for 3 months. Disease evaluation was started seven days after inoculation. The first symptoms of disease on infected clones were yellowish of central leaf at the apex and wilting. Average disease incidence as measured by percent infected and/or dead enset plants, which showed varied differences among test clones at different disease assessment period after inoculation. All *Xcm* inoculated enset clones extended infection symptoms to diverse intensity levels after 15 to 30 days inoculation (Figure 9). However, some enset clones proved comparative tolerance of the disease. A difference in progression of the disease also was apparent. In all disease assessment periods, the ranges of disease inci-

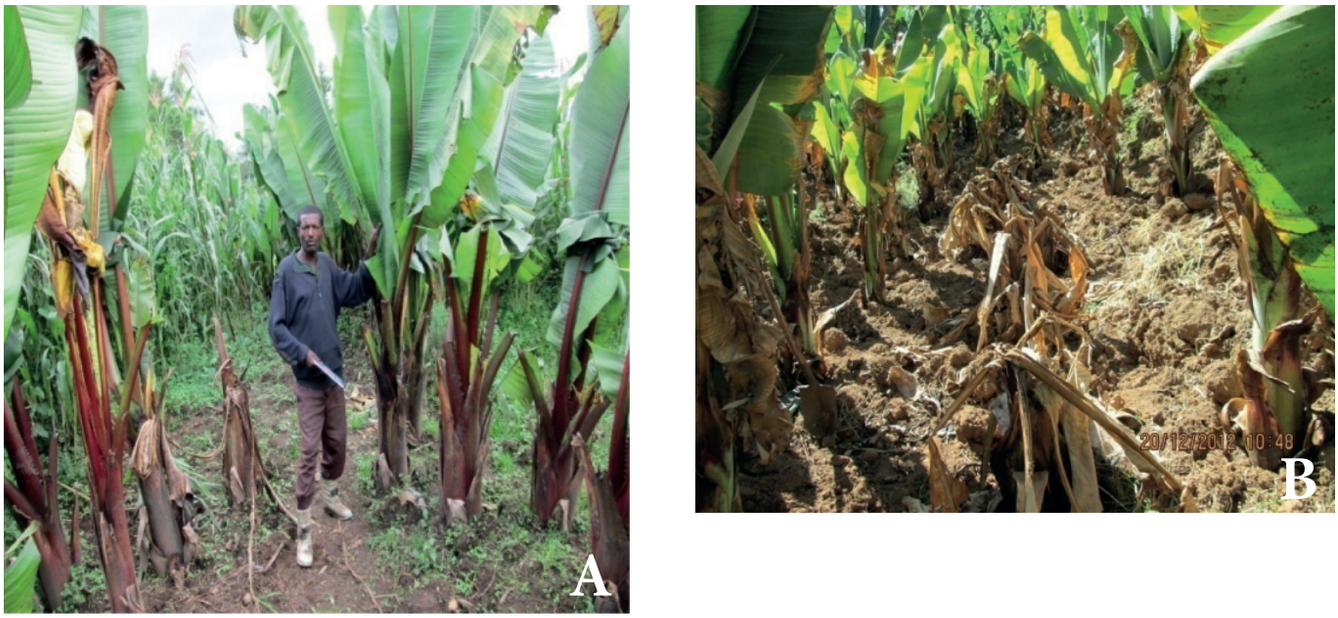

Figure 5. A & B. Transmission of EBW from infected enset plants to healthy plants through routine field activities.

Figure 6. A. enset pseudo stem with bacterial ooze. B. Enset corm with bacterial ooze.

dence were variable ranging from 20 to 100%. Out of the 20 enset clones, only 6 enset clones revealed a mean disease occurrence below 50 percent. Some of the clones were more severely affected within shorter period of time than others. But artificial inoculation of the bacterial suspension @1x107 and 1x108cfu/ml inoculated into the clones showed the disease incidence was not variable.

The enset clones 'Warke Bidu', 'Awenyi', and 'Kekar' confirmed 100 % disease signs at 30 days after inoculation and could be used as susceptible tests in further screening programmes. Disease symptoms were observed on 'Meziya', 'Hiniba', 'Bedadet' and Warke Dima between 21 and 30 days soon after the inoculation. These clones were the immune clones throughout the evaluation period. The remaining enset clones were relatively resistant /tolerant after inoculation of Xcm (Table 5). Among all, 'Meziya' was found to have the lowest percentage of disease incidence (19.31%) followed by 'Hiniba' (30.18%) and 'Bedadet' (34.26%). These results are in accordance with the earlier reports of 'Meziya' that was considered as better tolerant clone18,36. In the present study, the enset clones, 'Meziya', 'Hiniba', 'Bedadet' and 'Warke Dima' exhibited better resistant/ tolerant clones to the bacterial wilt, under artificial inoculation conditions @ both 107 and 108 dilutions Hence, 'Meziya', 'Hiniba', 'Bedadet' and 'Warke Dima' enset clones were considered as most tolerant/resistant clones to the pathogen and these four clones could be used as a bacterial wilt management component. Developing and use of resistant/tolerant enset

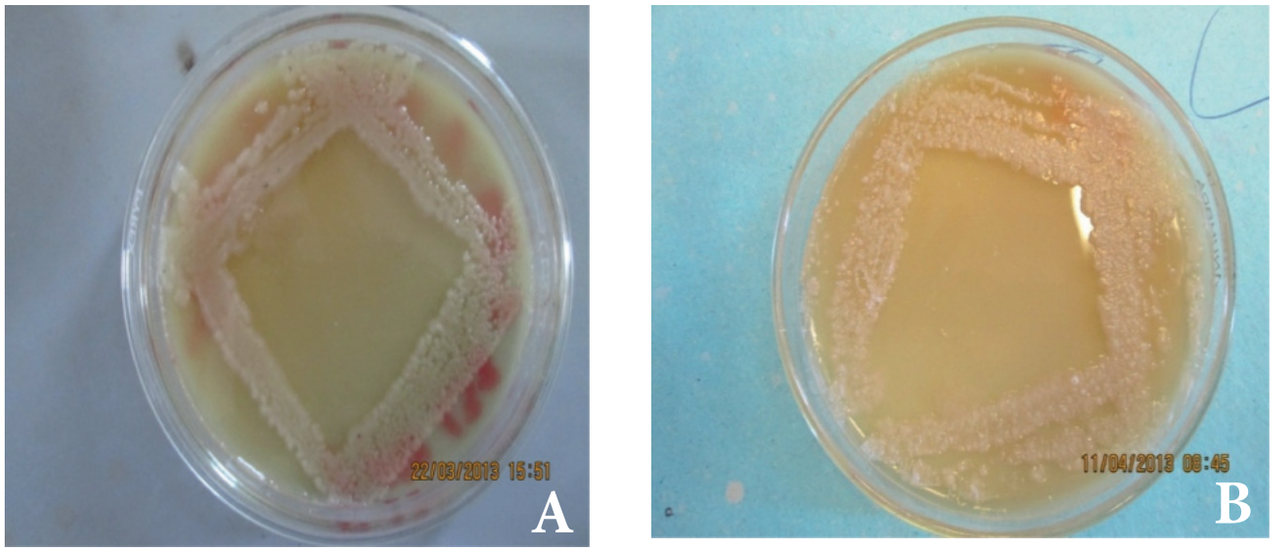

Figure 7. A & B. Pure colonies of *Xcm* on YDC agar culture plates.

Figure 8. Artificial inoculation for screening of enset clones against BW at experimental station.

clones is one of the best approaches in the management of EBW, cheaper to the farmers and safer to environment similarly, Variable stages of clonal response against the Xcm disease have been studied on field trials [1], [35].

4. Conclusion

The EBW infection was recorded in different levels at both districts. The results indicated that the disease was widely distributed with a very destructive incidence in survey areas and it was greatly concern and economically important disease to the community of Tikur Inchini and Jibat districts.

In the study of evaluation of 20 enset clones resistance/tolerance to Xcm using artificial inoculation under pot culture condition, among all, 'Meziya' was found to have the lowest percentage of disease incidence (19.31%) followed by 'Hiniba' (30.18%) and 'Bedadet' (34.26%) enset clones, The enset clones, 'Meziya', 'Hiniba', 'Bedadet' and 'Warke Dima' have exhibited better resistant/ tolerant clones to the bacterial wilt, under artificial inoculation conditions. Hence, 'Meziya', 'Hiniba', 'Bedadet' and 'Warke Dima' enset clones were considered as most tolerant/resistant clones to the pathogen and those clones could be applied as a bacterial wilt management component. Developing and use of resistant/tolerant enset clones is one of the best approaches in the management of EBW, cheaper to the farmers and safer to environments. The enset clones, 'Warke Bidu', 'Awenyi', and 'Kekar' noted 100 % disease signs at 30 days after inoculation and susceptible tests could be recommended in next screening trials. The farmers have also learned a lot from the collaborative experiments, they are very sure that the contaminated farming tools are the most important factor, play major role in disseminations of the pathogen in their enset fields. In these regards use of resistant / tolerant clones along with cultural practices and sanitary control measure is viewed to be the most feasible of the bacterial wilt management. The most advantageous control measure would be in view of the current study results, the use of resistant enset clones would be the most desirable control option. Hence, the enset clones that proved a resistant or tolerant reaction to the wilt pathogen can be studied in future against a large number of Xcm isolates under field conditions. In addition, a systematic effort to collect and evaluate other clones is immediately needed. Also, *Xcm*

Figure 9. Symptoms of EBW after artificial inoculation in enset clones after 30 days.

irregularity and virulence needs to be cautiously investigated. The current work alone cannot be conclusive; it is believed that the results obtained were facilitating further works for the satisfactory control of the bacterial disease of enset in the country. The presence of resistance/tolerance in enset clones to *Xcm* has been screened in some other enset clones; although no clone was found to be fully resistant, varying levels of tolerance to the disease was encountered. However, more research is needed considering the various enset clones from the different enset-growing regions. Future use of molecular techniques could be produced markers linked to tolerance in enset clones. Farmers are to be recommended to planting less susceptible clones with proper sanitation practice to minimize losses. Avoid contamination of implements such as knives, hoes, etc. by infected enset and hence, the work around enset plantations without thorough cleaning.

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