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Response of some selected enset varieties and its resistance to artificial inoculation of bacterial wilt disease of Enset (*Xanthomonas Campestris* Pv. *Musacearum*) at Kedida Gemella District, Kambata Tambaro Zone, Southern Ethiopia

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Abstract

The experiment was aimed with the objective to select and evaluate Enset varieties resistance to Enset bacterial wilt disease. This study was conducted in Kedida Gemella district in Durame Campus research demonstration site during main cropping season in 2021. A total of 16 selected Enset varieties were evaluated for resistance to *Xanthomonas campestris pv. musacearum* pathogen in the study area. The experiment laid out sixteen varieties in four replications in randomized complete block design (RCBD). Disease incidence rapidly increased thereafter artificial inoculation of the bacteria for first two months for most clones. Varieties Qegile, Sabera and wo'e showed greater than 23% disease symptoms at 95 days after inoculation. In contrary other varieties like manara, siskela, meriza, bishato, mandeluqa, xassa and gishira showed 0% or no symptom at days after 95 inoculation, but they showed initial symptom in all data collection times before 95 days after inoculation. However, their mean values showed relatively tolerant in the study area. In this research the two selected varieties namely bishato and mandeluqa showed resistance for bacterial wilt disease and these varieties were used for further multiplication purposes if the varieties.

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Introduction

Enset *(Enset ventricosum)* is a perennial, herbaceous and a monocarpic crop, belonging to the family musaceae and the genus Ensete. It is commonly known as "false banana" for its close resemblance to the domesticated banana plant. It is only in Ethiopia that enset has been domesticated and is cultivated for food, animal feed and fiber. About 25 species of Enset are equally distributed in Asia and Africa (Mesfin *et al.,* 2008). It is a classic multipurpose crop that every botanical part is used for numerous material cultures. It 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. The edible parts of Enset vary from place to place. In general, the pulp of the pseudostem, the young shoots, and the corm are eaten. It is also utilized for livestock feed, fuel wood, construction materials, containers, and to shade other crops. The processed pseudostem usually undergoes fermentation, which becomes flour and ultimately the basic ingredient of bread and porridge upon drying.

This crop contributes to food security (a traditional staple food crop) for more than 20% of Ethiopia's population notably southern and southwestern parts of Ethiopia (Ayele, A. and OmprakashS 2014). Enset has been known to play a role of a barrier food deficit for human and feed for animals during the dry spell and recurrent drought due to its resistance to fluctuating rainfall patterns after establishment. Each plant takes four to five years to mature, at which time a single root will give about 40 kg of food. Enset will tolerate drought better than most cereal crops (Hunduma T. *et al.,* 2015). Bacterial wilt, caused by the bacteria *Xanthomonas campestris* pv musacearum, is the worst to the Enset farming system. It is a serious loss for the farmers when a disease kills an Enset plant at any growth stages and late in its life cycle as they have already invested; labor, land, and resources for several years. The other shortcoming of this crop is it being poor in nutrient composition particularly protein and minerals. A serious outbreak of the disease with losses up to 70%. Many researchers (Endale *et al.,* 2003) reported that both the area and productivity of *Enset* is declining continuously due to this disease.

Enset in Kembata Tembaro zone is one of the major sources of food and income; and contributes significantly to household food security. Its production is declining due to a combination of abiotic and biotic production constraints; soil fertility decline being one of the major abiotic production constraints (Kelsa 1996). Out of the constraints, the most sensitive problem threatening Enset production at present is the bacterial wilt disease and lack of resistant variety problem. Therefore, the objectives of this research works was to select and evaluate Enset varieties resistance to Enset bacterial wilt disease in the study area.

Materials and methods

Description of Study area

This experiment was conducted in Kedida Gemella district in Durame Campus research demonstration site in 22 March 2021; which is one of the seven districts in Kambata Tambaro Zone, Southern Ethiopia. The total population of the district is about 106,867 of which 53,216 are male and 53,651 are female (CSA 2007). The district is approximately ranges altitude of 1600m – 3028m above sea level. According to the agricultural office of the district, the relief is 35% mountainous at the north, 37% slope and 28% flat at the south. The mean annual rainfall of the district ranges from 800mm -1400mm and the mean annual temperature ranges from 12-25^{o}C. The district is usually divided into two agro-ecological zones (Dega 5% and Weyna Dega 95%). Regarding land use of the district, about 79 percent of the total cultivated area is used for crop cultivation, 5.58 percent is forest, 5.53 percent grazing land and 10 percent Other Land use (settlements, river courses, etc). The soil texture is clay-to-clay loam. The major crops grown in the district are *enset*, cereals (wheat, barley and maize), pulses (beans and peas), vegetables and root crops. In general, Kedida Gemella district is the major producer of *enset* in Kambata Tambaro zone and *enset* production is considerable sources of cash in the district (KTZADO 2018).

Management of Experiment

Land preparation

The experimental field was prepared following the conventional tillage practice which includes 3 times plowed before planting of the Enset varieties. As per the specifications of the design, a field layout was prepared; the land was cleaned, leveled and made suitable for Enset crop establishment.

Experimental material

A total of 16 (sixteen) enset varieties were evaluated for resistance to Xcm pathogen in Kedida Gemella district in Durame Campus, Durame Ethiopia. Four one-year-old suckers of each of the 16 varieties were collected from the major Enset growing districts Kembata Tembaro Zone of Southern Region, Ethiopia viz., Kedida Gemella District. The Enset samples were collected from three model kebeles in random sample form. The 16*4 of total 64 suckers were used as an experimental material. These plants were planted in a randomized complete block design (RCBD) with 4 replications. The Enset varieties were collected from areas with the same environmental conditions from farmer fields. The suckers were developed from a single corm for each variety. The varieties were evaluated for their resistance to the pathogen under artificial inoculation.

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 0·1 mL *Xcm* cell suspension (10^8 cfu/ml). Then two milliliters of the bacterial cell suspension were injected into the leaves of tobacco seedlings using hypodermic syringe and needle. The control plants were inoculated with distilled water. Isolates, were show complete collapse of tissues around the injection point, were considered as positive for the test and identified as pathogenic isolates (Quimio 1992).

Inoculum Preparation

Bacterial oozes were collected from naturally infected Enset field around the area of farmer's field which used in the Hypersensitivity test. The pathogen, *Xanthomonas campestris pv. musacearum*, were collected at the cut end of petioles and leaf sheaths with the help of tooth pick and suspended in Sterilized Distilled Water (SDW). A loopful of the suspension was streaked on Yeast Dextrose Calcium Carbonate (YDC) plate (glass or plastic dish) for multiplication of inoculum. The plates were incubated at 28°C for 24 hrs. Pure bacterial colonies were show light yellow mucoid growth typical of Xcm from the plate and were incubated at 28°C for two days to produce enough bacterial culture for inoculation.

Inoculum Inoculation

Two months after transplantation (at 4-9 leaf stages), the enset clones were inoculated by using hypodermic syringe and needle with 2 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. Three suckers as replicates were inoculated with the pathogen and one sucker was inoculated with the same amount bacteria free of SDW as a control for each variety. Re-isolation of the pathogen from the inoculated plant were done at the end of the experiment which lasted for two months after inoculation.

Disease Assessment and data collection

Disease data was taken 20 days after inoculation, then at a 15-day-interval for three months. The number of suckers showed wilt symptom, the time of the initial symptom (incubation period) and the complete wilting date was recorded. The percentage of the wilted plants (wilt incidence) at each assessment period were calculated according to the following formula:

Incidence = (NW/NT) × 100

Where, NT = the number of total tested plants and

NW = the number of wilted plants.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) as per the experimental designs for each experiment using GenStat version 15.1, 18th edition of statistical software package. The Least Significance Difference (LSD) at 5%

level of probability procedure was used to determine differences between treatment means.

Result and discussion

Disease symptom and incidence

The analysis of variance of the means of all inoculated enset clones developed disease symptoms at various intensity levels at the study area (Table 2). Out of the 16 enset clones inoculated with Xcm pathogen, all of the clones showed different rate of 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. The first signs of infection (yellowing and chlorosis of the central leaf) were highly observed on sisqella, waaniqorotte, sheleqe and qegile at 20 days after inoculation. Disease incidence rapidly increased thereafter inoculation of the bacteria for first two months for most clones. Out of the 16 enset clones collected from the kerchicho kebele and used for the experimental field, only 2 enset varieties/clones showed a mean disease incidence of less than 5 percent. Clones Qegile and Sabera showed greater than 35% average disease symptoms at 95 days after inoculation and could hence be used as susceptible checks in future Xcm screening trials. In contrary other clones like manara, siskela, meriza, bishato, mandeluqa, xassa and gishira showed 0% or no symptom at days after 95 inoculation, but they showed initial symptom (Table 2).

The mean of the enset varieties such as bishato and mandeluqa showed relatively tolerant in the study area. The same result also reported by Gizachew (WM. *et al.,* 2008) the enset clones Anikefye, Eminiye, Lemat and Nechwe (1) from the Gurage collection showed a relative tolerance to Xcm. However, the enset clones Ado, Kembate, Hedesso, Soskila, Genticha and Abate were reported as having a relative tolerance to the disease. This may have been caused by a variation in Xcm isolates used for inoculation. None of the control plants for each of the tested enset clones showed wilt symptoms throughout the experimental period. The same results also reported by (Mekuria W *et al.,* 2016) who reported that in his study, 25 enset clones from Gurage zone were evaluated for their reaction to Xcm pathogen under artificial inoculation and produced varying reactions. Based on the evaluation of their reaction, none of the enset clones had a complete resistance to Xcm pathogen. The average wilt incidence over the assessment periods ranged from 29.17 to 70.58% (Table 2). The maximum average wilt incidence was recorded on 35 days after inoculation (70.58%) and the minimum average wilt incidence (29.17%) was recorder on 95 days after inoculation of bacterial wilt disease.

Table 1. Supplemental information for the field experiment.

No.	Name of Variety (clone)	Area of Collection	Date of Collection	Date of Planting	Plant Growth Stages	Field History
1	Waaniqqorotte	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
2	Godorote	Kerchicho kebele	20 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
3	Manara	Kerchicho kebele	20 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
4	Sisqeela	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
5	Sabera	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
6	Qegile	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
7	Sheleqe	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
8	Meriza	Kerchicho kebele	20 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
9	Bishaatto	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
10	Mandeluuqa	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
11	Addo	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
12	Wollaanche	Kerchicho kebele	20 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
13	Xassa	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
14	Gishira	Kerchicho kebele	21 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
15	Wo'e	Kerchicho kebele	20 March 2021	22 March 2021	One year old (dubbo'o)	Year 2020, maize field
16	Moche	Kerchicho kebele	12/07/2012 E.C	22 March 2021	One year old (dubbo'o)	Year 2020, maize field

In Bishato variety/clone in each block showed no infection at 20 days after inoculation and it showed low infection levels at days from 35 and 50 after inoculation of Xcm, hence indicating a high degree of tolerance to the disease (Table 2). Most clones showed the symptom of Yellowish and wilted leaves

were observed from 20 and 95 days after inoculation; however, some of the infected plants resumed normal growth at 65 days after inoculation (Table 2).

This apparent recovery may be explained by the un-systemic nature of the disease development after an artificial inoculation in the leaf petiole of a newly formed leaf. It could be possible that the bacteria stay confined to the leaf petiole and leaf sheath of this inoculated leaf. May be the bacteria cannot enter in the corm and hence cannot infect adjacent leaves as the vascular connection between leaves passes through the corm. This would result in the disappearance of the disease when the inoculated leaf eventually wilts and dies. This may be also the movement of the disease from the applied part to the next part by the vessels may be blocked in incorrect application. None of the control plants for each of the tested enset clones showed wilt symptoms throughout the experimental period. In other case it may be the nature of resistance of the plant.

Table 2. Plants for the different enset varieties/clones developing disease symptoms after artificial inoculation with Xcm

Name Of enset Variety (clone)	Area of Collection	Number of inoculated plans	Days after inoculation % infected plants						Mean
			20	35	50	65	80	95	
Waaniqqorotte	Kerchicho kebele	3	16.67	25	25	1.67	0	8.33	12.78
Godorote	Kerchicho kebele	3	10	10	10	5	0	3.33	6.39
Manara	Kerchicho kebele	3	10	10	10	3.33	0	0	5.56
Sisqeela	Kerchicho kebele	3	11.67	13.33	13.33	0	0	0	6.39
Sabera	Kerchicho kebele	3	10	8.33	8.33	3.33	86.67	96.67	35.56
Qegile	Kerchicho kebele	3	30	30	20	23.33	66.67	100	45
Sheleqe	Kerchicho kebele	3	10	15	15	8.33	11.67	20	13.33
Meriza	Kerchicho kebele	3	8.33	15	15	6.67	0	0	7.5
Bishatto	Kerchicho kebele	3	0	1.67	1.67	0	0	0	0.56
Mandeluqa	Kerchicho kebele	3	3.33	3.33	3.33	0	0	0	1.67
Addo	Kerchicho kebele	3	6.67	8.33	8.33	16.67	6.67	16.67	10.56
Wollaanche	Kerchicho kebele	3	1.67	1.67	1.67	13.33	5	33.33	9.44
Xassa	Kerchicho kebele	3	10	10	10	0	0	0	5
Gishira	Kerchicho kebele	3	10	16.67	16.67	10	3.33	0	9.44
Wo'e	Kerchicho kebele	3	10	10	10	1.67	45	66.67	23.89
Moche	Kerchicho kebele	3	5	11.67	11.67	3.33	6.67	20	9.72
Disease incidence			64.58	70.83	64.58	54.17	31.25	29.17	

This study shows that enset clones vary in their reaction to enset bacterial wilt. Some of the enset clones recover after initial disease symptom development. The clones that showed a tolerant reaction to the wilt pathogen should be further evaluated against a large number of Xcm isolates under field and greenhouse conditions. In addition, a concerted effort to collect and evaluate other clones is urgently needed. Also, Xcm variability and virulence needs to be thoroughly investigated. In this study, only one virulent pathogenic Xcm isolate was used. Future studies under field and greenhouse conditions should thus assess the reaction of enset clones to a large number of Xcm isolates collected from different growing areas. The clones with the highest disease infection rate could be used as susceptible checks during these future enset clonal Xcm screening studies for further conclusive recommendation of Xcm resistant varieties/clones.

In this study none of the control treatments of enset varieties were showed wilt symptom that treated with distilled water, but all treatments of the enset varieties showed curling of leaf and yellowing of the leaves starting from applied area to the other parts from 20 to 50 days after inoculation. Some varieties also forms the immune system after 70 days after inoculation and starts normal growth. This may be due to the genetic variability of the variety or it may be the potential activity of inoculated inoculum to infect the variety and un-systemic nature of the disease development after an artificial inoculation in the leaf petiole of a newly formed leaf. As a general result the varieties such as bishato and mandeluqa

showed relatively tolerant in the study area because their mean values of infection were less than 2%. So that this research were initiate the farmers to use bishato and mandeluqa for further production and dissemination around the kembata tembaro zone of southern Ethiopia.

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