African Crop Science Journal, Vol. 16, No. 1, pp. 89 - 95 ISSN 1021-9730/2008 $4.00

EVALUATION OF ENSET CLONES AGAINST ENSET BACTERIAL WILT

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ABSTRACT

Enset (*Ensete ventricosum* Welw. Cheesman) is an important food crop for over 20% of the Ethiopian population living in the southern and southwestern parts of the country. Enset farmers commonly grow combinations of clones in fields, but each clone is grown for its specific use. A large number of enset clones collected from the Sidama, Gurage, Kembata Tembaro and Hadyia zones were assessed for resistance/tolerance to enset bacterial wilt, *Xanthomonas campestris* pv. *musacearum* (*Xcm*) at the Awassa Agricultural Research Center, Awassa in Ethiopia, during the period 1994 to 2000. In addition, some enset clones that were reported by farmers and researchers as tolerant to *Xcm were* evaluated during the same period. The objective of the study was to screen field-grown enset clones collected from different zones of southern Ethiopia, for reaction against the wilt. All *Xcm* inoculated enset clones in each of the experiments developed disease symptoms to various intensity levels during the first 45 days after inoculation. However, several enset clones showed relative tolerance to the disease. The enset clones 'Astara', 'Buffare', 'Geziwet 2', 'Gulumo' and 'Kullo' showed 100% disease symptoms at 30 days after inoculation and could, hence, be used as susceptible checks in future screening trials. Disease symptoms were observed on 'Mezya', 'Hiniba', 'Sorpie' and 'Sigezasarum', between 21 and 75 days after inoculation. However, some plants resumed normal growth at 90 days after inoculation. The enset clones that showed a resistant and/or tolerant reaction to the wilt pathogen should be further evaluated against a large number of *Xcm* isolates under greenhouse and field conditions.

Key Words: Ensete, resistance, susceptibility, *ventricosum, Xanthomonas campestris* pv. *musacearum*

RÉSUMÉ

Enset *(Ensete ventricosum* Welw. Cheesman) est une importante récolte de nourriture pour plus de 20% de la population éthiopienne qui habite dans le Sud et les parties du Sud-Ouest du pays. Les agriculteurs d'Enset dévéloppent ordinairement des combinaisons de clones dans les champs, mais chaque clone sont grandis pour son usage spécifique. Plusieurs clones d'enset recueilli du Sidama, Gurage, Kembata Tembaro et les zones de Hadyia ont été évalué pour la résistance tolérance à enset bactérien flanche, *Xanthomonas campestris* pv. *Musacearum (Xcm)* à l'Awassa le Centre de Recherche Agricole, Awassa dans Ethiopie, pendant la période 1994 à 2000. Par ailleurs, quelque enset clone cela ont été rapporté par les agriculteurs et les chercheurs comme tolérant à Xcm ont été évalué pendant la même période. L'objectif de l'étude était de trier les clones d'enset champ-grandis recueillis des zones différentes du sud d'Ethiopie, pour la réaction contre le flanche. Tout *Xcm* a vacciné les clones d'enset dans chacune des expériences ont développé les symptômes de maladie aux divers niveaux d'intensité pendant le premier 45 jours après l'inoculation. Cependant, plusieurs enset clone la tolérance relative montrée à la maladie. L'enset clone « Astara », « Buffare », ‹ Geziwet 2 ›, « Gulumo » et « Kullo » ont montré 100% symptômes de maladie à 30 jours après l'inoculation et peut, donc, est utilisé comme les contrôles susceptibles dans avenir trier les procès. Les symptômes de maladie ont été observés sur « Mezya », « Hiniba », « Sorpie » et « Sigezasarum », entre 21 et 75 jours après l'inoculation. Cependant, quelques plantes ont repris la croissance normale à 90 jours après l'inoculation. L'enset clone a montré la réaction tolérante à un et/ou résistant au flanche le pathogène devrait être plus évalué contre plusieurs *Xcm* isole sous les conditions de serre et champ.

Mots Clés: La résistance, la susceptibilité, ventricosum, *Xanthomonas campestris* pv. *musacearum*

INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a monocarpic, herbaceous plant belonging to the Musacea family and the genus *Ensete*. Wild *E. ventricosum* is common and widespread in Ethiopia and along the rift valley in eastern Africa, all the way south to Mozambique (Simmonds, 1958). However, it is only in Ethiopia that enset has been domesticated and is cultivated for food, animal feed and fiber (Bezuneh *et al*., 1967). The cultivation of enset is concentrated in the southern and southwestern part of Ethiopia (Bezuneh and Feleke, 1966). There are over 200 enset vernacular names in Ethiopia (Tabogie and Diro, 1994, Addis, 2005). However, a particular clone may have different names in different geographic or language areas, while different clones could have same name (Tabogie, 1997). Tsegaye (2002) also mentioned that differences in names could be related to differences in the utilisation of a clone and the change in vernacular name after an enset germplasm exchange between communities.

Enset plantations are found at altitudes between 1,200 to 3,100 meters above sea level (Huffnagel, 1961). Most enset growing areas have an average annual rainfall of 1,100 to 1,500 mm, a mean temperature of 10-21^{0}C and a relative humidity of 63-80%. The ideal soils for enset cultivation are moderately acidic to alkaline (pH of 5.6 to 7.3) (Bezuneh and Feleke, 1966). The area of enset cultivation in Ethiopia is estimated at over 168,000 ha (CSA, 1997). Enset is the main source of food for over 12 million people (Belhu, 1991). The average annual yield of 'Kocho' (a non-dehydrated fermented product from mixtures of decorticated pseudostems and pulverized corms) is 10.3 t per hectare (Bezuneh and Feleke, 1966; Tabogie and Diro, 1994). Enset fiber accounts for more than 30% of the Ethiopian fiber production and its strength is equivalent to the fiber of Abaca (Brandt *et al.*, 1997). Fresh enset plant parts are fed to livestock and some enset clones are reported to have medicinal value to humans and domestic animals (Bezuneh *et al.*, 1967). Enset plantations prevent soil erosion and conserve soils, hence, contributing to the sustainability of the farming system (Welde-Tensaye, 1997). Enset is considered as a security food crop as it can withstand long periods of drought, heavy rains, and flooding, which normally devastates other crops (Degu and Workayehu, 1990).

The sustainability of enset agriculture is, however, threatened by a number of factors including population pressure, which is associated with more intense cultivation, degradation of the soil and the environment (Quimio and Tessera, 1996). Different types of diseases,. fungal, bacterial and viral, are also affecting enset production. Bacterial wilt of enset, which is caused by *Xanthomonas campestris* pv. *musacearum* (*Xcm*) is the most important disease affecting yield (Yirgou and Bradbury, 1968; Wondimagegne, 1981; Ashagari, 1985; Quimio, 1992; Quimio and Tessera, 1996; Welde-Michael, 2000).

Variable levels of clonal response against the *Xcm* disease have been observed under farmer's field conditions and while using artificial inoculation in on station trials (Anita *et al*., 1996; Welde Michael, 2000). Control measures which could prevent, reduce or eliminate the spread of *Xcm* in enset fields include the disinfection/ flaming of enset cutting tools after use on infected plants, preventing animals from browsing infected plants, fencing infected sites and the rigorous removal of infected plants (including the corms) (Quimio and Tessera, 1990).

The mechanical nature of Xcm transmission and the manner by which enset is propagated, raised and managed in a subsistence farming system, suggest that the wilt may be difficult to eradicate unless strict control options are implemented. Therefore, the use of resistant/ tolerant enset clones could offer a good approach to the control of bacterial wilt of enset (Ashagari, 1985; Quimio, 1992). Hence, studies were undertaken to evaluate large numbers of field-established enset clones for their reaction against enset bacterial wilt.

MATERIALS AND METHODS

Assessing enset clones from the Gurage, Hadyia, Kembata Tembaro and Sidama zones. A total of 103 enset clones were assessed under field conditions for their reaction to enset bacterial wilt using artificial inoculation. Fourty one clones

were collected from Gurage, 48 from Sidama, and 12 tolerant clones from the Hadyia and Kembata Tembaro zones. These 12 tolerant clones were selected from an initial 69 clones, which had been screened against *Xcm* during the period 1994-1995 (Anonymous, 1996). In addition, the enset clones 'Mezya' and 'Arkya' were included (Table 1).

The trail was established at the Southern Agricultural Research Institute (SARI), Awassa

TABLE 1. Plants for the different enset clones developing disease symptoms after artificial inoculation with *Xcm*

Clone name	Collection site	Number of inoculated plants	Days after inoculation								
			% infected plants								
			7[c]	15	21	30	45	60	75	90	120
Ado	Sidama	9	0	0	55	66	66	66	77	77	77
Astara	Sidama	9	0	0	18	100	100	100	100	100	100
Buacho	Sidama	9	0	22	44	44	100	66	66	77	44
Buffare	Sidama	9	22	55	55	100	100	100	100	100	100
Genticha	Sidama	9	0	0	55	77	88	88	88	88	88
Gulumo	Sidama	9	22	55	55	100	100	100	100	100	100
Kullo	Sidama	9	33	33	88	100	100	100	100	100	100
Serena (2)	Sidama	9	0	0	44	44	44	44	44	44	44
Wonigoro	Sidama	9	0	0	0	33	55	55	55	88	55
Achana	Gurage	9	0	0	0	44	77	77	88	88	44
Anikefye	Gurage	9	0	0	0	22	22	33	33	33	33
Astara	Gurage	9	0	0	0	22	44	44	44	44	44
Bazeriet	Gurage	9	0	0	11	44	55	33	11	11	11
Dere	Gurage	9	0	0	0	33	44	44	44	44	11
Eminiye	Gurage	9	0	0	11	55	77	77	66	88	33
Geziwet 2	Gurage	9	0	11	77	100	100	100	100	100	100
Lemat	Gurage	9	0	0	0	33	33	22	22	22	22
Nechwe (1)	Gurage	9	0	0	0	0	22	22	22	11	11
Weka	Gurage	9	0	0	0	22	33	33	44	88	44
Yeshirafre	Gurage	9	0	0	11	55	77	77	44	44	44
Hiniba	Hadyia	24	0	0	33	50	50	50	50	33	33
Kassiet	Hadyia	24	0	0	17	50	83	83	67	67	67
Sigezasarum	Hadyia	24	0	0	33	50	67	50	50	50	50
Sokide	Hadyia	24	0	0	33	50	50	50	50	50	50
Sorpie	Hadyia	24	0	0	17	50	50	50	50	33	33
Abata merza	Kembata Tembaro	24	0	0	17	50	50	67	67	67	67
Abate	Kembata Tembaro	24	0	0	0	33	33	33	33	33	33
Astara	Kembata Tembaro	24	0	0	17	50	50	67	67	67	50
Fugatessa	Kembata Tembaro	24	0	0	0	17	67	50	50	50	50
Gishera	Kembata Tembaro	24	0	0	0	50	50	50	50	50	50
Heila	Kembata Tembaro	24	0	0	0	50	33	33	33	33	33
Kembate	Kembata Tembaro	24	0	0	17	33	50	50	50	50	50
Mezya	Waka	24	0	0	50	50	50	50	50	33	33
Arkya	Wolyita	24	0	0	0	33	33	33	33	33	33

[c] = Percentage infected plants

Agricultural Research Center (7° 03'N, 38°30'E, 1,700 m above sea level); Awassa, Ethiopia in 1994 and the evaluations continued till 2000. The site has a mean annual rainfall of 1,046 mm, which is uni-modal distributed and a relative humidity of 60 percent.

Plantlets of the different clones were produced at the SARI, Areka Agricultural Research Center in Ethiopia. The plantlets were uprooted after one year and transported to the Southern Agricultural Research Institute (SARI), Awassa Research Center. For each of the enset clones collected from the Gurage and Sidama zones, 10 vigorous and uniformly sized plantlets were planted in holes of 50 cm deep and with a diameter of 50 cm. There were 3 replications of 3 plants and one control plant. On the other hand, 28 plantlets were planted per genotype from the Hadyia and Kembata Tembaro collections and for ' Mezya' and 'Arkya'. These plants were planted in a randomised complete block design with 4 replications. Each plot consisted of 1 row of 7 plants spaced 1.5 m from each other. There was a spacing of 1 m between rows and 1.5 m between plants in a row. There was one control plant in each replication.

Weeding was carried out every two months. A single application of 0.5 kg composted manure was applied per plant three months after planting. The plants were not irrigated during the dry season.

One year after transplanting, plants were inoculated with 3 ml of a 2 days old bacterial suspension with a cell concentration of 10^8 cfu/ml (adjusted to 0.3 OD at 460 nm using spectrophotometer). The plants were inoculated at the base of the newly expanding central leaf petiole using a 10 ml capacity sterile hypodermic syringe with metal needle. Numbers of live and, hence, injected plants per clone were recorded at the time of inoculation. The control plants were inoculated with the same volume of sterile distilled water. Disease assessment was done at 15, 21, 30, 45, 60, 75, 90 and 120 days after inoculation. The number of infected plants per clone at each disease assessment period was recorded.

Evaluation of previously reported tolerant/resistant enset clones. A set of 12 enset clones reported as tolerant to enset bacterial wilt by farmers and researchers (Ashagari, 1985) (Table 2) were evaluated for reaction to the pathogen. This experiment was conducted at the Awassa Agricultural Research Center between 1998 and 2000. Thirty plants were planted per clone in a randomised complete block design with three replications of 10 plants. Again, there was one control plant in each replication. The same

TABLE 2. Percentage of plants for the different enset clones (previously reported as tolerant/resistant) developing disease symptoms after artificial inoculation with *Xcm* (n= 27)

Clone name	Collection site	Days after inoculation								
		7[c]	15	21	30	45	60	75	90	120
YeshreKinke	Gurage	0	0	0	0	11	44	88	88	88
Abate Merza	Hadyia	0	0	0	22	33	55	77	77	77
Agade	Hadyia	0	0	0	0	22	22	55	55	55
Disho	Hadyia	0	0	0	11	11	22	22	22	22
Soskila	Hadyia	0	0	0	0	22	55	55	55	55
Unjamo	Hadyia	0	0	0	0	11	33	33	33	33
Kembate	Kembata	0	0	0	0	22	22	22	22	22
Ado	Sidama	0	0	0	22	22	22	22	22	22
Alanticho	Sidama	0	0	22	22	22	22	52	52	52
Genticha	Sidama	0	0	11	11	11	22	22	22	22
Hala	Wolyita	0	0	0	11	11	22	22	22	22
Mezya	Wolyita	0	0	0	0	11	11	11	11	11

[c]= Percentage infected plants

agronomic practices as in the other experiments were applied.

Eight months after transplanting, plants were inoculated with 3 ml of a 2 days old bacterial suspension using the same dilution and application protocol. Disease assesstment was done at 7, 15, 21, 30, 45, 60, 75, 90 and 120 days after inoculation.

The data collected were analysed using SPSS 12.0 for windows (SPSS, 2003).

RESULTS AND DISCUSSION

Evaluation of enset clones from the Gurage, Hadyia, Kembata Tembaro and Sidama zones. All inoculated enset clones developed disease symptoms at various intensity levels during the first 45 days after inoculation (Table 1). Disease severity rapidly increased thereafter for most clones. Out of the 89 enset clones collected from the Gurage and Sidama zones, only 13 enset clones showed a mean disease incidence of less than 50 percent. Clones Astara, Buffare, Geziwet 2, Gulumo and Kullo showed 100% disease symptoms at 30 days after inoculation and could hence be used as susceptible checks in future *Xcm* screening trials. Some clones like Buacho and Wonigoro from the Sidama collection and Bazeriet and Dere from the Gurage collection recovered from an initial infection (Table 1). The enset clones Anikefye, Eminiye, Lemat and Nechwe (1) from the Gurage collection showed a relative tolerance to *Xcm* (Table 1). Ashagari (1985) did not report any complete resistance in a *Xcm* screening trial with 60 enset clones. However, the enset clones Ado, Kembate, Hedesso, Soskila, Genticha and Abate were reported as having a relative tolerance to the disease. Contrary to Ashagari's report, Ado and Genticha were found to be susceptible to the disease in the present study. This may have been caused by a variation in *Xcm* isolates used for inoculation. Variations among isolates were observed in preliminary laboratory and field experiments (Bobosha, 2003).

The highest disease incidence for clones from the Hadyia and Kembata Tembaro collections was observed on Abate Merza and Kassiet (Table 1). In contrast, Abate, Arkya, Heila, Mezya and Sorpie showed low infection levels, hence indicating a high degree of tolerance to the disease (Table 1). Some clones surmounted from the infection. Yellowish and wilted leaves were observed on clones Mezya, Hiniba, Sigezasarum and Sorpie between 21 and 75 days after inoculation; however, some of the infected plants resumed normal growth at 90 days after inoculation (Table 1).

Quimio and Tessera (1996) also observed that artificially inoculated 'Genticha' plants recovered from a *Xcm* infection at 12 to 16 weeks after inoculation. 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. None of the control plants for each of the tested enset clones showed wilt symptoms throughout the experimental period.

Evaluation of previously reported tolerant/ resistant enset clones. All the evaluated enset clones developed wilt symptoms (Table 2). The first signs of infection (yellowing of the central leaf) were observed on Genticha and Alanticho at 21 days after inoculation. None of the control plants used for each of the tested enset clones showed wilt symptoms throughout the experimental period. Significant differences in disease infection rates were recorded. The highest infection rates were observed on Yeshrekinke and Abate Merza. However, Anita *et al*. (1996) reported clone 'Yeshrekinke' as a tolerant clone. Ado, Disho, Genticha, Hala, Kembate and Mezya showed low infection levels hence indicating a high degree of tolerance to the disease (Table 2). No recovery was observed for the clones assessed in this trial.

Previous reports had already indicated that the clones Ado, Genticha, Kembate and Mezya are tolerant to the disease (Ashagari, 1985 and Welde-Michael, 2000). Clone Soskila, previously reported as tolerant (Ashagari, 1985), was found

to be susceptible in the present study. This may have been caused by a variation in *Xcm* inoculum across studies. 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 in Ethiopia. The clones with the highest disease infection rate could be used as susceptible checks during these future enset clonal *Xcm* screening studies.

CONCLUSIONS

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. This recovery, in addition to the high labour requirements, makes farmers reluctant to apply the sanitary control measures. 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.

ACKNOWLEDGEMENTS

The study was made possible through the financial support provided by the Ethiopian Institute of Agricultural Research (EIAR) and the Southern Agricultural Research Institute (SARI).

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