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Evaluation of Enset (Ensete ventricosum (Welw.) Cheesman) Clone Suckers to Bacterial Wilt Disease Pathogen under Greenhouse Condition

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Abstract
Enset is an important food crop produced in Ethiopia with great role in food security especially for southern and south western parts of the country. The demand of the crop is increasing throughout the country. However, the production as the whole is decreasing due to devastation by enset bacterial wilt. Cultural practice, use of clean planting materials and resistant varieties/clones utilization are methods of the disease management techniques. Screening of varieties against the pathogen at open field level was conducted by different investigators with controversial outputs has been conducted for the last five decades. Artificial inoculation of healthy three month suckers of three clones (Arkiya, Digomerza and Mazia) by known source hagereselam strain inoculum was conducted to evaluate different clones resistance against the pathogen at sucker level under green house condition. The pathogen clone interaction response data was collected in seven days interval after infection for six weeks and the result showed that there was difference for disease symptom development up to four weeks (28 days) in which 100% of Arkiya suckers were diseased while 75% of Digomerza and 33% of Mazia clones have shown the disease symptom. Later after five weeks of inoculation suckers of all the three clones were diseased similarly with little severity difference that 100% of Arkiya and Digomerza suckers were highly diseased and some were died where as 80% of Mazia suckers were diseased.
Keywords: Enset, Clone, sucker, Xanthomonas, inoculum

INTRODUCTION

Among the total population of Ethiopia more than 85% lives in the rural area where crop production and animal husbandry are their main stay. There are different farming systems depending on different agro ecologies found in the country. Root and tuber crops play a major role in food production in southern, southwestern, western and central part of Ethiopia (Spring *et al.,* 1996).

Enset (*Ensete ventricosum* (Welw.) Cheesman*)* is a diploid (2n=18) herbaceous perennial edible species of the separate genus of the banana family, thus named 'false banana'. Enset is the vernacular name used in the Amharic language in Ethiopia for *Ensete ventiricosm* whose fruit is not edible and one of the indigenous root crops widely cultivated for its food and fiber values (Taye, 1996). Variation within the species to altitude, soil and climate has allowed widespread cultivation in the mid to highlands of western Arsi, Bale, the Southern Nations Nationalities and Peoples Regional State (SNNPRS) and western Oromia including West Shewa, Jimma, Ilubabora and Welega.

It is estimated that a quarter or more than 20 million of Ethiopia's population depends on enset as staple and co-staple food source, for fiber, animal forage, construction materials and medicines (Zerihun *et al,* 2014) and the area of enset production in Ethiopia is estimated to be over 321,362.43 hectares **(**CSA, 2013**)**. The average yield of kocho (non-dehydrated fermented product from the mixture of decorticated pseudo stem, pulverized trunk and corm) of superior food and fiber yielding cultivars such as Digomerza give yield over 43 ton per hectare bases per year (Atnafua *et al*., 2008). The fiber by-product of enset supplies more than 30 percent of Ethiopia's fiber need (Brandt *et al.,* 1997). Fresh enset parts are used as fodder for domestic animals during dry season and some enset clones are reported to have medicinal value to human beings and domestic animals (**Temesgen *et al*, 2014**). Enset is also well known to conserve soil and enrich plant nutrients through its dropped foliage (Kefale and Sandford, 1991) dried leaf sheath and petioles of enset are used for wrapping materials and other utensils (Endale and Mulugeta, 1994).

Enset cultivation is affected by a number of biotic and abiotic factors that contribute to low yield. Furthermore, diseases, insect pests and wild animals are also among the important production constraints of enset production system. Various diseases and insect pests of enset have been reported. Some of these are: leaf damaging fungal diseases, corm rot, sheath rot and dead heartleaf rot of enset with unknown causal agents and root knot, root lesion and black leaf streak nematode diseases (Quimio and Mesfin, 1996). However, based on the distribution and the damage incurred on enset production, enset bacterial wilt disease caused by *Xanthomonas campestris* Pv. *musacearum* is known to be the most threatening and important problem to enset production system.

The disease was first reported and described by Dangnachew and Bradbury in 1968 that attributed it to

Xanthomonas musacearum sp. (Eshetu, 1981). The disease was widely distributed in many enset growing regions of the country and affects the crop at all developmental stages and the results obtained from recent bacterial wilt disease assessment made in some enset fields in SNNPR showed losses of up to 100% under severe damage (Awassa Agricultural Research Center, 2008). Natural epidemics of the disease were also observed in banana fields at different enset growing areas. Even though, the disease is widely distributed and important, there is no intensive work which has been done on eradication of the disease except some cultural control measures which includes collective action campaign by farmers. On the contrary, the farmers are not familiar with the disease symptoms which are sometimes complicated with stress symptoms on plants.

Some efforts have been made to minimize the damage incurred by enset bacterial wilt. An eradication campaign of the disease was organized by Ministry of Agriculture some years back. In addition, screening of enset clones for their resistance to enset bacterial wilt is an ongoing project in southern Agricultural Research Institute. Resistance to pathogens is a genetically inherited character similar to other attributes such as height, yield and leaf size and it is used as a means to control losses caused by plant pathogens in most crops.

Enset farmers belief that certain enset clones such as Yeshirekinke in Gurage, Mezia in Wolaita, Ado and Genticha in Sidama, Siskela and Gimbo in Hadya and Nobo in Keficho have relatively high tolerance against bacterial wilt (Gizachew, 2000). Screening experiment conducted by Southern Agricultural Research Institute (SARI) involved 89 different clone one year old plants inoculation by 3 ml of two days old pathogen culture showed that all inoculated enset clones developed disease symptoms at various intensity levels during the first 45 days after inoculation. 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. The enset clones Anikefye, Eminiye, Lemat and Nechwe from the Gurage collection showed a relative tolerance to *Xcm*. Dereje (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 Dereje's report, Ado and Genticha were found to be susceptible to the disease in study conducted by Awassa research center (2000). 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 (Kidist, 2003).

There for this experiment was conducted under greenhouse using three month old suckers to evaluate the resistance level of representative clones believed as highly tolerant, moderately tolerant and most susceptible belong to Maziya, Digomerza and Arkiya clones respectively to verify the controversial reports from experiments conducted at open field by different workers.

Hence; the objective of the experiment is to evaluate resistance of different enset clones at early stage under green house condition.

MATERIALS AND METHODS

The Experimental Materials Used

Three month old greenhouse grown suckers of three enset clones: namely, Arkiya which is from Dawro collection, Digomerza from Kambata collection & Mazia from the same area as Arkiya and claimed to be highly susceptible, relatively tolerant and tolerant respectively for enset bacterial wilt disease pathogen *Xanthomonas compestrium musaceroum* as reported by farmers and genotype screening studies.

Experimental Design and Treatments

The experiment was laid out in a completely randomized design (CRD). Tewenty suckers of each clone were grown in pots from which 15 inoculated by pathogen inoculums and five by sterile distilled water as control.

Mother plant and pathogen inoculums preparation

Suckers of three month old of the clones generated from corms of 2 years old were collected from Areka enset clones maintenance and multiplication site. The suckers were planted in pots of 8 liter capacity with sterile soil mix of 1:1 loam soil and red ash respectively, under green house condition and maintained until they resume normal growth. Hagereselam strain of Xanthomonas, the most virulent strain type that can infect all clones of enset including those are claimed to be tolerant by farmers and some researchers. The inoculum was obtained from Hawassa research site by collecting bacterial ooze on plants that were inoculated by Hagareselam Xanthomonas strain purposely for evaluation of enset clones for disease resistance experiment. Bacterial suspension was prepared from these ooze in sterile distilled water and cultured on YPSA (Yeast Peptone Sucrose Agar) semi-selective media prepared from 5gm of yeast extract, 10gm of peptone, 10gm of sucrose and 15gm/l of bacteriological type agar. Then after 48 hours of incubation under 27^0C set incubator, grown bacterial colonies were harvested and bacterial suspension for inoculation at a cell concentration (10^8cfu/ml) as recommend by Hawassa research center pathology laboratory.

Inoculation of enset clones and disease assessment

As per the suggestion of Wolde-Michael *et al* (2008) healthy growing suckers of the three clones were inoculated with 3ml of two days young culture suspension prepared at the base of actively growing leaf using sterile hypodermic syringe. 15 suckers from each clone were infected by the sample suspension. Similarly, 5 suckers from each clone were inoculated by the same amount of sterile distilled water as a control.

Fig 1. Inoculation of healthy enset suckers by pathogen suspension

Disease evaluation was conducted at seven days of interval for one and half month as suggested by Wolde-Michael (2008) for number of diseased plants and severity after artificial

Data Recording

Number of diseased plants after artificial infection: Disease assessment was 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**.**

RESULTS AND DISCUSSION

The result of clone pathogen interaction after artificial infection showed that none of the three clones have shown any symptom of the disease before 14 days after inoculation (Table 1). Leaf of 50% of the Arkiya suckers inoculated have shown yellow color in the mid rib whereas, less number of inoculated plant leaf of Mazia and Digomerza shown the same symptom after 21 days which is the first symptom of the disease on infected plants. Later on 100% of Arkiya clone suckers inoculated were infected by the pathogen, 75% and 33% of Digomerza and Mazia clones respectively have shown the advanced disease symptoms of dark brown necrosis and complete wilting after 28 days of inoculation. After 35 days of inoculation hundred percent of Arkiya and Digomerza clones and eighty percent of Mazia were diseased which lead to the death of most of the infected suckers after 40 days of artificial inoculation (Table 1). On the contrary, the control suckers injected with the same volume of sterile distilled water were on healthy growth without any disease and stress symptoms like suckers before inoculation (Table 1 and Figure 2A-D).

This result shows that all the three clones were susceptible to the pathogen strain used since the disease was expressed equally even if its degree is different which was very early and highly on Arkiya but latently and weakly on the other two clones. The result of this experiment is in line with previous reports by Gizachew (2000) in his experiment on variation of Xanthomonas isolates and enset clones interaction in which he has reported that three month old suckers artificially inoculated under green house by the pathogen developed disease symptom in 10 to 21 days in highly susceptible (Ayna) clones and later on moderately tolerant (Yesha mazia) and tolerant clones (Mazia).

Table 2. Number of plants showing disease symptom after artificial infection by the pathogen

		Number of plants diseased after infection						
Clone	Number of plants infected	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days	% of PLDIS
Mazia	15	0	0	3	6	12	12	80
Control	5	0	0	0	0	0	0	0
Digomerza	15	0	0	5	10	15	15	100
Control	5	0	0	0	0	0	0	0
Arkiya	15	0	0	7	14	15	15	100
Control	5	0	0	0	0	0	0	0

PLDIS=Plants Diseased. Control= Suckers inoculated by distilled water

Figure 2. Pathogen clone interaction of the three clones after 35 days of inoculation:

A. Clean suckers of Digomerza before artificial inoculation, B. Diseased Mazia suckers, C. Diseased Digomerza suckers, D. Diseased Arkiya suckers.

SUMMARY

Enset *(Ensete ventricosum)* is the edible species of the separate genus of the banana family, thus named 'false banana'. Variation within the species to altitude, soil and climate has allowed widespread cultivation in the mid to highlands of western Arsi-Bale, the Southern Peoples Nations Nationalities Regional State (SNNPRS) and western Oromia including West Shewa, Jimma, Ilubabor and Welega.

Enset cultivation is affected by a number of biotic and abiotic factor that contribute to low yield. Among the biotic constraints disease are the ones in which several fungal, bacterial, viral and nematode diseases were known to affect enset.

Enset bacterial wilt which is caused by *Xanthomonas compestrum pv.musacearum* pathogen is a vascular disease that results in yellowing and wilting of leaves and yellowing of immature and mature fruits in case of banana. Foliar symptoms, yellowing and wilting quite often resemble those of fusarium wilt but the excretion of yellowish bacterial ooze from cut tissues is characteristic of enset/ banana bacterial wilt.

Previous efforts directed to eradicate the disease are using cultural practices and enset clonal screening against bacterial wilt indicated possibility of host plant resistance with controversial reports on same clone reaction against the pathogen

For this experiment greenhouse grown clean three month old suckers of enset clones Arkiya, Digomerza and Mazia were used for artificial inoculation. Hagereselam strain of *Xanthomonas* was collected from pockets of plant leaf sheath that were made to develop disease through artificial infection for resistance screening experiment at Hawassa research station. 3 ml of bacterial suspension with cell concentration of 10^8 cfu/cell was used to inoculate the clean suckers of the three clones. The result showed that there was difference for disease symptom development up to four weeks (28 days) in which 100% of Arkiya suckers were diseased while 75% of Digomerza and 33% of Mazia clones have shown the disease symptom. Later after five weeks of inoculation suckers of all the three clones were diseased similarly with little severity difference that 100% of Arkiya and Digomerza suckers were highly diseased and some were died where as 80% of Mazia suckers were diseased.

Based on the results of the present study the following recommendations are made:Screening of clones for *Xanthomonas* resistance should be reinvestigated since all the clones under this study become susceptible as opposite to previous reports and advanced disease diagnostic techniques have to be applied to confirm that plants are really clean from the pathogen before germplasm exchange used as planting materials.

REFERENCES

Atnafua B. and Endale T.., 2008. Food production and nutritional value of Enset. *Proceedings of Enset National workshop*, 9-14 August 2010, Wolkite, Ethiopia.

Awassa Agricultural Research Center. 2000. *Progress Report* 1999-2000

Awassa Agricultural Research Center, 2008. *Progress Report*, plant protection Research Division. Awassa, Ethiopia

Brandt, S.A., Spring, A., Hiebsch, C., McCabe, S.T., Endale,T., Mulugeta, D., Gizachew, W/M., Gebre, Y., Shigeta, M. and Shiferaw, T., 1997. The 'Tree Against Hunger'. Enset-based Agricultural Systems in Ethiopia. *American Association for the Advancement of science*. Pp 56.

Central Statistics Ageency (CSA). 2013. Report on Area and Crop Production forecast for Major Crops Dec., 2013. Addis Ababa, Ethiopia.

Dagnachew, Y., and Bradbury, J.F. 1968. Bacterial wilt of Enset (*Ensete ventricosum*) incited by Xanthomonas campestris sp. *Phytopathology* **59**:111-112

Dereje Ashagire., 1985. Studies on the bacterial wilt of enset (*Ensete ventricosum*) and prospects for its control. *Ethiopian Journal of Agricultural Science*. **7(1)**: 1-14.

Endale, T. and Mulugeta, D. 1994. Improvement studies on enset and sweet potato. In: *Proceedings of the second national horticultural workshop of Ethiopia.* Herath, E. and Desalegn, L. (Eds.), Pp. 63-64. 1-3 December 1992. IAR, Addis Ababa, Ethiopia.

Eshetu, W. 1981. The role of *Poecilocarda nigrinervis*, *Pentanolia nigronervosa* and *Plantococcus ficus* in the transmission of enset wilt pathogen Xanthomonas musacearum sp. in Wolaita, Ethiopia. In: Ethiopia (Tsedeke Abate, Clifton Hiebsch and Steve Brandt). *Proceedings of the first international workshop on Enset*. Dec 13-21 1993. IAR, Addis Ababa, Ethiopia. Pp.188-203

Gizachew W., 2000**.** Variation in isolates of enset pathogen (*Xanthomonas campestris* Pv. *musacearum*) and reaction on enset clones (*Ensete ventricosum* (Welw) Cheesman) to this disease. MSc.Thesis. Alemaya university, Ethiopia. Pp 61.

Kidist B., 2003. Characterization of Xanthomonas campestris PV. Musacearum isolates: causal agent of Enset Bacterial wilt disease. Msc Thesis, Addis Ababa University, Ethiopia. Pp. 100

Quimio, A. and Mesfine T. 1996. Diseases of enset. In: Tsedeke A., Hiebsch, C., Brandt, S.A., Seifu G.M. (Eds.), pp. 188-203. Enset Based Sustainable Agriculture in Ethiopia. *Proceedings of the International Work*

shop on enset. Addis Ababa, Ethiopia, 13-20 December 1993

Shigeta, M., 1996. Folk *in situ* conservation of ensete (*Ensete ventricosum* (Welw) Cheesman): towards the interpretation of indigenous agricultural science of the Ari, Southwestern Ethiopia. *African study monographs (Japan).* **10(3):** 93-107.

Spring, A., Hiebsch, C., Endale, T. and Gizachew, W/M., 1996. Enset needs assessment project *phase I Report*. Awasa, Ethiopia.

Taye, B., 1996. An Over view of enset research and the future technological needs for enhancing its production and utilization. *Proceedings of the First international workshop on Enset*. Dec 13-21 1993. IAR, Addis Ababa, Ethiopia. Pp. 1-14.

Taye, B. and Asrat, F., 1966. The production and utilization of the genus Enset. *Economic Botany* **20(1)** 65-70

Temesgen M., Bizuayehu T.**,** Marcello C. **and** Mario E., 2014. Indigenous knowledge, use and on-farm management of enset (*Ensete ventricosum* (Welw.) Cheesman) diversity in Wolaita, Hawassa University, School of Plant and Horticulture Science, Hawassa, Ethiopia. *Journal of Ethnobiology and Ethnomedicine.* Pp. 18

Wolde-Michael G., Bobosha K., Blomme G., Addis T., Mengesha T. and Mekonnen S., 2008. Evaluation of Enset clones against Enset bacterial wilt. Southern Agricultural Research Institute (SARI), Awassa Research Center, Awassa, Ethiopia. *African Crop Science Journal. **16(1**:* 89-95

Zerihun Y., Hussein M., Mulugeta D., Temesgen A. and Guy B., 2013. Enset (Ensete ventricosum) clone selection by farmers and their cultural practices in southern Ethiopia. *Genetic Resources and Crop Evolution.* 61(3): 1- 16