VARIATIONS IN ISOLATES OF ENSET WILT PATHOGEN (*Xanthomonas campestris Pv. musacearum*) AND REACTION OF ENSET (*Ensete ventricosum* (WELW.) CHEESMAN) CLONES TO THIS DISEASE.

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GIZACHEW WELDE MICHAEL

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BY

GIZACHEW WELDE MICHAEL (B.Sc. in Agriculture, Plant Science, AUA)

ADVISORS: TEMAM HUSSEIN (Ph.D., KANSAS STATE UNIVERSITY)
DEREJE ASHAGARI (Ph.D., UNIVERSITY OF MINNESOTA)

APPROVED BY THE BOARD OF EXAMINERS

Chairman	**Signature**
Major Advisor	**Signature**
Co-Advisor	**Signature**
External Examiner	**Signature**
Internal Examiner	**Signature**

BIOGRAPHY

The author was born on August 21, 1962, in Addis Ababa. He attended his primary school at Awassa Tabor Junior School in Awassa and Secondary School at Soddo Comprehensive High School at Wolayta Soddo. He joined the then Awassa Junior College Agriculture (AJAC) and graduated with Diploma in Plant Science and Technology in 1982. After working for 6 years in the Ministry of State Farms and Development as Research Co-coordinator he joined Alemaya University of Agriculture (AUA) as advanced standing student in 1989 and graduated with B. Sc. degree in plant sciences in 1992. In the same year, he was recruited by the then Institute of Agricultural research (IAR) and worked as Junior and Assistant Research Officer at Awassa Agricultural Research Center. In 1998, he joined the School of Graduate studies of AUA and studied his education leading to M. Sc. degree in plant pathology.

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VARIATIONS IN ISOLATES OF ENSET WILT PATHOGEN (*Xanthomonas campestris pv. musacearum*) AND REACTION OF ENSET (*Ensete ventricosum* (Welw.) Cheesman) CLONES TO THIS DISEASE.

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ABSTRACT

Enset (Ensete ventricosum (Welw.) Cheesman.) is a herbaceous monocot plant used as a staple food crop for over 15 million Ethiopians. Bacterial wilt caused by Xanthomonas campestris pv. musacearum is the major factor that limits enset production in Ethiopia today. The objective of this study was to determine pathogenic variation of this pathogen and to evaluate some enset clones for resistance to the disease. Samples of bacterial ooze were collected from wilt infected enset plant in six "Woredas" of Gurage and North Omo Zones. Sixty-five yellow colored bacterial colonies were isolated from the collected samples. After conducting hypersensitivity and pathogenicity tests, isolates were tested for their cultural, morphological, physiological and biochemical properties. All pathogenic isolates fit the characteristics of Xanthomonas campestris pv. musacearum (Xcm). A total of eighty-one enset clones of Waka collections obtained from Areka Agricultural Research Center were evaluated for resistance to the enset wilt pathogen under field condition. Resistance was found only in one clone, Mezya. All other enset clones developed disease symptoms at varied disease incidence levels. Significant difference was observed between Mezya and all other test clones; between Yesha Mezya, Hala Mezya, Anko Mezya, and Bota Mezya and all other infected clones ($p<0.005$). Among the 80 infected clones, Yesha Mezya, Hala Mezya, Anko Mezya, and Bota Mezya showed relatively better tolerance than others. The remaining seventy-five enset clones were found to be highly susceptible. Pathogenic variations of Xcm isolates were tested on twelve enset clones obtained from Areka Agricultural

Research Center. Four pathogenic isolates of Xcm collected from naturally wilting enset plants of Gurage, Sidama, North Omo and Kembata Zones were used for artificial inoculation in the greenhouse. All the twelve-enset clones developed disease symptom typical of enset wilt regardless of isolates used. There was no significant difference (P<0.005) between four Xcm isolates and twelve enset clones within 60 to 75 days after inoculation.

On the other hand, interaction between Xcm isolate and enset clone was significant (P<0.005). Among the 48 isolate-clone combinations, the lowest disease incidence 87.44% was recorded on clone Ayna by isolate K13 and the highest disease incidence was recorded on 32 of the isolate-clone combinations. Among the four isolates, isolate G13 was able to cause 100% disease incidence to all enset clones. However, only one clone, Yilga, was found to be 100% infected by all the four isolates. The remaining isolate-clone interactions showed varying degrees of infection ranging from 91.44 to 95.78%. In this study all the four Xcm isolates induced a compatible reaction on the twelve-enset clones used for the variability study irrespective of their geographic origin. With the limited number of isolates and enset clones, it was not possible to detect much variability in the pathogen populations as expected. In the future further work needs to be undertaken by employing a large number of isolates and clones from different geographic regions.

1. INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Cheesman.) is a herbaceous monocot plant. It belongs to the family *Musaceae* and the genus *Ensete*. The center of diversity for enset is located in the low land and mountain areas of Uganda, Tanzania, Sudan and Asia (Smeds, 1955). However, it is only in Ethiopia that enset is cultivated for food (Westphal, 1975). The cultivation of enset is concentrated in the southern and southwestern part of the country (Taye and Asrat, 1966).

Enset plantations are found at altitudes between 1200 to 3100 masl (Huffangel, 1961; Westphal, 1975; Taye, 1980; Bezuayehu *et al.*, 1993). Most enset growing area receive an annual rainfall in the range of 1100 to 1500 mm (Smeds, 1955). The average temperature and relative humidity of enset growing area is reported to be between 10 to 21^{0}C and 63 to 80%, respectively (Taye, and Asrat, 1966). Enset grows on most types of soils if sufficiently fertile but it prefers nitosol than vertisol (Huffangel, 1961).

The total Ethiopian population that depends on enset as a staple food crop is estimated to be about fifteen million (Anita *et al.*, 1996). The area of enset production in Ethiopia is estimated to be over 180,000 hectares (Central Statistical Authority (CSA 1994). The average yield of "Kocho" (non-dehydrated fermented product from the mixtures of decorticated pseudostem, pulverized trunk and corm) was 10.3 tons per hectare per year basis (Taye and Asrat, 1966; Endale and Mulugeta, 1994).

. The fiber, by-product of enset, supplies more than 30 percent of Ethiopia's fiber need (Huffangel, 1961). Fresh enset parts are used as fodder for domestic animals during dry season and some enset clones reported to have medicinal value to human beings and domestic animals (Taye and Asrat, 1966). Enset is also well known to conserve soil and enrich plant nutrients through its dropped foliage (Kefale and Sandford, 1991). Dried leaf sheaths and petioles of enset are used for wrapping material and other household utensils (Endale and Mulugeta, 1994).

Enset is affected by a number of biotic and abiotic factors that contribute to low yield. Among the biotic constraints, diseases are the ones. Several fungal, bacterial, viral and nematode diseases were known to affect enset (Archaido and Mesfin, 1993). However, bacterial wilt of enset is considered to be more important disease than others (Mesfin, 1990).

The disease was first reported and described by Dagnachew and Bradbury in 1968 who attributed it to *Xanthomonas musacearum* sp.n. (Eshetu, 1981). The disease is widely distributed in many enset growing regions of the country and affects the crop at all stages (Dereje, 1985). Natural epidemics of the disease were also observed in banana fields at different enset growing areas (Archaido and Mesfin, 1993).

Eventhough the disease is widely distributed and important, very little work has been done on the etiology of the causal pathogen and management aspect of the disease. Previous efforts directed to enset clonal screening against enset bacterial wilt indicated the possibility of using host plant resistance (Dereje, 1985). Different workers have reported variable level of disease incidence in enset field at different enset growing regions of the country (Dereje, 1985; Anita, *et al.,* 1996).

However, information on the extent of pathogenic variation in *Xanthomonas campestris pv. musacearum* (*Xcm*) population in the country is not available.

Host plant resistance is believed to be most effective and economical control measures for this disease. Although the development of resistant enset clone (s) has remained difficult, available reports related to clonal screening against bacterial wilt have indicated the possibilities of using host plant resistance (Dereje, 1985).

Hence, this project is proposed with the following specific objectives:

1. To determine pathogenic variation of *Xanthomonas campestris pv. musacearum* isolates.

2. To evaluate some enset clones for resistance to this disease.

2. LITERATURE REVIEW

2.1 The Pathogen

2.1.1 Classification

The causal agent of enset bacterial wilt was reported to be *Xanthomonas musacearum* (Dagnachew and Bradbury, 1968). The International Committee on Systematic Bacteriology (ICSB) formed in 1980, for the first time compiled approved list of bacterial nomenclatures which included only names of bacteria (Dye *et al.*, 1980). They also suggested several species of the genus *Xanthomonas* to be reduced to the pathovar level under the type species *Xanthomonas campestris* and the pathovars were distinguishable with only host range (Dye *et al.*, 1980) rather than by the usual biochemical tests used in Bergy's manual (Schaad and Stall, 1988). *Xanthomonas campestris* consisting of over 120 pathovars was considered as one of the 5 species of the genus *Xanthomonas* that are easily differentiated by a few key tests (Young, *et al.,* 1978; Moore, 1988; Schaad and Stall, 1988). Accordingly, the correct name for the causal agent of enset wilt is *Xanthomonas campestris pv. musacearum* .

2.1.2 Disease Symptom

Initial symptoms appear on the central heart leaf or on one of the inner leaves of enset whose tip becomes yellowish, limp and droop. A cut made through the petioles of a newly infected enset plant reveals browning of the vascular strands and yellowish or grayish masses of bacteria ooze out from strands. Cross sections at the base of the pseudostem and corm show discoloration of

the vascular strand with large bacterial pocket and grayish or yellowish exudate with brownish to black spot, respectively (Eshetu, 1981; Dereje, 1985). In a more advanced stage of disease development, most of the leaves wilt, breaks at the petiole and wither. Eventually, the whole plant dies and rots to the ground (Archaido, 1992).

2.1.3 Morphological and Cultural Characteristics

Cells of *Xcm* are gram negative rod shaped, measuring on the average 0.8 μm by 1.9 μm. They are motile with a single polar flagellum. On glucose peptone agar, colonies of the bacteria appear distinct after 48 hours incubation at 25 to 28^0C. Cultures on nutrient agar were light yellow. On King's medium B, colonies are deeper yellow, but produce no diffusable fluorescent pigment. On potato plugs honey-colored, slimy growth and sudanophilic inclusions are also observed in cells grown on either sucrose or glucose peptone agars (Eshetu, 1981).

2.1.4 Physiological and Biochemical Characteristics

Xanthomonas campestris pv. musacearum produces acid without gas from glucose, mannose, galactose, sucrose but not from lactose, xylose, maltose, manitol sorbitol or salicin with in one month time. Gelatin is slowly liquefied; H_2S and NH_3 are produced; lipase activity and aesculin are hydrolyzed. Starch is not hydrolyzed, nitrate is not reduced nor nitrite is decomposed. Indole and acetoine are not produced. Tests for methyl red reaction, pectinase, lecuthinace or egg yolk reaction, Kovac's oxidase and arginine dehydrolase are negative. Litmus milk become white within three days and then turns pink. It is neither coagulated nor cleared. Growth in the nutrient broth is

retarded by 3% and suppressed by 4% NaCl. The optimum temperature for its growth is between 25 and 28^{0}C and absent at 39^{0}C (Eshetu, 1981).

2.2 Variation in Disease Incidence and Possibilities for Disease Management

The occurrence of variation in pathogenicity in pathovars of plant pathogenic bacteria with in the same crop type has been known long ago (Robert and Stelle, 1956). Considerable variation has been observed among the isolates of *Xanthomonans campestris* in relation to pathogenicity or virulence including biochemical and physiological characteristics (Gupta *et al.*, 1986; Noda *et al*, 1990). A strain that causes diverse variability with respect to pathogenicity and other characteristics has been observed in other pathovars of *Xanthomonas campestris* (Singh, 1984; Stall *et al.*, 1986; Sijam *et al.*, 1992). The variants may occur suddenly and cause a great damage in crop production (Russel, 1978).

Results of some studies indicated obvious variations in the occurrence and incidence of enset bacterial wilt across the main enset growing regions in the country (Dereje, 1985; Archaido, and Mesfin, 1993; Anita *et al.*, 1996). This suggests the probable existence of variability within *Xcm* population in the country. As has been observed in other pathovars of the genus *Xanthomonas* (Gupta *et al.*, 1986; Noda *et al.*, 1990), the apparent pathogenic specialization of *Xcm* in the country may result the appearance of virulent variants and complicate the use of resistant enset clones. Hence, the knowledge on variations within *Xcm* population in respect to pathogenicity and investigating the variations within the available enset clones in their reaction to this pathogen seems to be feasible to effectively utilize host plant resistant in enset wilt management strategy.

In developing long-term strategies for enset bacterial wilt management, it is important to assess

variations in virulence within pathogen populations of *Xcm*, that may be achieved by screening the available enset clones to different pathogenic isolates.

2.3 Economic Importance

Eventhough yield loss on enset due to enset wilt is not well quantified, variable level of disease incidences had been reported to occur in most enset growing localities (Dereje, 1985). Moreover, in some areas, enset fields were completely destroyed due to this disease and farmers were forced to replace the field with other crops (Archaido, 1991).

On the other hand, related bacterial diseases on banana namely bacterial wilt of banana (Moko disease) caused by *Pseudomonas solanacearum* and bacterial head rot or 'tip-over' caused by *Erwinia cartovora* were reported as a serious yield-limiting diseases of banana in some major banana growing areas of Asia, Africa and America (Buddenhagen, 1961; Stover, 1972).

In Honduras, Pacific coast of Costa Rica and Panama banana zones of 30,000 acres, less than 1% of the plant are lost yearly due to Moko disease. However, this low level of loss is maintained by an expensive system of prevention and control costing nearly 400,000 dollars annually. Loss in peasant plantings of banana and plantain have been high in Central America, Colombia, Venezuela and Peru (Wardlaw, 1972). In the same country, damage due to head rot or 'tip over' was serious and an incidence of 10 to 15% of the mats was common and rises up to 50% in localized areas (Stover, 1972).

Susan (1972) reported banana wilt to be a great potential menace to large-scale banana growing

areas of Central America than the most important Panama disease.

Therefore, considering the similar nature of infection incited by these two different bacterial pathogens on enset and banana, enset wilt might cause more or less a comparable yield loss on enset plant to that of banana wilt at times of heavy disease out break.

2.4 Disease Distribution

Enset bacterial wilt disease was first reported by Dagnachew in the year 1967 (Dagnachew and Bradbury, 1968). Since then it has been one of the most commonly reported disease of enset (Eshetu, 1981; Dereje, 1985; Archaido and Mesfin, 1993; Anita *et al.*, 1996).

The disease was widely distributed in high, mid and lower altitude areas of the Central, Southern and Southwestern enset growing regions of the country (Anita *et al.*, 1996). Intensive survey involving 29 "woredas" (Amharic terminology denoting an administrative unit equivalent to sub-district) revealed that the disease to be severe in 23 woredas (Dereje, 1985). Disease survey conducted in the three selected enset growing woredas of Gurage, Hadiya and Sidama zones showed the bacterial wilt to be the most serious disease (Anita *et al.*, 1996).

2.5 Host Range

Enset bacterial wilt infects banana under natural conditions. A natural epidemic of the disease was reported on the banana hybrid cultivar, Du Case, in Kaffa Province (Dagnachew and Bradbury, 1974). Archaido and Mesfin (1993) also observed infected bananas growing side by side with enset

clones in Sidama and North Omo regions. Studies made to identify host range for *Xcm* under artificial inoculation conditions, the pathogen failed to initiate wilt symptom in 27 plant species which encompassed legumes, cereals, solanaceous and other crops including weeds commonly found in enset growing areas (Dagnachew and Bradbury, 1968).

In another study, Dereje (1985) observed the pathogen to be infective on *Canna orchoides*, the only host outside of Musa family, and decorative weed commonly found associated with enset plant.

2.6 Survival and Dissemination

Infected enset plant debris and to some extent infested soils were suggested to be the source of inocula for natural disease epidemics (Dagnachew and Bradbury, 1968). According to Dereje (1985), infectivity of the pathogen was maintained for four days when infected plant parts were incubated in moisture saturated soils under field conditions. He further observed that infectivity of the bacterium was retained up to three and four days when the contaminated knives were incubated under humid and dry conditions, respectively.

Mechanical transmission of enset bacterial wilt pathogen and the importance of wounds in disease initiation was demonstrated by cutting enset leaf sheaths with contaminated knife and inoculating plants with suspension of bacterium (Dereje, 1985). In addition, hundred percent disease transmissions was obtained when bacterial suspension was directly injected or when petioles were cut with contaminated knives (Dereje, 1985). In a similar study made on the transmission of banana wilt, it was reported that the disease is readily transmitted from plant to plant with knives and machete used in pruning operations (Sequeira, 1958).

Enset and banana have shown wilt symptoms when bacteria were applied to wounded leaves; when roots were injured and then planted in infested soil; and when roots were dipped into a bacterial suspension (Dereje, 1985). Dagnachew and Bradbury (1974) anticipated transmission of the pathogen by cattle browsing on infected enset and banana plants. Eshetu (1981) reported that mealy bug (*Planococcus ficus*), banana aphid (*Pentalonia nigronervosa*) and leaf hopper species (*Poecilocarda nigrinervis*) to be the most frequently observed insect species in enset field. However, the latter was assumed to be more important as vector due to its active flying ability. Similarly, insect dispersal of banana wilt pathogen (*Pseudomonas solanacearum*) was reported by Kelmant (1963).

Eventhough detailed data is not available, Mesfin (1989) noted the frequent association of some nematodes mostly *Meloidogyn*e species and *Pratylenchus goodeyi* with infected enset plants and suspected that they may play a role in the dispersal of the pathogen.

Transmission may occur through contaminated soil, but it is not clearly known for how long it can survive in the soil. In a test using susceptible enset suckers as indicator plants, *Xcm* infested soil samples incubated for more than four days prior to planting failed to produce the disease symptom six to eight weeks after inoculation (Dereje, 1985).

2.7 Disease Control Methods

2.7.1. Sanitary and Cultural Control Measures

Sanitary control measures which would prevent, reduce or eliminate the spread of *Xcm* in enset field include flaming of enset cutting tools after using them on infected plants; preventing animals from browsing and straying into infested fields; fencing in the infested site after digging out the corm and roots and chopping them up together with the pseudostem and leaves (Archaido and Mesfin, 1993)

Cultural practices such as deep tillage and turning over the soil to expose the soil under during dry period prior to planting; proper spacing and planting layouts, manuring the soil of planting holes; and spot rotation of the infested sites in the field with non-host crops were suggested to play role in reducing the re-spread of the pathogen in the field (Archaido, 1992).

Local quarantine system that would restrict the movement of planting materials like suckers and corms should be effected (Archaido, 1992). Early detection and prompt destruction and disposal of the diseased plants is believed to reduce the chance of *Xcm* inoculum spread in the field and thereby suppress the development of bacterial wilt.

Since enset is a semi perennial crop and plantations are found adjacently covering wider areas, access to disease establishment, survival and respread of the pathogen all round the year became apparent. Once the pathogen reach to healthy enset field it can multiply and disseminate to non infected adjacent enset fields.

Sanitary control measures are anticipated to be effective option in reducing the disease to acceptable level if exercised regularly by all enset farmers at community level (Archaido and Mesfin, 1993). This is because of that if some wilt infested enset fields left without such action in a given locality, the infested field will be source inocula for respread of the disease.

2.7.2 Host Plant Resistance

It is estimated that there are over 200 different enset clones described by vernacular names in Ethiopia (Endale and Mulugeta, 1994). Enset farmers commonly grow combinations of clones in their enset field and each clone is basically grown for its specific use. The selection of relatively disease tolerant clones reported by the farmer as one of the other criteria for enset bacterial wilt management decisions (Anita *et al.*, 1996). Stover (1972) screened 59 varieties of bananas in Central America against bacterial wilt of banana using pseudostem inoculation method and found varieties with different levels of resistance. Among the 59 tested varieties, 57 were found to be highly susceptible while the remaining two were found to be partially resistant. Wardlaw (1972) also reported the existence of clonal variability to disease resistance in Musa species and suggested their potential in future utilization as sources of resistance. Similarly, Dereje (1985) observed that five enset clones namely Ado, Kembate, Genticha, Hedesso and Soskella appeared to have better tolerance than others do in artificial test involving sixty enset clones. Archaido and Mesfin (1993) also observed artificially inoculated Genticha clone recovering from the disease 12 to 16 weeks after inoculation.

Variable levels of clonal response against disease have been observed in enset fields under natural condition. Farmers also reported to use some relatively tolerant or resistant enset clones known by them to replace infected enset field. In Gurage, enset clones such as Yeshrekinke, Bedadet and Yekeswe (local name for enset clones given by Gurage farmer) are reported by the farmer to have relative tolerance to wilt than others in their order listed (Anita *et al.,* 1996).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The field experiment was conducted during 1998/99 main crop season at the experimental field of Awassa Agricultural Research Center (AARC) located in the Southern Nations, Nationalities and Peoples Regional State. The AARC is located at $7^0$40'N latitude and $38^0$31'E longitude and an altitude of 1680 masl. The type of soil is of volcanic origin and is classified in the fluvisoil and/ or andosol units. Fifteen years average rainfall of the center is 1110 mm (Desta, 1982).

Greenhouse and laboratory experiments were conducted at the National Soil Research Center, Soil Microbiology Section in Addis Ababa.

3.2 Experimental Procedure

3.2.1 Study on Isolate Variation

3.2.1.1 Sample Collection

Samples of diseased specimen were collected from two main enset-growing regions of Ethiopia namely: Gurage and North Omo Zones representing different enset cropping systems. In each zone, 3 woredas, 9 Peasants Associations (PAs) were targeted for sample collection (Table 1).

Samples of bacterial ooze were collected from the cut ends of infected petiole and leaf sheath by tooth pick and placed into a 10 ml screw cupped tube half filled with sterile distilled water. The tubes with bacterial suspension were gently shaked, placed in icebox and transported to the laboratory (Blanchard and Tatter, 1981). The samples were labeled with date of collection, locality, altitude, enset clone name, age and color of bacterial ooze.

Table 1 Geographic origin and number of bacterial isolate used for morphological and biochemical study

Designation	Geographic origin				
	Zone	Wereda	PAs	Altitude masl	Number of Isolates
NOMGM1	North Omo	Mareka gena	Mari	2600	9
NOMGM2			"	2600	
NOMGM3			"	2600	
NOMGM4			"	2600	
NOMGM5			"	2500	
NOMGM6			"	2500	
NOMGM7			'	2500	
NOMGM8			"	2500	
NOMGM9			"	2400	
NOMGDY10			Dakayeli	2400	9
NOMGDY11			"	"	
NOMGDY`12			"	"	
NOMGDY13			"	"	
NOMGDY14			"	"	
NOMGDY15			"	"	
NOMGDY16			"	"	
NOMGDY17			"	"	
NOMGDY18			"	"	
NOMGGS19			Gososhasha	2300	9
NOMGGS20			"	"	
NOMGGS21			"	"	
NOMGGS22			"	"	
NOMGGS23			"	"	
NOMGGS24			"	"	
NOLBGB25		Loma bossa	Gassa bosa	2150	9
NOLBGB26			"	"	
NOLBGB27			"	"	
NOLBGB28			"	"	
NOLBGB29			"	"	
NOLBGB30			"	"	

Table 1. Continued

Designation	Geographic origin				
	Zone	Wereda	PAs	Altitude masl	No. of Isolates
NOLBGB31			"	"	
NOLBGB32			"	"	
NOLBGB33			"	"	
NOLBTB34			Tulema bero	2100	6
NOLBTB35			"	"	
NOLBTB36			"	"	
NOLBTB37			"	"	
NOLBTB38			"	"	
NOLBTB39			"	"	
NOLBTT40		"	Tulema tama	2150	6
NOLBTT41			"	"	
NOLBTT42			"	"	
NOLBTT43			"	"	
NOLBTT44			"	"	
NOLBTT45			"	"	
NOBSBK46		Boloso sore	Boyna kere	1900	3
NOBSBK47			"	"	
NOBSBK48			"	"	
NOBSD49		"	Dolla	1850	3
NOBSD50		"	"	1850	
NOBSD51		"	"	1850	
GCAA52	Gurage	Cheha	Atuncha	2150	3
GCAA53		"	"	"	
GCAA54			"	"	
GCYE55		"	Yefekterek	2200	3
GCYE56			"	"	
GCYE57			"	"	
GCM58		"	Miger	2200	9
GCM59			"	"	
GCM60			"	"	
GCM61			"	"	
GCM62			"	"	

Table 1. Continued

Designation	Geographic origin				
	Zone	Wereda	Peasant Association	Altitude masl	Number of Isolates
GCM63			"	"	
GCM64			"	"	
GCM65			"	"	
GCM66			"	"	
GYWS67		Yesane welene	Sheremo	2450	6
GYWS68			"	"	
GYWS69			"	"	
GYWS70			"	"	
GYWS71			"	"	
GYWS72			"	"	
GYWG73		"	Geche	2200	6
GYWG74			"	"	
GYWG75			"	"	
GYWG76			"	"	
GYWG77			"	"	
GYWG78			"	"	
GYWA79		"	Agena	2550	3
GYWA80			"	"	
GYWA81			"	"	
		Gumer			
GGYD82			Yebuder dender	2800	3
GGYD83			"	"	
GGYD84			"	"	
GGK85		"	Kebul	2650	3
GGK86			"	"	
GGK87			"	"	
GGA88		"	Abeke	2800	3
GGA89			"		
GGA90			"		

3.2.1.2 Isolation

One loopful of bacterial suspension was taken from each sample in the vials and streaked onto the surface of Yeast Peptone Sucrose Agar (YPSA) medium and plates were incubated at 27^{0}C for 48 to 72 hours (Schaad and Stall, 1988). After 48 to 72 hours of incubation colonies showing light-yellow mucoid growth typical of *Xcm* were transferred to individual YPSA slant and maintained at 4^{0}C in refrigerator for further studies (Schaad and Stall, 1988).

3.2.1.3. Hypersensitivity Test

In order to separate the pathogenic isolates from nonpathogenic ones, hypersensitivity test was conducted on tobacco plant (*Nicotiana tabacum* var. white burley) (Kelmant, 1963). The inoculum was prepared from 48 hours old cultures in sterilized distilled water. A two months-old tobacco plant grown in clay pot was injected with 2ml of bacterial suspension (spectrophotometrically adjusted to 10^8 cfu/ml) into the intercellular space of leaves by a 5ml sterile hypodermic syringe and needle. The control plant was inoculated with sterile distilled water. All inoculated plants were then maintained in the glasshouse at 25 to 30^{0}C until symptom development. Isolates, which showed complete collapse of tissue around the point of infection after 24 hours, were recorded as a positive test.

3.2.1.4 Pathogenicity Test

Bacterial isolates that induced hypersensitive reaction on tobacco plants were subjected to pathogenicity test on susceptible enset clone, Mandaluka (Dereje, 1985). Clay pots of 8 kg capacity

were filled with sun dried mixtures of soil, manure and sand in 3:1:1 ratio, respectively (Archaido, 1992). Enset suckers were transplanted in wet soil and kept in glasshouse at 25 to 30^{0}C and 15 to 18^{0}C day and night temperatures, respectively. Plants were watered uniformly at 3 to 5 days interval.

For each isolate, three enset suckers of three months-old were inoculated with 3ml of bacterial suspension (10^8 cfu/ml) at the base of the newly expanded central leaf petiole using a 10 ml sterile hypodermic syringe and needle. The same quantity of sterile distilled water was injected to control plants (Eshetu, 1981; Dereje, 1985). The test was replicated three times. Observations for symptom development were made at seven 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 pseudostem. Re-isolation of the pathogen was made from infected leaf petiole and sheaths of inoculated plants. Following the same steps used for the original isolates, hypersensitivity and pathogenicity tests were conducted on the re-isolates.

3.2.1.4 Morphological and Biochemical Tests

Isolates which induced hypersensitive reaction on tobacco leaves and pathogenic on susceptible enset plant were subjected to the following morphological and biochemical tests: gram reaction, motility, mucoid growth, colony color on YPSA, gelatin liquefaction, casein and starch hydrolysis, citrate and malonate utilization, hydrogen sulfide and indole production, nitrate reduction and fermentation of carbohydrate. (For details of the techniques please see Appendix 1)

3.2.2 Evaluation of Enset Clone

3.2.2.1 Enset Clones

A total of 81 enset clones of Waka collections were obtained from the Areka Agricultural Research Center, (ArARC) that is responsible for National Enset, Root and Tuber Research Program (Table 2). The clones were evaluated for their reaction to enset wilt pathogen under artificial condition at the experimental field of Awassa Agricultural Research Center (AARC) during 1998/99 crop growing season. Six months old, vigorous and uniformly sized enset suckers were uprooted and transplanted into holes (50 cm deep by 50 cm width).

The experiment was laid out in 9x9 simple lattice design. From each clone, 7 suckers were transplanted in a single row and a row was considered a plot. Spacing was arranged 1 meter by 2 meters between plants and row, respectively.

Table 2 List of enset clones commonly cultivated in Mareka and Loma weredas of North Omo Zone used for clonal evaluation study.

No. Clone Name	No. Clone Name	No. Clone Name	No. Clone Name
1. Ankogena	21. Buba	41. Delulya	61. Gashashya
2. Fenchere yepa	22. Berguda	42. Mecha shododina	62. Delya
3. Onsa	23. Alewa	43. Bota mezya	63. Yesha mezya
4. Kosya	24. Yilga	44. Berja	64. Erpa-3
5. Zergessa	25. Ado	45. Zera mezya	65. Yesha
6. Chichya	26. Bedadya	46. Erpa-20	66. Bombe
7. Rentya	27. Atuma boza	47. Utula	67. Tsela
8. Keretya	28. Erpa-7	48. Dirbo	68. Shelekumya
9. Bosena	29. Donkola	49. Sanka	69. Hasa bedadya
10. Guluma	30. Tena	50. Dorta	70. Amarete
11. Sutya	31. Gesa	51. Chemertya	71. Yore
12. Banga arkya	32. Argema	52. Bulla	72. Anko mezya
13. Digo merza	33. Sirrara	53. Agunsa	73. Erpa-8
14. Gimira arkya	34. Arkya	54. Shutatya	74. Koche arkya
15. Shebera	35. Mecha boza	55. Erpa-13	75. Bota bukunya
16. Mezya	36. Budunswa	56. Akacha	76. Hala mezya
17. Tuzuma	37. Astara	57. Trey	77. Shengya
18. Shasha	38. Ayna	58. Wonshe	78. Hoendya
19. Erpa-6	39. Mashasha	59. Kekere	79. Nekaka
20. Goshendya	40. Banga	60. Bukunia	80. Gena
			81. Shododina

3.2.2.2. Sample Collection and Inoculum Preparation

A total of 12 bacterial ooze samples were collected from Hagere Selam (HS11, HS12, HS13, HS21, HS22 and HS23) and Aleta Wondo (AW11, AW12, AW13, AW21, AW22, and AW23) farmers' enset fields. Bacterial oozes exuding from cut ends of infected petioles and leaf sheaths samples were picked with tooth pick and placed into 5 ml capacity test tubes half filled with sterile distilled water (SDW).

From each sample, a loopful of suspension was taken with sterile wire loop and streaked on to YPSA plate under aseptic condition and incubated at 27^0C for 48 hours (Archaido, 1992).

A total of 8 isolates, HS11-1, HS13-2, HS21-2, HS22-1 and AW12-2, AW13-3, AW22-2, AW23-3, with yellow colored colony and assumed to be *Xcm* were selected. A single colony of each culture was transferred to a test tube slant of the same media and incubated at 27^0C for 48 hours. A 48 hours old pure slant culture of each isolate was suspended in sterile distilled water and shaken vigorously for 1 minute. Bacterial cell concentration was spectrophotometrically adjusted to 10^8 cfu / ml (0.3 optical density at 460 nm).

These isolates were subjected to hypersensitivity and Pathogenicity tests following the same procedure described in sections 3.2.1.3 and 3.2.1.4. Among these isolates, HS12-2, the most virulent one, was selected, multiplied on YPSA plate and served as inoculum source for clonal screening experiment.

3.2.2.3. Inoculation of Enset Clones and Disease Assessment

Five months-old enset plants were inoculated with 3 ml of 48 hours-old bacterial suspension, whose cell concentration was adjusted to 10^8 cfu/ml. From each enset clone, 6 plants per row were inoculated at the base of the newly expanding central leaf petiole using 10ml sterile hypodermic syringe and needle. The same amount of sterile distilled water was inoculated to the control plants.

Disease evaluation was conducted at 7 days interval for one month and 15 days interval for 2 months. Dates of disease symptom development, number of infected plants per clone, number of wilted and healthy leaves were recorded. Data obtained from individual plant per clone was averaged for statistical analysis. Before undertaking analysis, row data were transformed and arcsine transformation was used and the variance is homogenized (Gomez and Gomez, 1984).

3.2.3. Study on Variations of *Xcm* Isolates in Pathogenicity

3.2.3.1 Sample Collection and Inoculum Preparation

A total of 21 bacterial ooze samples were randomly collected from newly wilt infected enset fields of Sidama, North Omo, Kembata and Gurage Zones (Table 3). Bacterial oozes were taken from the cut ends of infected petiole and leaf sheath samples with tooth pick, put into test tube half filled with sterile distilled water, placed inside ice box and transported to laboratory. From each sample, a loopful of suspension was streaked onto YPSA plate and incubated at 27^0 C for 48 hours. After 48 hours, yellow colored colonies presumptive to *Xcm* were sub-cultured. The

pure cultures were then maintained on YPSA slant at 4^0C in refrigerator until used. Following the procedure described in section 3.2.1.3 and 3.2.1.4, pathogenic isolates were separated from non-pathogenic ones. As a result, four isolate, NO23, S13, G23 and K23 were used as source of inoculum for isolate clone pathogenic variations study (Table 4).

Table 3 Bacterial ooze samples collected from naturally wilting enset plants of Gurage, Kembata, North Omo and Sidama, enset growing zones for variation in pathogenicity study.

Code	Zone	Woreda	PAs	Altitude(masl)	Clone name	Age (year)	Color of ooze
S1	Sidama	H/Selam	Loya	2600	Gulumo	2	Light yellow
S2	"	"	"	"	Ado	3	"
S3	"	"	"	"	Derasity	2	Yellow
NO1	North Omo	Sodo zurya	Wareza gerera	1850	Chiche	3	Yellow
NO2	"	"	"	"	Ketene	2	Light yellow
NO3	"	"	"	"	Lembo	4	Yellow
NO4	"	Boloso sore	Dolla	1850	Tuzuma	3	Yellow
NO5	"	"	"	"	ketene	3	Yellow
NO6	"	"	"	"	delulya	4	Light yellow
K1	Kembate	Angacha	Ancha sedcho	2600	Siskela	3	Light yellow
K2	"	"	"	"	Dego	4	Light yellow
K3	"	"	"	"	Siskela	5	Yellow
K4	"	Kacha bira	Hobichaca	2100	Dirbo	4	Yellow
K5	"	"	"	"	Siskela	4	Yellow
K6	"	"	"	"	Siskela	5	Yellow
G1	Gurage	Gumer	kebul	2800	Ayshere	3	Yellow
G2	"	"	"	"	Agade	4	Light yellow
G3	"	"	"	"	Agade	3	Yellow
G4	Gurage	Cheha	Girrar	2250	Nechwe	5	Yellow
G5	"	"	"	"	Agade	3	Yellow
G6	"	"	"	"	Yeregye	4	Yellow

Table 4 List of four Xcm isolates used for pathogenicity variation study under Greenhouse condition

No.	Designation	Origin (Zone)
1	NO23	North Omo
2	S13	Sidama
3	G23	Gurage
4	K23	Kembata

3.2.3 2 Inoculation and Disease Assessment

Four isolates, NO23, S13, G23 and K23 were inoculated to 12 enset clones (Table 5) following the same procedure as described in section 3.2.1.4. The experiment was laid out in a split plot design with the enset clones as the main plot treatments and bacterial isolates as the sub-plot treatments. The design of main plot was a Randomized Complete Block Design (RCBD) with 3 replications. Enset clones were randomly assigned to the main plots within each block and isolates were randomly assigned to sub-plot within each main plot (Gomez and Gomez, 1984).

From each clone, 5 plants of 4 months- old were inoculated with each isolate. Observation for symptom development was started 7 days after inoculation. Data on date of infection, number of infected plants and leaves (percent plant and leaves infected) were recorded at seven days interval for one month and every fifteen days for two months after inoculation. Data on percent infected plant and leaves were transformed using arcsine and square root transformation before undertaking analysis of variance. Disease incidence values from for isolate clone interaction was subjected to analysis of variance using a computer package MSTAT and the specificity of isolates' infectivity to different enset clones was determined. Mean separations were made using LSD value at 5% level of significance.

Table 5 List of twelve enset clones used for pathogenicity variation study under Greenhouse condition.

No	Enset Clones	Designation
1	Agna	Ag
2	Astara	As
3	Ayna	Ay
4	Banga	Ba
5	Bosena	Bo
6	Chichya	Ch
7	Delya	**DE**
8	Dirbo	Di
9	Erpa-7	Er
10	Nekaka	Ne
11	Sutya	Su
12	Yilga	Yi

4. RESULTS AND DISCUSSION

4.1. Morphological and Biochemical Characteristics

A total of ninty bacterial ooze samples were collected from naturally wilting enset plants of two zones, six weredas and twenty three PAs in some enset growing localities of Ethiopia (Table 1). Seventy-nine of them produced light-yellow colored bacterial colonies on YPSA medium and these bacterial colonies were suspected to be causal pathogen of enset wilt.

Among the seventy-nine suspected bacterial isolates inoculated on leaves of tobacco plant (*Nictiana tabacum* variety White burley), only sixty-five of them induced hypersensitive reaction within 24 to 48 hours after inoculation. The treated area surrounding inoculated spot dried with in one week after inoculation. The same isolates also resulted in a positive pathogenic reaction on enset plants within 15 to 30 days after inoculation. The remaining 14 isolates did not induced hypersensitivity and pathogenicity reaction on tobacco and enset plants, respectively. These isolates were excluded from morphological and biochemical tests.

Those isolates, which were positive for hypersensitivity and pathogenicity tests were subjected to some morphological and biochemical tests for the purpose of characterization according to biotypes (Table 6 and 7). Tests conducted on cultural and morphological characteristics revealed similar results among the pathogenic bacterial isolates (Table 6).

Bacterial isolates grown on solid YPSA medium, appeared visible, light yellow, very mucoid and highly convex after 48 hours at 28^0C incubation. The light yellow pigment formed was non-water

soluble typical of the bacterial colonies of the genus *Xanthomonas* (in contrast to the greenish fluorescent water-soluble colonies of *Pseudomonas* (Zehr, 1970)). The bacteria are aerobic and gram negative. When observed under microscope, cells were found to be rod. With respect to movement, they have single polar flagellation (Table 6).

All isolates were capable of utilizing the protein, casein. None of the isolates were able to hydrolyze starch four weeks post inoculation. All isolates were negative for nitrate reduction from tryptone broth. Gelatin was slowly liquefied by all isolates invariably. Hydrogen sulfide was produced by all isolates (Table 6).

The bacteria produced acid with out gas on utilization of carbohydrate sources from arabinose, fructose, galactose, glucose, mannose and sucrose but acid is not produced from glycerol, lactose, maltose and manitol within one-month time (Table 7). The result obtained from the current study thus confirm the previous findings that both hypersensitivity and pathogenicity tests on tobacco and enset plant simultaneously can be used to rapidly identify pathogenic isolates of *Xcm* (Eshetu, 1981; Dereje, 1985; Archaido, 1992).

Table 6 Results of morphological and biochemical characteristics of *Xcm* isolates.

	Geographic locations					
Zone	North Omo				Gurage	
Woreda	[a] Ma	Lo	B S	Ch	Yw	Gu
	[b]1,2,3,5,7,9,10,12,14,15,17,18,19,20,24	25,27,28,30,31,32,33,35,36,37,38,39,40,41,42,43,44,45	46,47,48,50	52,53,54,56,57,60,61,62,63,64,65	68,69,70,71,72,73,74,75,76,77,80	82,83,84,86,88,89
TESTS						
Gram reaction	–	–	–	–	–	–
Motility	+	+	+	+	+	+
Yellow colony on YPSA	+	+	+	+	+	+
Mucoid growth	+	+	+	+	+	+
Citarate utilization	–	–	–	–	–	–
Gelatin liquefaction	+	+	+	+	+	+
Nitrate reduction	–	–	–	–	–	–
Starch hydrolysis	–	–	–	–	–	–
Casein hydrolysis	+	+	+	+	+	+
Malonate utilization	+	+	+	+	+	+
Hydrogen sulfide production	+	+	+	+	+	+
Indole production	–	–	–	–	–	–

– = Negative reaction, + = Positive reaction

[a] = Weredas under each zone:Ch, Cheha; YW, Yesana Wolene; Gu Gumer, Ma, Mareka ; Lo, Loma; BS, Boloso Sore

[b] = *Xcm* isolates designated by number under respective zone and wereda.

Even though these isolates were collected from different enset growing regions covering relatively wider range of altitudes and varied enset cropping systems, most of them tended to show similar results on morphological and biochemical properties regardless of geographic localities. The result obtained in this test suggests that population of *Xcm* pathogens could be characterized by similar morphological and biochemical properties.

The findings of this study are in agreement with the previous study related to confirmatory work in identifying the enset wilt pathogen by Dagnachew and Bradbury (1968) and by Eshetu (1981). However, Eshetu (1981), observed differences in biochemical characteristics for some isolates in the utilization of lactose, gelatin liquefaction, starch hydrolysis and lipase activity. There was, however, no evidence to prove that these bacterial isolates collected from test enset plants belong to another species. As regard to these tests, all our isolates invariably utilized lactose, liquefied gelatin and decomposed starch (Table 6 and 7).

Under this study we have considered relatively small numbers of bacterial isolates that might have resulted in our failure to detect variability on the morphological and biochemical properties with in these set of test isolates. However, the result obtained from this study suggests that the population of *Xcm* in most enset growing regions of the country in general and sampled areas in particular might be dominated by those isolates capable of hydrolyzing casein, gelatin, malonate and utilizing hydrogen sulfide but incapable of producing acid from lactose, maltose and manitol.

Table 7 Mode of utilization of carbohydrate by *Xanthomonas campestris pv musacearum* isolated from naturally wilting enset plant.

	Geographic locations					
Zone	North Omo			Gurage		
Wereda	[a] Ma	Lo	B S	Ch	Yw	Gu
Isolates	[b]1,2,3,5,7,9,10, 12,14,15,17,18 ,19,20,24	25,27,28,30,31,3 2,33,35,36,37,38 ,39,40,41,42,43, 44,45	46,47, 48,50	52,53,54,56, 57,60,61,62, 63,64,65	68,69,70,71, 72,73,74,75, 76,77,80	82,83,84, 86,88,89
Carbohydrate Source						
Arabinose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Galactose	+	+	+	+	+	+
Glucose	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+
Mannose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Lactose	_	_	_	_	_	_
Maltose	_	_	_	_	_	_
Manitol	_	_	_	_	_	_

- = Negative reaction, + = Positive reaction

[a] = Weredas under each zone:Ch, Cheha; YW, Yesana Wolene; Gu Gumer, Ma, Mareka ; Lo, Loma; BS, Boloso Sore

[b] = *Xcm* isolates designated by number under respective zone and wereda.

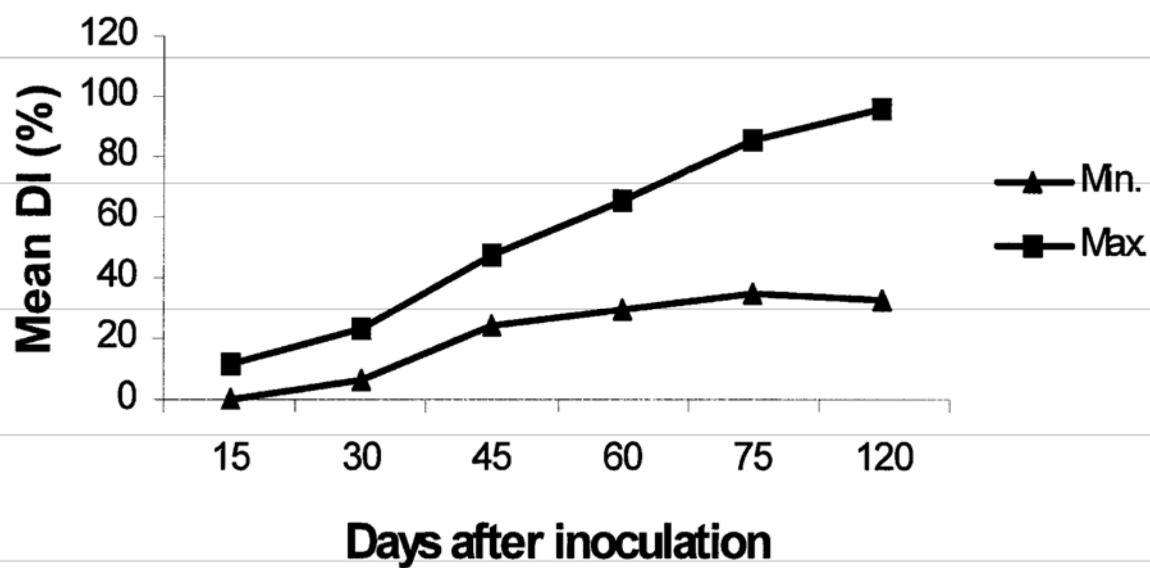
4.2. Enset Clone Evaluation

Eighty-one enset clones originally collected from Mareka and Loma Werdas, North Omo Zone and maintained at ArARC were evaluated for their resistance to enset bacterial wilt pathogen using artificial inoculation under field condition (Table 2).

Five months old enset plants at seven to ten leaf stages were inoculated. Disease evaluation was started seven days after inoculation. The first symptoms of disease on infected clones were yellowish of central leaf at the apex. Average disease incidence as measured by percent infected and /or dead enset plants, showed varied difference among test clones at different disease assessment periods after inoculation. The period of time between inoculation and initial wilt symptom development (incubation period) was variable, ranging from 10 to 21 days.

A difference in the progression of the disease also was apparent (Figure 1). Dead or infected plants were not observed in any of the control plants through out the evaluation periods. In all disease assessment periods, the range of disease incidence, except for Mezya, were variable ranging from 0 to 100% (Figure 2). The highest disease infection (100%) was recorded for some clones at 45 days after inoculation. Mezya was the only immune clone through out the evaluations period. However, Mezya, Yesha Mezya, Yesha, Anko Mezya, Hala Mezya and Hoendya did not show disease symptom until 21 day after inoculation (Table 8).

Figure 1 Mean maximum and minimum infected and /or dead plants at seven assessment periods



Disease incidence rapidly progressed 30 days after inoculation (Figure 2). At the 120-day evaluation, except Mezya, all test clones in variably showed wilt symptom. However, four clones namely Hala Mezya, Yesha Mezya, Anko Mezya, and Bota Mezya, with disease incidence 16.67%, 25%, 33.33% and 33.33%, respectively, showed better tolerance next to Mezya; eleven clones showed infection ranging from 61% to 76.66%. The remaining sixty-five enset clones showed the highest disease infection and considered to be highly susceptible (Table 8).

Figure 2 Mean proportion of enset clone with infected and/or dead plants at five disease incidence categories and different disease assessment periods.

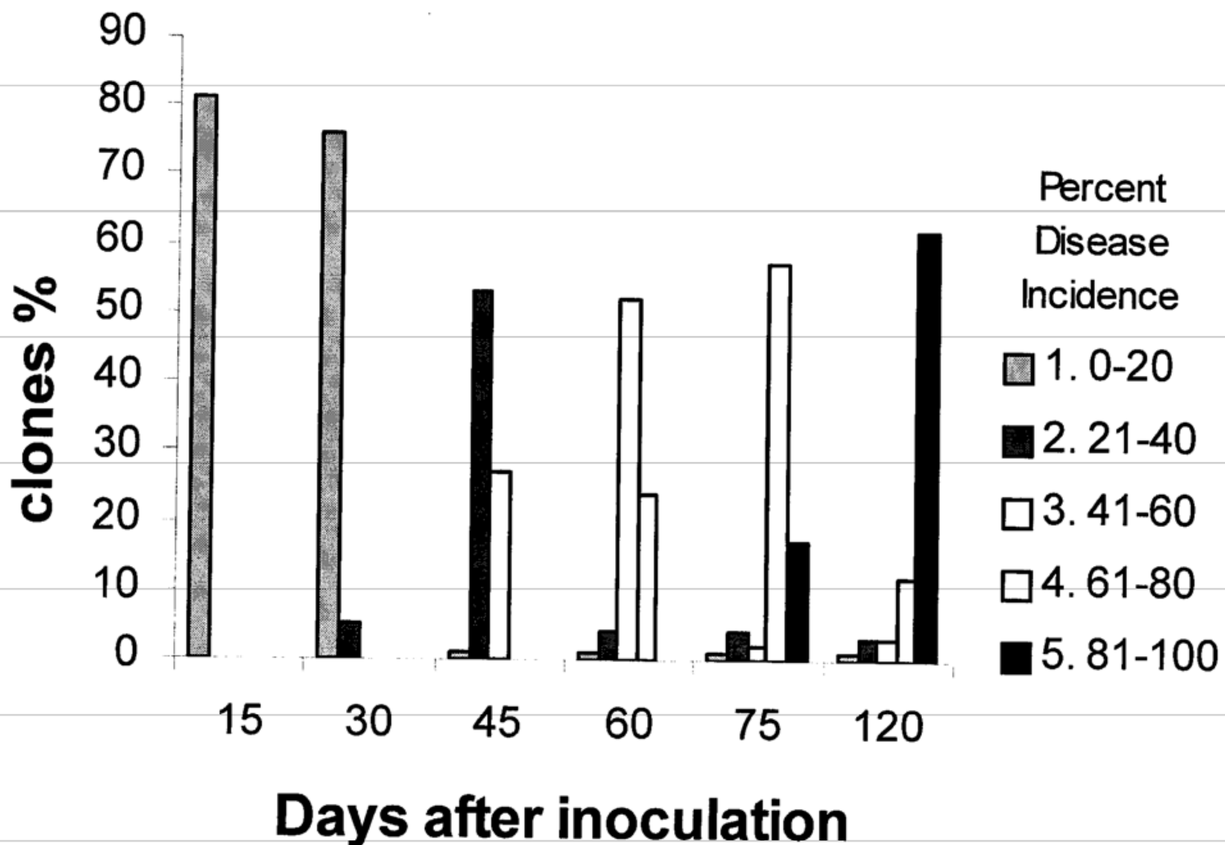


Table 8 Reactions of 81 enset clones to one pathogenic Xcm isolate at different disease assessment periods

No[a] Clone name	[b] Mean Disease Incidence (DI)							
	[c]7	15	21	30	45	60	75	120
1.ANKO GENA	0	16.67	50.00	66.67	83.33	100	91.67	91.67
2.Fenchereyepa	0	16.67	50.00	83.33	83.33	83.33	100	100
3Onsa	0	16.67	50.00	83.33	91.67	100	100	100
4.Kosya	0	16.67	58.33	83.33	83.33	83.33	91.67	100
5.Zergesa	0	20.00	50.00	83.33	83.33	91.67	100	100
6.Chichya	0	16.67	50.00	83.33	100	100	100	100
7.Rentya	0	16.67	33.33	66.67	83.33	91.67	91.67	100
8.Kekerya	0	16.67	33.33	50.00	83.33	83.33	91.67	91.67
9.Bosena	0	16.67	50.00	83.33	91.67	100	100	100
10.Guluma	0	16.67	50.00	83.33	91.67	100	100	100
11.Sutya	0	16.67	66.67	83.33	91.67	100	100	100
12. Banga arkya	0	20.00	50.00	83.33	83.33	91.67	91.67	91.67
13.Digo merza	0	16.67	58.33	83.33	83.33	100	100	100
14.Gimira arkya	0	16.67	66.67	83.33	83.33	100	100	100
15.Shebera	0	16.67	66.67	75.00	83.33	83.33	100	91.67
16.Mezya	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17.Tuzuma	0	33.33	50.00	83.33	91.67	100	100	91.67
18.Shasha	0	16.67	58.33	83.33	83.33	83.33	100	100
19.Erpa-6	0	0.00	8.33	8.33	16.67	16.67	16.67	16.67
20.Goshendya	0	20.00	50.00	83.33	91.67	100	100	91.67
21.Buba	0	20.00	66.67	83.33	83.33	83.33	91.67	100
22.Bergudya	0	16.67	50.00	83.33	83.33	83.33	100	100
23.Alewa	0	33.33	66.67	83.33	91.67	100	100	91.67
24.Yilga	0	16.67	58.83	83.33	83.33	91.67	100	100
25.Ado	0	20.00	66.67	83.33	66.67	91.67	100	100
26.Bedadya	0	16.67	33.33	41.67	58.33	66.67	83.33	91.67
27.Atuma boza	0	16.67	25.00	33.33	50.00	50.00	66.67	75
28.Atuma boza	0	16.67	33.33	50.00	66.67	50.00	83.33	91.67
29.Donkola	0	33.33	50.00	83.33	91.67	100	100	100
30.Tena	0	16.67	50.00	83.33	83.33	91.67	100	100
31.Gessa	0	16.67	33.33	83.33	83.33	83.33	100	100
32.Argema	0	33.24	66.67	83.33	91.67	100	100	100
33.Sirrara	0	20.00	66.67	83.33	83.33	100	100	100
34.Arkya	0	16.67	66.67	83.33	91.67	100	100	91.67
35.Mecha boza	0	16.67	58.33	83.33	83.33	83.33	100	100
36.Budunswa	0	16.67	66.67	83.33	83.33	83.33	100	91.67
37.Astara	0	33.33	50.00	83.33	83.33	100	100	100
38.Ayna	0	33.33	58.33	83.33	91.67	100	100	100
39.Mashasha	0	16.67	66.67	83.33	83.33	83.33	100	91.67
40.Banga	0	16.67	58.33	83.33	83.33	100	100	100
41.Delulya	0	16.67	58.33	83.33	83.33	100	100	100
42.Mecha shododinya	0	33.33	50.00	83.33	91.67	100	100	91.67
43.Bota mezya	0	16.67	66.67	83.33	83.33	83.33	91.67	91.67
44.Berjya	0	16.67	50.00	66.67	83.33	83.33	83.33	100.00
45.ZERA MEZYA	0	16.67	25.00	50.00	50.00	50.00	66.67	91.67
46.Erpa-20	0	16.67	50.00	83.33	83.33	100	100	100

Table 5. continued

47. Utula	0	16.67	50.00	83.33	83.33	83.3	91.67	100
48. Dirbo	0	33.33	66.67	83.33	91.67	100	100	100
49. Sanka	0	33.33	50.00	75.00	83.33	100	100	100
50. Dorta	0	33.33	66.67	83.33	100	100	100	100
51. Chemeretya	0	33.33	58.33	83.33	100	83.33	100	100
52. Bulla	0	20.00	66.67	83.33	91.67	100	100	91.67
53. Agunsa	0	16.67	33.33	66.67	66.67	66.67	83.33	100
54. Shutatya	0	33.33	66.67	83.33	83.33	100	100	100
55. Erpa-13	0	16.67	66.67	83.33	83.33	83.33	100	100
56. Akacha	0	16.67	50.00	66.67	66.67	66.67	91.67	91.67
57. Trey	0	16.67	33.33	66.67	66.67	66.67	100	100
58. Wonshe	0	18.00	73.33	91.67	91.67	91.67	91.67	100
59. Kekere	0	20.00	66.67	83.33	100	100	100	100
60. Bukunya	0	33.33.	66.67	83.33	100	100	100	100
61. Gashashya	0	33.33.	50.00	83.33	91.67	100	100	100
62. Delya	0	33.33	66.67	83.33	100	100	100	100
63. Yesha mezya	0	0.00	8.33	8.33	25	16.67	33.3	25.00
64. Erpa-3	0	20.00	66.67	83.33	100	100	100	100
65. Yesha	0	0.00	8.33	8.33	33.33	33.33	33.33	58.33
66. Bombe	0	16.67	66.67	83.33	100	100	100	100
67. Tsela	0	16.67	66.67	83.33	100	100	100	100
68. Shelekumya	0	16.67	58.33	83.33	83.33	83.33	100	100
69. Hassa edadya	0	20.00	50.00	83.33	91.67	91.67	100	100
70. Amarate	0	16.67	66.67	83.33	83.33	83.33	100	100
71. Yore	0	33.33	66.67	83.33	91.67	100	100	100
72. Anko mezya	0	0.00	16.67	16.67	25.00	33.33	33.33	33.33
73. Erpa-8	0	16.67	50.00	83.33	91.67	100	100	100
74. Koche arkya	0	33.33	66.67	83.33	100	100	100	100
75. Bota bukunya	0	16.67	16.67	50.00	66.67	66.67	83.33	91.67
76. Hala mezya	0	0.00	16.67	8.33	33.33	33.33	33.33	33.33
77. Shengya	0	16.67	41.67	83.33	66.67	66.67	91.67	91.67
78. Hoendya	0	0.00	25.00	33.33	50.00	50.00	50.00	75.00
79. Nekaka	0	16.67	41.67	58.33	83.33	100	100	100
80. Gena	0	16.67	41.67	66.67	83.33	83.33	83.33	100
81. Shododina	0	20.00	33.33	58.85	100.	100	100	100
cv (%)		14.17	13.66	13.59	12.94	7.71	8.41	11.43
Lsd (5%)		7.11	13.71		17.05	11.19	13.25	18.48

[a] = Vernacular name of enset clones given by enset farmers of Mareka and Loma wereda of North Omo Zone

[b]= Mean Disease Incidence (%) measured as percentage of plant infected and /or dead.

[c] = Days at which disease assessment was undertaken after artificial inoculation.

Variability within enset clones in reaction to wilt pathogen was very clear and easy to detect. Except clone Mezya, all test clones developed disease symptom variably. In the current study, Mezya proved to be the only resistant clone, considering only one *Xcm* pathogenic isolate. However, the response of this clone to large number of *Xcm* isolates has to be confirmed under natural disease epidemics in different hot spot areas and artificial inoculation in the field and greenhouse conditions. Moreover, the relatively tolerant clones such as Yesha Mezya, Anko Mezya, Anko Mezya, and Bota Mezya, have to be evaluated further under field condition. Clones with the highest disease incidence and lowest incubation periods could be used as a susceptible check during clonal evaluation study (Figure 3).

In our study, the reaction of 81 enset clones to enset wilt pathogen under field inoculation condition was observed. Significant differences in the field inoculation were recorded between these clones (Table 9).

Table 9 Average percentage of infected and/ or dead plants of 81 clones at 120 days after inoculation

Enset Clone	Mean DI(%)
1. Anko gena	91.67bc
2. Fenchereyepa	100a
3. Onsa	100a
4. Kosya	100a
5. Zergessa	100a
6. Chichya	100a
7. Rentya	100a
8. Kekerya	91.67bc
9. Bosena	100a
10. Guluma	100a
11. Sutya	100a
12. Banga arkya	91.67bc
13. Digo merza	100a
14. Gimira arkya	100a
15. Shebera	**91.67BC**
16. Mezya	0.00e
17. Tuzuma	91.67ab
18. Shasha	100a
19. Erpa-6	91.67ab
20. Goshendya	100a
21. Buba	100a
22. Bergudya	91.67ab
23. Alewa	100a
24. Yilga	100a
25. Ado	91.67ab
26. Bedadya	75.00bc
27. Atuma boza	91.67a
28. Erpa-7	100a
29. Donkola	100a
30. Tena	100a
31. Gessa	100a
32. Argema	100a
33. Sirrara	91.67a
34. Arkya	100a
35. Mecha Boza	91.67a
36. Budunswa	100a
37. Astara	100a
38. Ayna	91.67a
39. Mashasha	100a
40. Bangya	100a
41. Delulya	91.67a
42. Mecha Shodenya	91.67a
43. Bota mezya	25.00d
44. Berjya	100a
45. Zera mezya	91.67a
46. Erpa-20	100a

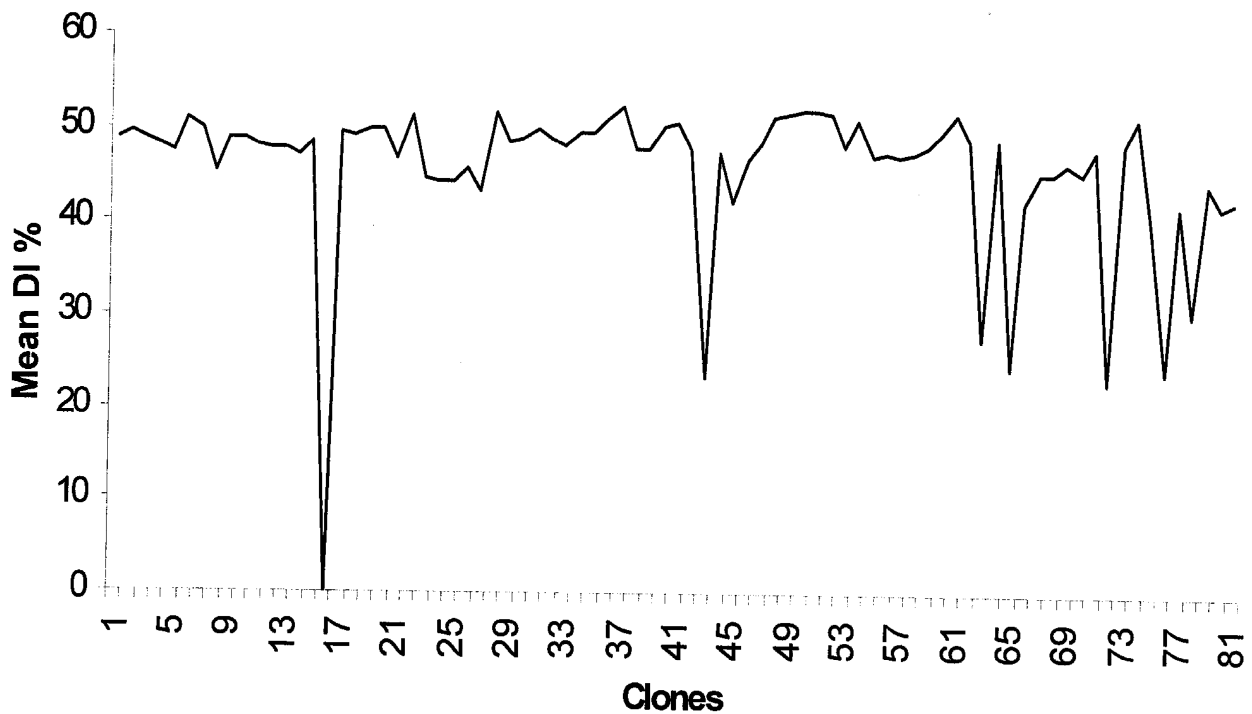
Table 9. continued

Enset clone	Mean DI
47. Utula	100a
48. Dirbo	100a
49. Sanka	100a
50. Dorta	100a
51. Chemeretya	100a
52. Bulla	91.67a
53. Agunsa	100a
54. Shutatya	100a
55. Erpa-13	100a
56. Akacha	91.67a
57. Trey	100a
58. Wonshe	100a
59. Kekere	100a
60. Bukunya	100a
61. Gashashya	100a
62. Delya	100a
63. Yesha mezya	25.00d
64. Erpa-3	100a
65. Yesha	58.33c
66. Bombe	100a
67. Tsela	100a
68. Shelekumya	100a
69. Hassa bedadya	100a
70. Amarate	100a
71. Yore	100a
72. Anko mezya	33.33d
73. Erpa-8	100a
74. Koche arkya	100a
75. Bota bukunya	91.67a
76. Hala mezya	33.33d
77. Shengya	91.67a
78. Hoendya	75.00bc
79. Nekaka	100a
80. Gena	100a
81. Shododina	100a
CV(%)	11.43
LSD(5%)	18.48

Means followed by the same letter within a column are not statistically different at 5% level.

The initial progression of the disease was more pronounced starting from 30-day evaluation (Figure 2). We observed that some of the clones were most severely affected within shorter period of time than others (Figure 1). The high-level of disease infection and early symptom development as exhibited by some of the test clones may be compounded by susceptibility to the pathogen (Figure 3). It was observed that highly susceptible, relatively tolerant, and resistant clones gave uniform reactions through out the evaluation period indicating the efficiency of inoculation procedure and uniformity of inoculum challenge.

Figure 3 Average percentage of infected and/or dead plants of each clone over seven disease assessment periods.



The result of the present study, for the most part, was in agreement with results obtained from previous enset clone screening experiment involving 60 clones (Dereje, 1985). In the latter, none of the 60 clones were resistant to the pathogen. However, some clones were observed to have relatively better tolerance reaction than others. Similarly, we have also observed that Hala Mezya, Bota Mezya, Yesha Mezya and Anko Mezya to have better tolerance reaction to this disease. Archaido and Mesfin (1993) reported that enset clone, Genticha revived 12 to 16 weeks after infection.

Generally, the results obtained from previous related study on enset and banana supports the current results observed in 81 enset clones. Moreover, the presence of one resistant enset clone, Mezya, seems to be promising as far as enset clonal evaluation against enset wilt is concerned.

4.3. Variability of *Xcm* Isolates

Pathogenic variation of *Xcm* isolates was tested on twelve enset clones obtained from ArARC (Table 8). Four pathogenic *Xcm* isolates collected from naturally wilting enset plants of Gurage, Sidama, North Omo, and Kembata Zones were inoculated to these twelve enset clones in the greenhouse (Table 9).

Analysis of variance on disease incidence on the twelve enset clones revealed no significant interaction ($p<0.005$) between four *Xcm* isolates and twelve enset clones (Table 10). The main factor effects of enset clones and *Xcm* isolates were also non significant ($p <0.001$).

4.3.1 Comparison of *Xcm* Isolates and Enset Clones

Four of the test *Xcm* isolates (NO23, S13, G23, and K23) used in the variability study induced compatible reaction to twelve enset clones (Table 10). Average disease incidence as measured by percent plant infected ranged from 87.44% by isolate S13 on clone Ayna to 100% by four isolates on most of the enset clones. There was no significant difference within four *Xcm* isolates and within twelve enset clones.

There was, however, significant difference between isolate-clones interactions (Table 10). Significant differences were observed between isolate S13 with clone Ayna (S13- Ayna) and all other isolate–clone interactions. Isolate K23 - Erpa-7, NO23 -Dirbo and Erpa-7, K23 and NO23-Astara significantly differ from all other isolate-clone interactions but they are statistically similar with interactions Agena -NO23, Sutya with S13, Nekaka with K23, Bosena-S13, Delulya - S13, Bosena-NO23, Banga – K23, Bosena –K23, Agena -S13. Among all the interactions, Ayena - S13 was found to have the lowest percent of disease incidence (87.44%) followed by Erpa-7 -K13, Dirbo - NO23, Astara - K13 and NO23, but they are not statistically different among themselves. On the other hand, 32 of the interaction treatments showed 100% disease incidence and Agena - S13, Bosena - K13, Banga – K23, Bosena - NO23, Chichya Bosena and Delulya, - S13, Nekaka and Sutya with K23 and Agena with NO23 showed 95.78% disease incidence.

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Table 10 Analysis of variance on percent infected and/ or dead plant + after inoculating twelve enset clones with four Xcm isolates under greenhouse condition.

Source	Degrees of freedom	Mean square
Replication	2	35.25
Clone(A)	11	23.21
Error	22	18.75
Isolate(B)	3	66.73
A5B	33	20.05*
Error	72	
Total	143	

CV (Clone): 4.41%

CV (Isolate): 4.57%

$^+$ Average of 3 replication, in each plot five plants were tested for disease reaction

* Significant at 5% level of significance

Isolates G23 was able to cause 100% infected by all enset clones. In contrast, clone Yilga was found to be 100% disease incidence by all isolates (Table 11). All the control enset plants used to monitor the entire procedure were not infected.

Table 11 Reaction of twelve enset clones to four Xcm isolates in pathogenicity variation study under greenhouse inoculation condition.

	Percent plant infected (100%)[a]												
Clones	Ag[b]	As	Ay	Ba	Bo	Ch	De	Di	Er	Ne	Su	Yi	Av.
Isolate[c]													
NO23	95.78ab	91.55bc	100a	100a	95.78ab	100a	100a	91.44bc	91.44bc	100a	100a	100a	97.17
S13	95.78ab	100a	87.44c	100a	95.78ab	95.78ab	95.78ab	100a	100a	100a	95.78ab	100a	97.20
G23	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100a	100
K23	100a	91.55bc	100a	95.78ab	95.78ab	100a	100a	100a	91.44bc	95.78ab	100a	100a	97.53
Av.	97.89	95.76	96.86	98.95	96.84	98.95	98.95	97.86	95.72	98.95	98.95	100	

[a] = Mean disease incidence value (%)

[b] = Names of enset clones listed in table 8.

[c] = *Xcm* isolates listed in table 9.

Means followed by the same letter within a column are not statistically different at 5% level

In spite of the fact that we used a limited number of *Xcm* isolates and enset clones, the result obtained clarifies the variation in virulence in some of the four *Xcm* isolates to some of the twelve enset clones used in this study. Variations in isolate–clone interaction are suggestive of the need to evaluate the response of several number of *Xcm* isolates before one can draw meaningful inferences about the characters of enset clones and genotypes of *Xcm* isolates.

Therefore, there seems a need for further work by including more *Xcm* isolates and enset clones with known genetic background of resistance to wilt pathogen so that clear understanding of the variability situation in *Xcm* population might be determined in the major enset growing regions of the country.

In the current study, none of the twelve clones were immune to four *Xcm* isolates. All clones were infected to varying degree by all isolates. The method employed in this study revealed the presence of similarity within *Xcm* isolates regarding ability to infect enset clones tested. None significant clone-isolate interaction gave an evidence for the susceptibility of twelve clones irrespective of four *Xcm* isolates tested. Even though study with regard to specificity of *Xcm* isolate to infect enset clones is not worked out previously, our finding confirms that the non-different reaction of four *Xcm* isolates on twelve enset clones. In this study, we have used a limited number of *Xcm* isolates and enset clones.

5. SUMMARY AND CONCLUSION

Among the ninty bacterial ooze samples collected from naturally wilting enset plants in six enset growing weredas of Gurage and North Omo Zones, sixty-five of them were identified as *Xcm* regardless of their geographic origin.

These isolates produced a positive hypersensitive reaction on two months-old tobacco plant *(Nicotiana tabacum var. white burley)* within 24 to 48 hours after inoculation to the intracellular space of leaves between two veins using a 5 ml hypodermic syringe with needle. In addition, these isolates induced pathogenic reaction on five months-old susceptible enset clone Mandaluka within 15 to 21 days after inoculation to the base of the central leaf petiole using a 5 ml hypodermic syringe with needle.

All of these pathogenic isolates tended to show similar reactions to all of the in-vitro tests carried out. All isolates showed positive reaction in the utilization of malonate, production of hydrogen sulfide and hydrolysis of protein like casein. The same isolates showed negative results in the utilization of citrate, reduction of nitrate, hydrolysis of starch and production of indole. Besides, all isolates showed similar mode of utilization of most carbohydrate sources including arabinose, fructose, galactose, glucose, glycerol, mannose and sucrose. Acid with out gas was produced by all isolates from these sugars but not from lactose, maltose and manitol.

Eighty-one enset clones of Waka collection obtained from ArARC tended to show varied level of reaction to a pathogenic *Xcm* isolate under field inoculation conditions. Except one clone, Mezya, none of the other clones were immune to this pathogen within 120 days after inoculation.

Significant differences in disease incidence were observed between clones. Mezya, being resistant clone, was significantly different from all clones. Significant difference was also observed between Yesha Mezya, Hala Mezya, Anko Mezya, and Bota Mezya and the remaining infected clones ($P<0.005$). Among the infected clones, Yesha Mezya, Hala Mezya, Anko Mezya, and Bota Mezya showed relatively better tolerance than the others. The remaining seventy-five clones with highest disease incidence were considered to be highly susceptible.

In order to determine the variation in pathogenicity of *Xcm* isolates to enset clones, four pathogenic *Xcm* isolates were selected from bacterial samples. These samples were collected from enset growing zones of Gurage, Kembata, Sidama and North Omo. All isolates induced disease symptom typical of enset wilt to twelve clones between 10 and 21 days after inoculation under greenhouse condition. None of the clones reacted differentially to any of the four isolates used.

Analysis of variance of disease incidence on the twelve enset clones revealed significant interactions ($p<0.005$) between isolates and clones. Disease incidence varied from 87.44% on clone Ayna by isolate S13 to 100% on 32 of the other isolate-clone combinations. Among all the four isolates, isolate G23 was able to cause the highest (100%) disease incidence to all the twelve enset clones. On the other hand, one clone, Yilga was found to be 100% infected by all the four

isolates. Isolate NO23 caused infection ranging from 91.44 to 95.78% on Agna, Astara, Bosens, Dirbo, and Erpa-7, isolate S13 on Agena, Bosena, Chichya, Delulya and Sutya; and isolate K23 on Astara, Banga, Bosena, Erpa-7 and Nekaka.

Even though the bacterial isolates were collected from different ecological zones, populations of pathogenic *Xcm* were similar in many of their morphological, biochemical and physiological characteristics. The expression of such similar properties may suggest low rates of mutation of the genes controlling such properties. However, this conclusion may not hold true for all isolates and needs a careful consideration; because, in the current study, we considered relatively limited number of isolates and tests. More studies are required in the future by including many isolates from different enset growing regions of the country and many characteristics of the pathogen.

Enset clones with varied level of reactions to wilt pathogen were noted among the commonly cultivated enset of Mareka and Loma weredas of North Omo Zone. The presence of one resistant enset clone, Mezya, seems promising as compared with the previous results obtained from clonal screening work. However, the occurrence of relatively better tolerant enset clones observed in this study is in agreement with the previous findings obtained from clonal screening involving different set of enset clone (Dereje, 1985).

Similar study made on banana and plantain in identifying resistance varieties to banana wilt pathogen *Pseudomonas solanacearum* revealed that nearly all varieties of banana and plantain to be susceptible, however, some of them showed moderate degree of inherent field resistant (Wardlaw, 1972). Moreover, Stover (1972) in his comparative study involving 345 accessions,

observed Papila to be highly resistant and 34 of them to be moderately resistant under artificial inoculation condition.

Differential pathogenicity caused by different isolates of *Pseudomonas solanacearum* resulted in slight and considerable disease intensity in *Musa* spp. In addition to this, different bacterial isolates showed variability in rapidity of infection development and death of host (incubation period) after inoculation in which some cause during 7 to 10 days, whereas others may require 30 or more days.

In view of the previous work and present study results, the use of resistant enset clone would be the most desirable control option. Hence, enset clones that showed resistant and/ or tolerant reaction to wilt pathogen should be further evaluated against a large number of *Xcm* isolates under field and greenhouse conditions. A more concerted effort in the collection and evaluation of more additional clones against wilt pathogen seems to be a critical task. In addition to this, some works related with survival, host range, transmission, virulence and some characteristics of the pathogen needs to be thoroughly investigated.

The current work alone can not be conclusive, it is believed that the results obtained will facilitate future works for the satisfactory control of the bacterial disease of enset in the country.

6. REFERENCES

Anita, S., Clifton, H., Endale, T., and Gizachew, W.M. 1996. Enset needs assessment project phase 1 report. Awassa, Ethiopia

Archido, J. Q. 1991. First Quarter Report of the Plant Pathologist: July 1, to September 30, 1991. Enset Team Support Project. Sidamo Gamo Goffa Peasants Agricultural Development Program- PADEP-III. Awassa Research Center (IAR), Awassa, Ethiopia.

Archido, J. Q. 1992. Annual Report of the Plant Pathologist: July 17, 1991 to July 16, 1992. Enset Team Support Project Sidamo Gamo Goffa Peasants Agricultural Development Program- PADEP III. Awassa Research Center (IAR). Awassa, Ethiopia.

Archido, J. Q., and Mesfin, T. 1993. Diseases of Enset. *In.* Proceedings of the First International Workshop on Enset pp . (Tsedeke Abate, Clifton Hiebsch and Steve Brandt eds.). Dec. 13-21. IAR, Addis Ababa, Ethiopia.

Bezuayehu, H., Mulugeta, D., and Endale, T. 1993. Agronomy Research on Enset. *In.* Proc. of the First International Workshop on Enset. pp. 235-241. (Tsedeke Abate, Clifton Hiebsch and Steve Brandt eds.). Dec 13-21. IAR, Addis Ababa, Ethiopia.

Blanahard, R. D. and T. A. Talter. 1981. Field and laboratory guide to tree pathology. Academic press, New York.

Buddenhagen, I.W. 1961. Bacterial wilt of banana: History and known distribution. Trop. Agric. (Trinidad) 38:107-125.

Central Statistical Authority (CSA). 1994. Annual report of 1991/1992. Addis Ababa, Ethiopia.

Dagnachew, Y. and Bradbury, J.F. 1968. Bacterial wilt of enset (*Enset ventricosum* L.) incited by

Xanthomonas musacearum sp.n. Phytopathology 59: 111-112.

Dagnachew, Y., and J. F. Bradbury. 1974. A note on wilt of banana caused by enset wilt organism. *Xanthomonas musacearum.* East African Agricultural and Forestry Journal 40: 111-114.

Dereje Ashagari. 1985. Studies on the bacterial wilt of Enset (*Ensete ventricosum)* and prospects for its control. Ethiop. J. Agric. Sci. 7(1): 1-14.

Desta , Beyene. 1982. Diagnosis of Phosphorus Deficiency in Ethiopia. Soil Science Bulletin No. 3, p 18. IAR, Addis Ababa, Ethiopia.

Dye, D. W., J. F. Bradbury, M. Goto, A. C. Hayward, R. A. Lelliot, and M. N. Schroth. 1980. International standards for naming pathovars of phytopathgenic bacteria: a list of pathovars names and pathotype strains. Review of Plant Pathology. 59: 153-168.

Endale, T., and Mulugeta, D. 1994. Improvement studies on enset and sweet potato pp. 63-64. In Proceedings of the second national horticultural workshop of Ethiopia (Edward Herath and Lemma Desalegn eds.) 1-3 Dec. 1992. IAR, Addis Ababa, Ethiopia.

Eshetu, W. 1981. The Role of *Poecilocarda nigrinervis, Pentanolia nigronervosa* and *Planococcus ficus* in the transmission of enset wilt pathogen *Xanthomonas musacearum* sp. n. in Wolyita, Ethiopia. M.Sc. Thesis, Addis Ababa university.

Gomez, K. A., and A. A. Gomez. 1984. Statistical procedure for agricultural research. 2 nd ed. John Wily and Sons, New York.

Gupta, A. K., Sharma, S. C., and Saini, R. G. 1986. Variation in pathogenicity of some Indian isolates of *Xanthomonas campestris Pv. oryzae*. Phytopathology 76: 881-883.

Huffangel, H. P. 1961. Agriculture in Ethiopia. FAO. Rome.

Kefale, A., and S, Sandford. 1991. Enset in north Omo region. Farmer's research technical

pamphlet, No. 1. Addis Ababa, Ethiopia.

Kelmant, A. 1963. Rapid detection of the pathogenicity of phytopathogenic *Pseudomonas*. Nature 199: 299-300.

Mesfin, T. 1989. Enset (*Ensete ventricosum*) wilt syndrome: monopathogenic origin or complex? P. 17. *In*. Proc. Ethiop. Phytpath. Comm. 14 th Ann. Meet., 30-31 March, 1989. Ambo, Ethiopia.

Mesfin, T. 1990. Status of Enset Diseases in Ethiopia. *In*. Proc. EPC 15 th Ann. Meet., 13-14 March, 1990. Seid Ahmed and Yaynu Hiskias (eds.). EPC, Addis Ababa, Ethiopia.

Moore, L. W. C. I. Cado and Bouzar, H. 1988. Agro bacterium. p. 16-36. *In.* N. W. Schaad (ed.). Laboratory guide for identification of plant pathogenic bacteria. 2 nd edition. APS Press St. Paul, Minnesota.

Noda, T., Horino, O., and Ohuchi, A. 1990. Variability of pathogenicity races of *Xanthomonas campestris Pv. oryzae* in Japan. Jpn. Agric. Res. Q. 23(3): 182-189.

Robert, E. B., and Estelle, D. B. 1956. Bacteriology 4 th ed. Mackmillan Comp., London pp. 55 - 75.

Russell, G. E. 1978. Plant breeding for pest and disease resistance. Butterwrths, London.

Schaad, N. W. and R.E., Stall. 1988. *Xanthomonas*. Pp. 81- 84. *In*. N. W. Schaad (ed.). Laboratory guide for identification of plant pathogenic bacteria 2 nd ed. APS Press t. Paul, Minnesota.

Seqeira, L. 1958. Bacterial wilt of banana: Dissemination of the pathogen and control of disease. Phytopathology. 48: 64-69.

Sijam, K., C. J. Chang., and R. D. Gitaitis. 1992. A medium for differentiating tomato and pepper strains of *Xanthomonas campestris Pv. vesicatoria*. Can. J. Plant Pathol. 14: 182 -

184.

Singh, R. S. 1984. Introduction to principles of plant pathology. 3 rd ed. Oxford and IBH Publishing Co., New York.

Smeds, H. 1955. The planting culture of Eastern Sidama, Ethiopia, Acta Geographica,Helsinki (14).

Stall, R. E., D. C. Loschke and J. B. Jones. 1986. Linkages of copper resistance and avirulence loci on a self-transmissible plastid in *Xanthomonas campestris Pv. vesicatoria*. Phytopathology. 76: 240 - 243.

Stover, R, H. 1972. Banana, Plantain and Abaca Diseases. CMI, Kew, Surrey, England.

Susan, D. F. 1972. Pest control in bananas Manual No.1. London p. 37-38.

Taye, B. 1980. Morphology of *Ensete* embryo in vitro culture. Ethiop. J. Agric Sc. 2 : 23-28.

Taye, B., and Asrat. F. 1966. The production and utilization of the genus *Ensete* in Ethiopia. Econ. Bot. 20 (1): 65-70.

Wardlaw, C. W. 1972. Banana diseases including Plantain and Abaca (2nd ed.), Longman Group Ltd. London.

Westphal, E. 1975. Agricultural Systems in Ethiopia. Wageningen Agricultural University, The Netherlands: Center for Agricultural Publication and Documentation.

Young, J. M., D. W. Dye, J. F. Bradbury, C. G. Panagopoulos, and C. F. Robbs. 1978. A proposed nomenclature and classification for plant pathogenic bacteria. New Zealand Journal of Agricultural Research. 21: 153-177.

Zehr, E. I. 1970. Cultural, physiological and biochemical properties of isolates of Philippines *Pseudomonas solanacearum*. 6 (1): 29-43.

APPENDIX 1 PROCEDURES FOLLOWED, MEDIA AND REAGENTS USED TO UNDERTAKE CULTURAL, MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF *XCM* ISOLATES.

1. Gram reaction

As described by Schaad and Stall (1988), on a clean slide, thinly spread bacterial film was dried in air with out heat. Then the underside of slide was lightly flamed to fix the bacteria to the slide. The smear was flooded with iodine solution for 1 minute.

Solutions

A. Hucker's ammonium oxalate crystal violate

Solution A

Crystal violet (Difcco, certified) 2.0 g

Ethyl alcohol (95%) 20.0 ml

Solution B

Ammonium oxalate (Fisher) 0.8 g

Distilled water 80.0 ml

These solutions were mixed and filtered through paper into storage bottle.

B. Gram's modification of Lugol's solution

Iodine 1.0 g

Potassium iodide 2.0 g

Distilled water 300.0 ml

The iodine solution was allowed to dissolve several hours or overnight in a dark place.

test.

3. Mucoid growth

One loopful of bacterial suspension was streaked on to YPSA media plates and incubated at 27^0c for 48 hours (Schaad and Stall, 1988). *Xcm* colonies, which was not separated easily when, rose from the colony using a wire loop was considered as a positive test.

4. Colony color on YPSA

Xcm isolates were allowed to grow on YPSA plates by streaking a loopful of *Xcm* suspension onto YPSA plates and incubated at 27^0c. The color for each colony was observed after 48 hours 48 hours. Colonies with light yellow color was considered as a positive test (Shaad and Stall, 1988).

5. Gelatin liquefaction

Forty eight hours old slant cultures of *Xcm* isolates were stab- inoculated into the tubes of gelatin medium containing (beef extract, 3 g; peptone, 5 g; gelatin, 120 g; distilled water, 1000 ml (Schaad and Stall, 1988). Uninoculated deep tube was served as a control. The inoculated and uninoculated deep tubes were incubated at 27^0 C for 3, 7, and 14 days. Tubes were examined for gelatin hydrolysis at each mentioned date after keeping tubes at 4 ^{0}C for 30 minutes. The medium that flowed readily as tubes were gently tipped denoted a positive reaction for the test and compared with uninoculated medium.

6. Casein hydrolysis

A single line streak was made from 48 hours old culture on the surface of the skim milk agar medium plate (skim milk powder, 100g; Peptone, 5g; Agar, 15g; and P^H adjusted to 7.2. The same media but uninoculated one was included as control. Inoculated and unioculated plates were incubated at 27 ^{0}C in an inverted position for 2 to 14 days. Appearance of clear zone around the colonies was considered as a positive reaction for extra cellular protease secretion (Schaad and Stall, 1988).

7. Citrate utilization

Utilization of citrate was tested in Sodium citrate agar slant medium (Ammonium dihydrogen phosphate (K H_4 PO_4), 1 g; Dipotassium Phosphate (K_2PO_4), 1.0 g; Sodium chloride (NaCl), 5g; Sodium citrate or Citric acid, 2 g; Magnesium sulfate (Mg $SO_4.7H_2O$), 0.2g; agar, 15g; bromthymol blue, 0.08g; Distilled water, 1000 ml). One loopful of 48 hours old culture was inoculated on Sodium citrate slant and incubated at uninoculated control was included. The result was observed after 48 hours incubation at 27^{0}C for 48 hours. Appearance of visible growth on the surface of the slant and change of color of the medium to blue was considered as positives test (More, 1988).

8. Malonate utilization

A forty eight hours old culture was loop inoculated into malonate broth tubes containing $(H_4)_2$ SO_4, 2g; K_2 HPO_4, 0.6g; KH2 PO_4, 0.6g; Yeast extract, 1g; Distilled water, 1000 ml; Bromthymol blue (0.2% solution) , 12.5 ml and incubated at 27^{0}C for 48 hours . Uninoculated tubes were included as

control. Appearance of blue color after 48 hours incubation was recorded as positives test.

9. Indole production

Decomposition of amino acids and other nitrogen compounds were tested in tryptone broth (tryptone, 10g; Calcium chloride, 0.03M; Sodium chloride, 5g). As described by More (1988), to this medium a loopful of 48 hours old bacterial cultures was inoculated and then incubated at 27^0C for 48 hours. After 2 and 5 days later, 0.5 ml of Kovak's reagent (dimethylamine bezaldehyde) was added to each tube and the tubes were shaken gently. The development of a cherry-red in the top layer of the tube was indicative of indole production

10. Nitrate reduction

Tubes of medium containing KNO_3, 1g; Peptone, 5g; Yeast extract, 3g; Agar, 3g; distilled water, 1000 ml were stab-inoculated with 48 hours old culture and the tubes were sealed with 3 ml of 3% sterile agar and incubated at 27^0C for two weeks. The presence of gas bubbles that were trapped beneath the agar seal was recorded as a positive test (Shaad and Stall, 1988).

11. Starch hydrolysis

A loopful of 48 hours old culture was streaked once across the surface of the starch agar medium (Starch (soluble), 20g; Peptone, 5g; Beef extract, 3g; Agar, 15g; distilled water, 1000 ml) and incubated at 27^{0}C. After 2 to 14 days, reactions were observed by flooding to the surface of medium with gram's iodine solution (Iodine, 1g; Potassium Iodide, 2g; Distilled water, 300 ml) for 30 minutes. Clear zone formed around the line of bacterial colony growth was recorded as an indicative test for starch hydrolysis.

12. Hydrogen sulfide production

A loopful of 48 hours old culture was inoculated to a nutrient broth (Peptone, 5g; Beef extract, 3g; NaCl, 5g; Distilled water, 1000 ml). A strip of filter paper impregnated with lead acetate was placed between the wall and cork of tube and inoculated at 27^{0}C for 2 days. After two days blackening of the paper strip was indicative for H_2S production test

13. Fermentation of Carbohydrates

As described by Moore (1988), to a basal medium (NH_4 H_2 PO_4,0.5g ; K_2 HPO_4, 0.5g; Mg $SO_4.7H_2O$, 0.2g; NaCl, 5g; Yeast extract, 1g;Agar,12g; Bromcresol purple, (1.5% alcohol solution, 0.7ml). The carbohydrate source used in these tests were arabinose, fructose, galactose, glucose, glycerol manose sucrose and saccharose. Tubes of each medium were stab inoculated with a loopful of 48 hours old culture and incubated at 27^{0}C and examined for acid production after 2, 4, 7, 14 and 21 days. A yellow color formation was considered as an indicative test for fermentative degradation of carbohydrates.

DECLARATION

I, the undersigned, declare that this thesis is my work that all sources of materials used for the thesis have been duly acknowledged.

Gizachew Welde Michael

Signature:

Date: