# CHARACTERIZATION OF *Xanthomonas campestris* pv. *musacearum* ISOLATES: CAUSAL AGENT OF ENSET BACTERIAL WILT DISEASE

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## Table of Contents

## LIST OF TABLES

## LIST OF FIGURES

**Page**

## LIST OF APPENDICES

**Page**

## ABSTRACT

Enset bacterial wilt disease is a major constraint in enset production system in Ethiopia. The causal agent for the disease is *Xanthomonas campestris* Pv. *musacearum*. Earlier, variation among isolates was observed in some preliminary laboratory and field experiments. However, further detailed study on variation of isolates was found necessary. Therefore, this study was conducted with the objective of detection of variation among isolates and evaluation of crude extracts from the plant *Pychnostachis abyssinica* against the isolates. Thirty-two bacterial samples were collected from 12 different enset growing weredas found in different enset growing zones of the Southern and Oromiya regions. Forty-eight different bacterial colonies were isolated and tested for their Gram-staining reaction and for their incapability of utilizing asparagine as a sole source of carbon and nitrogen. Isolates with negative gram staining reaction and those that did not grow on asparagine medium were subjected to biochemical, hypersensitivity and pathogenicity test. Following these tests, all isolates including those not able to grow on asparagine, those able to grow on asparagine and two reference strains (DSMZ 1050 and DSMZ 1350) were subjected to repetitive PCR analysis using ERIC1 and ERIC 2 primers. The different band patterns yielded from rep-PCR were analyzed using SPSS. Variations in reaction to different biochemical tests were observed among isolates. There were also isolates with similar reaction like YCMTge and LIHSsw2 from Yirgachefie and Limo and FGMTge and YCMSas from Fisseha genet and Yirgachefie, respectively. The hypersensitivity test on tobacco leaves was positive to all isolates tested. However, pathogenicity test was found negative to all, which needs further investigation on the loss of the virulence of the bacterium on the host plant. The results from discriminant function analysis, principal component analysis and cluster analysis based on the band patterns of the isolates resulted from repetitive PCR showed variation and similarities among isolates. The same isolates which showed similarity in biochemical tests (YCMTge and LIHSsw$_2$) were

also grouped together with 95% similarity in cluster analysis. Isolates FGMSto, $HSHSda_2$ and $ANHTmz_1$ were also near to the reference strains with better similarity than the other isolates. The only sample taken from banana (GIMB) was found to be highly isolated from other isolates in cluster analysis, which might indicate the presence of a different strain of *Xanthomonas campestris* Pv. *musacearum* attacking banana naturally. The region Areka was also found highly variable in discriminant function analysis in grouping regions that might be due to the presence of corm rot in the analysis, which might be caused by a different bacterial species. The crude extracts from *Pychnostachis abyssinica* were also evaluated against isolates where the bract and leaf extracts showed growth inhibition on 13 isolates. In general, the study has produced results that are important for further research on the causal agent of enset bacterial wilt.

# 1. INTRODUCTION

Ethiopia (about 65 million people) mainly depends on agriculture. Over 85% of its population lives in the rural area where crop production and animal husbandry is their main stay. There are different farming systems depending on different agroecologies 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 one of the indigenous root crops widely cultivated for its food and fiber values in the above-mentioned parts of the country (Taye, 1996).

It is estimated that a quarter or more than 15 million of Ethiopia's population depends on enset as staple and co-staple food source, for fiber, animal forage, construction materials and medicines (Brandt *et al.*, 1997) and the area of enset production in Ethiopia is estimated to be over 180,000 hectares (CSA, 1994).

According to Admasu *et al*. (1998), based on the level of priority given to enset cultivation in different zones and regions, three enset based farming systems have been identified. Enset is the main food source in Gurage, Kembata, Sidama, Gedio, Hadya, Jemjem and Arero zones. It is a second important crop as co-staple food in Wolaita, Gofa, Kafa zones and Yem special wereda. It is planted as the third most important food crop in Wollega, Illubabor and in some parts of Southern region. In the second and third farming systems, cereals and other root crops take the primary and secondary importance.

Due to unsatisfactory research attention given to enset crop, its production system is still traditional and tiresome. Different management practices starting from propagation to

harvesting and processing demand high labor. Further more, 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). There are also viral diseases of enset known as mosaic and chlorotic leaf streak diseases. Insects damaging enset leaves such as Jassid fly, spider mites, mealy bugs and wild animals such as porcupine, molerat and wild pigs have been reported (Brandt *et al.,* 1997). 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.

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. According to Awasa Agricultural Research Center Progress report (1999/2000), enset farmers were trained on application of sanitary measure and a participatory application of the measure which involved researchers, extension agents and enset farmers was done in the high and mid lands of Gurage recently. In addition, screening of enset clones for their resistance to enset bacterial wilt is an on going project in Awasa Agricultural Research center.

The disease eradicating efforts couldn't be effective because of gaps in the basic research on the disease. The nature of the disease, the host-pathogen interaction, mode of transmission etc. have not been exhaustively studied. The nature and use of the crop is also one of the reasons that affect the effectiveness of sanitary measure and resistant clone screening activities. This is because enset is a semi-perennial crop with an average length of 10 meter and pseudostem

circumference of 1meter and it is very difficult to easily practice sanitary measure. Uprooting and burying of infected plants will not be a simple task for the farmers. In addition, selecting tolerant clones and distributing among enset growing farmers will not be easy because of the fact that farmers use different enset clones for different purposes. For example, enset clones such as Arkiya and Suite (wolaita) are preferred for their corm, Astara (Gurage) for its medicinal value and Hala (Wolaita) for its good kocho yield; Yeshirekinke (Gurage) and Genticha (Sidama) are preferred for their kocho and especially for their disease tolerance (Awasa Agricultural Research Center Progress report, 2000).

Despite the existing situation, sanitary measure and screening enset clones for resistance to the disease seem to be the way out in addition to looking for appropriate control measures. In order to work on several other possible control strategies such as evaluation of antibiotics and plant extracts against the disease, conventional breeding to come up with resistant and high yielding enset clones and even to transfer genes of valuable traits, characterization of the causal agent is very critical.

Some studies indicated variations in the occurrence and incidence of enset bacterial wilt in major enset growing areas. This is perhaps the implication of the existence of variability within *Xanthomonas campestris* Pv. *musacearum* population. Therefore detection of this variation could contribute for the development of technologies to control enset bacterial wilt (Gizachew, 2000).

Recently evaluation of plant extracts against many *Xanthomonas* species is becoming an important area. Thus a focus should also be given to indigenous practices of the farmer to look for their effectiveness. Especially the indigenous knowledge on medicinal plants is important.

For example, *Pychnostachis abyssinica* (Fresen) locally called Fanfo is a herbaceous plant where farmers of Hadya and Silte plant it near infected enset with expectation that it prevents transmission of the disease. Such practices need to be proven through scientific methods.

Generally, in development of control strategies against plant diseases, the main problem is the presence of different strains within a given pathogen population and low capacity to use different modern techniques to detect this variation. For example, in enset bacterial wilt disease, enset clones that are tolerant to some isolates of the causal agent become susceptible to other isolates (Awasa Agricultural research Center Progress report, 2000). Therefore, there is a need to identify this variation using proper techniques for devising better control strategies against the disease. Hence this study was carried out with the following objectives:

**OBJECTIVES**

1. Detection of variation among isolates of *Xanthomonas campestris* pv *musacearum* from infected enset plants collected from different enset growing zones using biochemical methods and repetitive PCR technique.

2. Evaluation of crude extracts from leaf, stem, root and bract of Fanfo (*Pychnostachis abyssinica*) against *Xanthomonas campestris* Pv. *musacearum* isolates.

# 2. LITREATURE REVIEW

## 2.1 Taxonomy and History of Enset

Enset (*Ensete ventricosum* (Welw) Cheesman) is a Monocot that belongs to order *Schistaminae* and family *Musaceae*. Enset was considered as member of the genus *Musa* as it strongly resembles banana morphologically (Taye, 1980) and because of this some of the species names formerly given to enset were *Musa ensete* and *Musa ventricosa* (Lye and Edwards, 1997). It was Cheesman (1947) who separated enset from banana on the basis of differences in pseudostem morphology and chromosome numbers.

Other than *Ensete ventricosum*, there are species in the genus *Ensete* but there is some ambiguity regarding their number. Cheesman (1947) revised the *Musacearum* family and transferred about 20 species from the genus *Musa* to the genus *Ensete* and listed 24 species, which are distributed in Africa and Asia. But Simmons (1962) and Pursglove (1972) recognized 6 and 7 species under this genus respectively. Taye (1984) has also stated that 7-8 species are known in the genus. This shows that further research is needed on the taxonomy and distribution of enset species (Brandt *et al.*, 1997). In any ways all authors agree that *Ensete ventricosum* is the only cultivated species in Ethiopia and has great economic importance.

Different hypothesis are proposed on the origin of enset agriculture. Agronomists and biogeographers have long considered the Ethiopian high lands to be the primary center of origin for enset agriculture. In relation with this, anthropologists, archaeologists, historians and

other scholars have also developed hypothesis that argue for the domestication of enset in Ethiopia as early as 10,000 years ago (Brandt *et al*., 1997).

Currently enset distribution is restricted to south, southwest and central part of Ethiopia and it is not known as food crop in the northern part of Ethiopia. However, historical evidences suggested that enset may have once played a much more important role in the agricultural practices of central and northern Ethiopia before the mid 19$^{th}$ Century (Brandt *et al.*, 1997). The possible reasons for total disappearance of enset culture in the North could be disease, drought and instability in the sociopolitical events between mid 1700 and mid 1800.

## 2.2. Enset Morphology and Ecology

Enset looks like a large, thick, single- stemmed banana plant. Usually it is larger than banana and 6-12 meters tall. The leaves are 5-7 meters tall and 1 meter in diameter and are more erect than a banana plant (Kefale and Sandford 1991; Brandt *et al.*, 1997). The stem has three parts. The pseudostem, which is made of tightly clasping leaf sheaths, is 2-3 meters in height and with an average of 1meter diameter. It contains an edible pulp and quality fiber. The underground corm is an enlarged lower portion of the stem with an average of 0.7meter length and diameter. The fibrous rooting system of enset grows out from this part. The true stem is between the pseudostem and corm near the ground. Usually it grows up during maturity and initiates a single flower head, which forms multiple flower fruits and seeds. The small banana like fruits produce several irregularly shaped black seeds. Most wild enset plants are produced from seeds unlike the domesticated ones, which are propagated from suckers (Brandt *et al.*, 1997).

Enset cultivation was accepted to restrict at altitudes ranging from 1600-3100 m.a.s.l (Taye and Asrat, 1966; Westphal, 1975). But recent investigations indicated that it grows in areas as low as 1200m.a.s.l (Endale, 1990). However, the best elevation for enset cultivation is between 2000 and 2750 m.a.s.l with an annual rainfall of 1100 to 1500 mm where the majority falls between March and September (Brandt *et al.*, 1997). According to Taye and Asrat (1966), enset can with stand relatively long period of drought (about 5 months), and unlike species in genus *Musa*, enset tolerates short period of frost.

The average temperature of enset growing areas is between 16 to 20°c and the relative humidity 60 to 80 % (Yohannes and Mengel, 1994). Soil types in the enset growing areas of Ethiopia are moderately acidic to slightly basic with a pH reaction ranges from 5.6 to 7.3. These soils contain 0.10 to 0.15% total Nitrogen and 2 to 3% organic matters (Taye and Asrat, 1966).

## 2.3. Economic and Ecological Uses of Enset

Enset is a multipurpose crop of which every part is thoroughly utilized (Shigeta, 1996). It is a good source of starch. The corm and the pseudostem are the most important sources of food (Kefale and Sandford, 1991). The types of food from these parts are known as ‘Kocho’, ‘Bulla’ and ‘Amicho’ (Spring *et al.*, 1996). Kocho is the bulk of the fermented starch obtained from the decorticated (scraped) leafsheathes and grated corm. Bulla is obtained by squeezing out the liquid containing starch from scraped leafsheathes and grated corm and allowing the resultant starch to concentrate into white powder. Amicho is boiled corm of young enset plants known for best quality of corm. It is prepared and consumed in a similar manner to preparation of other root and tuber crops (Brandt *et al.*, 1997). Fiber is the by-product of enset that is left after

decorticating the leaf sheathes. Its strength is found to be equivalent to the important fiber crop *Musa texstalis* (abaca) (Taye, 1984). Fiber is used for making bags, ropes, twines, cordage, mats, etc where the variety, the age of the plant, and the way in which the fiber is extracted and stored determine its length and quality (Kefale and Sandford, 1991; Yohannes and Mengel, 1994).

Enset is one of the major crops that can significantly help to ensure food security in a country like Ethiopia (Brandt *et al.*, 1997). The average yield of refined enset product kocho ranged from 7 to 12 tons/ha/ year. The amount of food attainable from 50-60 enset plants per year could provide enough food for an average family of 5-6 persons (Zeweldu and Ludders, 1996). Enset products are available through out the year and can be stored in pits for long periods of time without spoiling.

Enset is rich in carbohydrate and mineral substances like calcium and iron (Shigeta, 1990; Taye and Asrat, 1966). The energy yield of enset is by far higher than that of several cereals. A mature enset plant could yield 20 $x10^6$ cal / ha/ year which is 20 times higher than that of barley (Olmstead, 1974; Terefe, 1991). Enset energy yield was also reported to be higher than potato, sweet potato and banana (Pijls *et al.*, 1995). This shows that cultivation of enset can significantly improve food security at household and at national level.

Some clones and parts of enset plants are reported to have medicinal value for both human and animals. These clones are claimed to heal bone fractures, for treatment of diarrhea and delivery problems i.e. assisting to discharge the placenta (Spring *et al.*, 1996). Even some farmers believe that eating bulla from clones like Boliae (Wolaita) after taking traditional medicines against tapeworm protects the liver from the side effect of the medicine. Bulla supplemented

with milk and milk products is also known as important for quick recovery of women after child delivery (Kefale and Sandford, 1991).

The fresh and dried leaves of enset have various uses. They are used as food wrappers, serving plates and pit liners during kocho storage. Dried petioles and midribs are used as fire wood, to make mats and tying materials (Brandt *et al.,* 1997). In the dry season, the fresh leaves are used as cattle feeds. Fekadu and Ledin (1997) reported that the degradability of *Ensete ventricosum* lamina given for rumen animals was found to be better than that of straw and banana leaf and similar with that of stoker and *Chloris gayana* hay. Other parts of enset such as leaf midribs, pseudostem sheath, pseudostem core and corm were all found to have high degradability than green *Chloris gayana*, setaria grass, elephant grass and Guatemala grass.

Further more enset contributes for higher reduction of losses of plant nutrients particularly nitrogen loss through leaching as compared to annual crops (Brandt *et al.,* 1997; Lee-R and Zawdie, 1997). Research conducted on fields where enset is continuously cultivated has revealed that there is a higher soil nutrient status in the enset fields than in other crops. This indicates that enset cultivation is sustainable system with regard to maintaining the soil fertility (Asnaketch, 1997). Eyasu (1998) has also confirmed that soil fertility is maintained and even increased around the garden area, enset and taro fields.

## 2.4. Major production constraints of enset

The agronomic practices from field preparation and propagation to harvesting and processing are laborious and time consuming. It is mainly women who carry out enset harvesting and

processing using local tools. However, diseases and insect pests are the major constraints in the production system. Some major diseases and insect pests of enset are discussed below.

### 2.4.1. Enset Diseases

There are many diseases that attack different parts of enset caused by fungi, bacteria and nematodes. Their importance also varies depending on the damage they incur. According to Quimio and Mesfin (1996), fungal foliar diseases are numerous and widespread. Some are destructive on suckers, seedlings, young transplants and rapidly growing plants up to two years old. However, infected plants normally tolerate these diseases. *Phyllostica* Sp., *Piricularia* Sp., and *Drechslera* Sp. are suspected to cause these leaf spot diseases on suckers and young plants and *Cladosporium* Sp. and *Deightoniella* Sp. infect older plants. *Mycosphaerella musicola*, which causes sigatoka in banana, is also known to cause destructive leaf spot on enset.

Bacterial wilt caused by *Xanthomonas campestris* Pv. *musacearum* (Dagnachew and Bradbury, 1968) Dye is the major enset production constraint (It is discussed in topic 2.5). Following enset bacterial wilt, bacterial corm rot caused by unidentified bacterium is reported in 1991 as important bacterial disease affecting the enset production (Quimio and Mesfin, 1996; Brandt *et.al*., 1997). It attacks both young and mature plants and in advanced stage of the disease, the plant easily topples down when pushed and a rotten corm is observed (Quimio and Mesfin, 1996). Another reported bacterial disease is sheath rot of enset, which is manifested by patches of watery rot in the outer leaf sheaths (Quimio, 1991).

The common nematodes that attack enset are the root lesion nematode, *Pratylenchus goodeyi* and the root knot nematode, *Meloidogyne* Sp. where *Pratylenchus goodeyi* is often found in

association with bacterial wilt. Therefore, it is suspected in transmission of enset bacterial wilt disease (Peregrine, 1992). The leaf nematode disease of enset caused by *Aphelechoides* Sp. was discovered in 1991 (Quimio, 1992). It attacks leaves of suckers and young seedlings and characterized by linear black leaf streaks usually occurring on leaf margins and near the base of the newly expanded leaves (Quimio and Mesfin, 1996).

The mosaic and chlorotic streak viral diseases were first observed in 1991 and resemble those of mosaic and infectious chlorosis of banana caused by strains of cucumber mosaic virus. The mosaic is more destructive than chlorotic streak as it causes severe stunting of affected plants (Quimio and Mesfin, 1996).

### 2.4.2. Insect pests and Wild animals

According to Terefe and Endale (1989), banana aphid, leafhopper, spider mites and mealy bug were frequently observed on both healthy and wilting enset plants and Jassid flies in virus-infected plants. Usually these insects were suspected in transmitting bacterial wilt. However, recent survey on enset root mealy bug damage has revealed that it is incurring great loss in enset production especially in Gedio and Sidama zones. These soft bodied insects feed on the corm and roots and the infested enset plants show stunted growth (Brandt *et.al*., 1997). The wild animals usually feed on the corm and pseudostem of enset and among them mole rat is reported to cause considerable damage.

## 2.5. Characteristics of *Xanthomonas campestris* Pv. *musacearum* and the nature of Enset bacterial wilt disease

### 2.5.1. Genus *Xanthomonas*

The genus *Xanthomonas* is classified under the family *Pseudomonadaceae* and the yellow-pigmented plant pathogen of this family have been unified in this genus (Schiegel, 1995). Cells are straight rods usually within the range 0.4 - 0.7 wide X 0.7 – 1.8 um long. They are gram negative, aerobic and motile by a single polar flagellum. The optimum temperature for growth is usually 25-30 $^{o}$C. Colonies are yellow, smooth and butyrous or viscid (Bradbury, 1984).

The oxidase test for genus *Xanthomonas* is negative or weak positive and catalase test is positive. They are chemoorganotrophic, able to use a variety of carbohydrates and salts of organic acids as sole carbon sources. Asparagine is not used as a sole source of carbon and nitrogen and this is used as a diagnostic test for *Xanthomonas* (Dye, 1962 cited in Bradbury, 1984). Certain species especially *Xanthomonas campestris* and *Xanthomonas fragriae* are known to produce large amount of extracellular polysaccharides or xanthan gums when grown in media containing usable carbohydrates (Bradbury, 1984; Schiegel, 1995). Although not well understood, the role of these extracellular polysaccharides is to maintain viability of bacterial cells in exudate and plant material under dry conditions and to protect them from enzymatic degradation (Schiegel, 1995). Xanthans have a wide range of uses in food industry and in other industries because they are not toxic to man and animals. They are mainly used to increase the viscosity of aqueous solution such as diet soups, packed desserts, printing inks, paints etc. (Schiegel, 1995; Becker *et.al*., 1998).

Xanthomonadins (Yellow pigments) are brominated aryl polyenes found in all species of *Xanthomonas* and important characteristics for identification. However, there are non-pigmented strains, which sometimes occur. Therefore absence of Xanthomonadins does not exclude an organism from the genus (Bradbury, 1984).

Phenotypic and genetic variations were observed in different *Xanthomonas campestris* pathovars. For example, biochemical and pathogenic variation was observed in strains of *Xanthomonas campestris* Pv. *mangiferaindicae* collected from southern India. The isolates were different in their ability to liquefy gelatin, reduce nitrate and utilize carbon sources (Dayakar and Gnanamanickam, 1996). Pathogenic variability was also observed in isolates of *Xanthomonas campestris* Pv. *glycines* collected from soybean cultivars from 6 states of USA and National Collection of Plant Pathogenic Bacteria in England. Based on the variability in pathogenicity, the isolates were classified into five races among which some avirulent strains were found (Hwang *et al*., 1998). Phenotypically distinct strains of *Xanthomonas campestris* Pv. *mangiferaeindicae* as yellow pigmented and apigmented were isolated in Brazil (Pruvost *et al.*, 1998). White pathovars of *Xanthomonas campestris* were also observed (Sugimori and Oliveira, 1994). Genetic variations in different isolates of several *Xanthomonas campestris* pathovars were also observed and measured using different molecular techniques such as RFLP and rep-PCR (Bragrad *et al*., 1995; Louws, *et al*., 1995; Norman *et al*., 1999; Restrepo *et al*., 2000).

Antibacterial substances from *Xanthomonas* species have been described. For example, antibacterial substances were produced from 32 isolates of *Xanthomonas oryzae* Pv. *oryzae* when treated with chloroform vapor, heat and UV light (Huang *et al*., 1996). The production of a bacteriocin called glycinecin from *Xanthomonas campestris* Pv. *glycines* that was

antagonistic to related bacteria *Xanthomonas campestris* Pv. *vesicatoria* was also reported (Jung *et al*., 1998).

### 2.5.2. Characteristics of *Xanthomonas campestris* Pv. *musacearum*

The etiological agent of enset bacterial wilt disease was first reported and described by Dagnachew and Bradbury who named it as *Xanthomonas musacearum* (Dagnachew and Bradbury, 1968). Later, however, it was renamed as *Xanthomonas campestris* Pv. *musacearum* (Dagnachew and Bradbury) Dye 1978 (Dye *et al*., 1980) as just a variety of *Xanthomonas campestris.* The bacterium is a gram-negative rod with an average length of 1.9µm, 0.8µm width and it has a single polar flagellum. Distinct colonies appear after 48 hours incubation at 25-28$^{o}$C on glucose peptone agar. Different colony colors were observed on different media; such as light yellow growth on nutrient agar, deep yellow on King's medium B and honey color on potato plugs. Further more, slimy growth is also observed on sucrose or glucose peptone agars (Eshetu, 1981).

According to Eshetu (1981), *Xanthomonas campestris* Pv. *musacearum* produces acid with out gas from glucose, mannose, galactose, and sucrose but not from lactose, xylose, maltose, manitol and sorbitol within one-month time. It liquefies gelatin slowly; produces $H_2S$ and $NH_3$ and hydrolyzes aesculin but not starch. The bacterium does not reduce either nitrate or nitrite and does not produce indole and acetoin. Its growth in the nutrient broth is retarded by 3% and suppressed by 4% Sodium chloride.

Although not confirmed, variations in isolates of *Xanthomonas campestris* Pv. *musacearum* were observed in some preliminary laboratory and field experiments. There were isolates from

Hagereselam wereda, which were resistant to antibiotics rifampicin, and streptomycin (Quimio, 1992) and variation in symptom development of different isolates was also observed in field on the same enset clone.

### 2.5.3. Disease Host range, Distribution and Damage

Other than enset, the disease affects banana under natural condition and epidemics of the disease was reported in the former Kaffa province (Dagnachew and Bradbury, 1974). Wilt symptom was also observed in banana through artificial inoculation of the disease (Dereje, 1985) and recently a screening trial on 45 banana cultivars for resistance to enset bacterial wilt disease revealed that all cultivars were found susceptible (Awasa Agricultural Research Center Progress Report, 2000). Dagnachew and Bradbury (1968) inoculated 27 plant species other than enset and banana and all failed to develop the symptom. Dereje (1981) also inoculated 11 plant species and disease symptoms were observed only on *Canna orchoides*, which is not in *Musaceae* family.

Surveys conducted in the early 1980's and 1990's in major enset growing zones revealed the occurrence of enset bacterial wilt disease in all zones with different degree of severity (Dereje, 1985; Spring *et al*., 1996; Awasa Research Agricultural Center, Progress report, 1997). It is very destructive as it kills enset plants at all growth stages including 4 to 7 years old plants ready to harvest (Quimio and Mesfin, 1996). Once it appears in a field, it is easily transmits from infected enset plant to healthy plants through different mechanisms and in some areas where the severity of the disease and loss is high, farmers are obliged to abandon the whole field and replace it with another crop.

Bacteria cannot enter plants via intact cuticles, and entry is either through wounds or natural openings such as hydathodes and stomata (Manners, 1993). For example, *Xanthomonas campestris* Pv. *campestris* enters to *Arabidopsis thaliana* leaves through hydathodes (Hugouvieux, *et al*., 1998). With regard to *Xanthomonas campestris* Pv. *musacearum,* mechanical damage as an entrance for initiation of enset bacterial wilt disease was demonstrated by cutting enset leaves with contaminated knives (Dereje, 1985 and Awasa Research Center Progress report, 2000). Once the bacteria enter into the plant, they multiply in the intercellular spaces and move through the tissues. Cell death of the plant may follow due to toxins or pectolytic enzymes produced by the bacteria. In general the rate of spread of the disease depends on the rate of multiplication of the pathogen, its motility, its ability to produce pectolytic enzymes and the structure of the host. All the parameters are also affected by the environmental conditions especially on temperature and on the extent to which the host produces stimulants or inhibitors for bacterial growth and activity (Manners, 1993). Usually, vascular bacterial pathogens multiply rapidly in the xylem vessels adjoining the point of entry, and are then passively dispersed in the transportation stream (Suhagda and Goodman, 1981 cited in Manners 1993). Regarding *Xanthomonas campestris* Pv. *musacearum* the mechanism for pathogenesis is not yet studied.

According to Dagnachew and Bradbury (1968), the disease is transmitted from infected plants to healthy plants by mechanical means mainly through implements used in enset cultivation. In addition birds, insects and nematodes are suspected in transmitting the disease through mechanisms not confirmed yet. Eshetu (1981) reported that among the commonly observed insects in enset fields, leafhopper species seems to be important as vector due to its active flying ability. The leaf streak nematode and the root lesion nematode have also been found in

association with bacterial wilt. This is a clue for suspecting nematodes as vectors of the disease (Pergrine and Bridge, 1992, Swart *et al*., 2000 and Mesfin, 1989).

The disease could be disseminated with agents like wind and rain (Agrios, 1988) as the ooze like exudates from the bacteria filling the parenchyma tissues of the enset easily flow out from infected plants to healthy ones. Contaminated soil and infected debris are also possible dissemination agents as survival studies on other *Xanthomonas campestris* pathovars in soil and plant debris strengthened the possibility. For example, according to Thaveechai *et al*. (1993) study, survival of populations of *Xanthomonas campestris* Pv. *manihotis* strain CSg in plant tissue and soil samples were evaluated by using semi selective medium and they were found to survive for 7-14 days in rhizosphere soil of cassava weeds and for 21-49 days in infected cassava tissues buried in the soil. The results suggested that cassava debris may be important as a source of inoculum than rhizosphere soil of weeds for the dissemination of cassava bacterial blight. *Xanthomonas campestris* Pv. *glycine* were also remained viable for 110 days in infected soybean leaves placed on soil surface and for 29 days in infected leaves buried 15 cm. deep (Khare and Khare, 1995).

## 2.6. Potential control measures of enset bacterial wilt

### 2.6.1. Cultural Control measures

Bacterial wilt constitutes a potential threat to the cultivation of enset in Ethiopia (Tigist, 1986). Because of its severe damage to enset, farmers undertake a variety of traditional practices (smoking bones, tires, burning porcupine body including local spiritual believes such as 'Dua' prayer ceremony and slaughtering black goat etc.) for the control of the disease. Some farmers

also uproot and throw away-infected plants on the road or near the enset farm which further spread the disease. Farmers in Peasant associations where some training on sanitary measure was advocated also do not practice the measure correctly (Million *et al*., 1999).

According to Agrios (1988), sanitary measure is the first control measure, which should be taken against bacterial diseases. In an experiment done in Netherlands on survival and carry over of *Xanthomonas campestris* Pv. *campestris* in soil, it was found that good crop and soil management impeded survival of inoculum from one year to the next (Kocks *et al.*, 1998). Therefore, sanitation has been recommended for enset bacterial wilt by several researchers (Dagnachew and Bradbury, 1974; Dereje, 1985; Quimio, 1992; Brandt *et al*., 1997). This measure includes the use of disease free suckers as planting material, uprooting and burying of diseased plants far from the field, cleaning and flaming of equipment that has come in contact with diseased plants and rotation of crops if the damage is severe (Brandt *et al*., 1997). Such measures should be taken in a manner of campaign and as regular practice in all enset-growing areas.

### 2.6.2. Host plant resistance

Resistance to pathogens is a genetically inherited character similar to other attributes such as height, yield and leaf size (Dixon, 1984) and it is used as a means to control losses caused by plant pathogens in most crops.

Enset farmers know 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. This was partially confirmed in screening trials

by Dereje (1985) and by Awasa Agricultural Research center (Awasa Agricultural Research center progress report, 2000). These tolerant clones are potential genetic resources for future enset breeding work.

### 2.6.3. Chemical and Biological control measures

So far no bactericide has been recommended against enset bacterial wilt. Various in-vitro trials were done on antibiotics and plant extracts against *Xanthomonas campestris* pathovars that cause diseases in different crops. For example, streptomycin, oxytetracycline, chloroamphenicol and rifampicin were tested for the control of black rot of cauliflower caused by *Xanthomonas campestris* Pv. *campestris* and streptomycin was the most effective, giving 100% control followed by oxytetracycline (Lenka and Ram, 1997). In- vitro test was also done on *Xanthomonas campestris* pv *mangiferaeindicae* and all the chemicals and antibiotics used, aureofungin, bavistin, erythromycin, streptocycline, streptomycin and tetracycline inhibited the bacterium (Talwar *et al*., 1996). Efficiency of copper oxychloride and a mixture of oxytetracycline + streptomycin sulfate were also evaluated in controlling angular leaf spot of cotton caused by *Xanthomonas campestris* Pv. *malvacearum* in fields and 643.3 g/ha oxytetracycline + streptomycin sulfate was recommended (Araujo and Siqueri, 1999). Nisin 50 µg/ml was also reduced the growth of *Xanthomonas campestris* Pv. *campestris* by greater than 90% (Wells et al., 1998).

Several studies have also indicated the potential of plant extracts in the control of diseases caused by *X. campestris* in several important crop plants. Akhtar *et al.*, (1995) tested about 208 diffusates from various plants such as forest trees, shrubs, herbs, fruit seeds etc. against *Xanthomonas campestris* Pv. *citri*. and diffusates from various parts of *Phyllanthus emblica,*

*Acacia nilotica*, *Sapindus mukorossis* and *Terminalia chebula* exhibited an inhibition zone 4.83-6mm at 50 g/liter appeared to be the most effective. Extracts from *Acacia arabica*, *Achras zapota*, and from other 6 higher plants were also found inhibitory to various pathovars of *Xanthomonas campestris* (Satish *et al*., 1999). *Chamomilla recutita* and *Chamaemelum nobile* extracts also inhibited the growth of *Xanthomonas campestris* Pv. *citri* strains causing citrus bacterial canker disease (Csizinszky, *et al*., 1993). Patil and Ghoderao (1997) also evaluated some medicinal and aromatic plants against cotton bacterial blight infection and *Azadirachta indica* and *Ipomoea carnea* extracts were found effective at reducing the incidence and intensity of the disease. Root and leaf extracts of *Adhatoda zeylanica* showed in-vitro inhibition of *Xanthomonas campestris* Pv. *vignicola* by producing inhibition zones of 1.35 cm. and 1.52 cm respectively (Thammaiah *et al*., 1995).

In some enset growing areas in Ethiopia, particularly in Gurage and Hadya, farmers plant a herb locally called 'Fanfo', 'Yeriyo' or 'Olomo' in Silte, Oromiya and Wolaita, respectively near infected enset plant roots and in the middle of the enset field. Farmers believe that, the plant prevents transmission of the disease from infected to healthy enset plants (Personal communication). The plant is *Pychnostachis abyssinica* (Fresen) and classified under the family *Labiatae*. In some areas it is known as a repellant to termites, ants and related insects (Herbarium Sheet). Testing and evaluation of extracts from such plants against the causal agent of the disease could support the research on devising control measures against the disease.

## 2.7. Detection of Genetic Variation

According to Gabriel and De Feyter (1992), accurate strain identification of pathogens is important for epidemiological and ecological monitoring purposes where highly refined genetic technologies facilitate high-resolution distinctions. The availability of powerful analytical tools such as DNA sequencing, DNA-DNA hybridization, Multi loci isozyme analysis and RFLP hybridization analysis are influencing changes towards phylogenetic based taxonomies.

Recently rep-PCR is becoming an important tool in measuring the variation among strains. The technique was demonstrated for epidemiological studies of several mammalian pathogens and also used to differentiate pathovars and strains of *Xanthomonads* and *Pseudomonads*. According to Veracruz *et al.*, (1996), it is based on the amplification of DNA with oligonucleotide primers from three families of unrelated repetitive sequences corresponding to repetitive extragenic palindromic (REP), enterobacterial repetitive intergenic consensus (ERIC) and BOX elements. The primers amplify DNA between the dispersed elements and these fragments are likely to be different in size so that differences between closely related strains might be detected. Since rep-PCR is simpler, faster and cheaper than RFLP, it should be useful in the analysis of genomic variation within pathogen populations. The variation detected by each primer usually differs. For example, in measurement of haplotypic variation in *Xanthomonas oryzae* Pv. *oryzae*, ERIC primers resulted the most consistent group of strains and the easiest to recognize visually followed by REP and BOX the least (Veracruz *et al.*, 1996). The combined analyses of the yields from the three primers including yields from RFLP could give refined groups of strains (Veracruz *et al.*, 1996).

# 3. MATERIALS AND METHODS

## 3.1. Sample collection

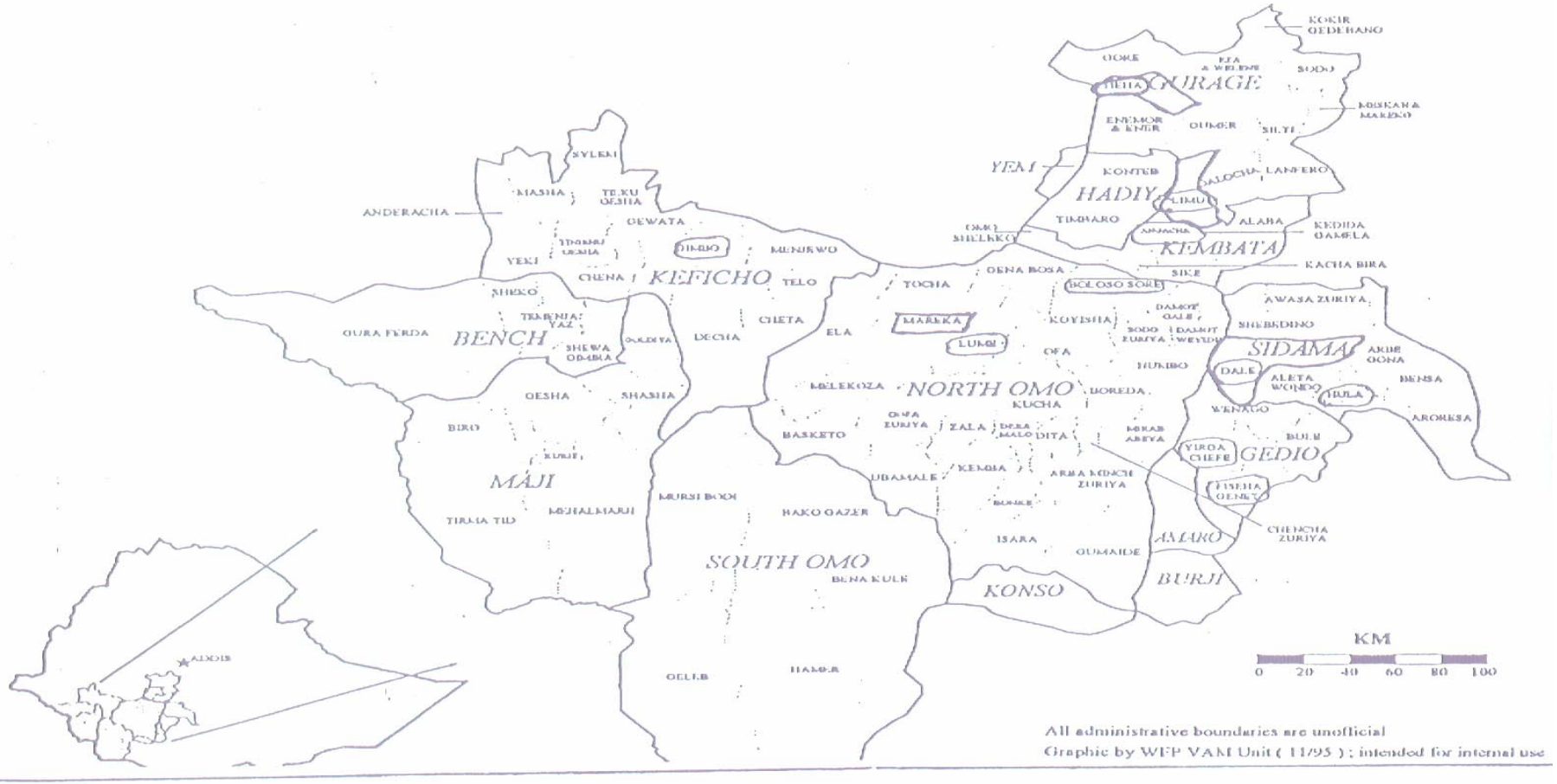
Bacterial samples were collected from October 11-18, 2001 from different zones of enset growing areas. These were Sidama, Gedio, Wolaita, Kembata and Tembaro, Hadya, Gurage, Dauro, Keffa and Jimma zones taking altitude and types of enset clones into consideration (Table.1 and Fig.1). Samples were taken from infected enset plant leaf petioles, which show early stage of the disease symptom to avoid some saprophytic microorganisms that grow in tissues killed by the primary pathogen (Quimio, 1994). The vascular wilt bacteria like *Xanthomonas campestris* Pv. *musacearum* ooze out from the diseased tissue. Therefore, 32 ooze/exudate samples were taken in the field using toothpick and then suspended in sterilized distilled water in half filled screw-capped vials according to Quimio (1994). Each sample was labeled and all information including date, location, altitude and clone name were recorded.

Table 1. Enset bacterial wilt disease samples and locations for collection

| S/N | ISOLATES | CODE | Zone/Wereda/PA | Altitude | CLONE |
|---|---|---|---|---|---|
| 1. | Angacha Gishera | ANHSgi | KAT-Angacha-Anchasedicho | 2490 | Gishera |
| 2. | Angacha Merza | ANHTmz | KAT-Angacha-Anchasedicho | 2490 | Merza |
| 3. | Angacha Siskela | ANHSsi | KAT-Angacha-Anchasedicho | 2490 | Siskela |
| 4. | Areka Nekaka | ARMSnk | Wolaita-Bolososore-Dubo | 1800 | Nekaka |
| 5. | Boloso Gefetenewa | BOMSgf | Wolaita-Bolososore-Dola | 1790 | Gefetenewa |
| 6. | Boloso Genticha | BOMTge | Wolaita-Bolososore-Dola | 1790 | Genticha |
| 7. | Boloso Silkent | BOMSsi | Wolaita-Bolososore-Dola | 1790 | Silkent |
| 8. | Boloso Tuzuma | BOMTtu | Wolaita-Bolososore-Dola | 1790 | Tuzuma |
| 9. | Cheha Nechiwe | CHMSne | Gurage-Cheha-Sese | 1850 | Nechiwe |
| 10. | Cheha Yeshire kinke | CHMTye | Gurage-Cheha-Sese | 1850 | Y/Kinke |
| 11. | F/Genet Genticho | FGMTge | Gedeo-F/genet-Kisha | 2300 | Genticha |
| 12. | F/Genet Torecho | FGMSto | Gedeo-F/genet-Kisha | 2300 | Torecho |
| 13. | Gessa Bedadiya | GEMSbe | Dauro-Gessa-Gessachere | 2200 | Bedadiya |
| 14. | Gessa Bukunia | GEMSbu | Dauro-Gessa-Gessachere | 2200 | Bukunia |
| 15. | Gessa Shedodinya | GEMTsh | Dauro-Gessa-Gessachere | 2200 | Shedodiniya |
| 16. | Gimbo Banana | GIMB | Keffa-Gimbo | 1700 | KenyaBanana |
| 17. | Goma Ariko Dima | GOMSad | Jimma-Goma-EfoYachie | 1730 | Ariko dima |
| 18. | Goma Geno | GOMSgn | Jimma-Goma-EfoYachie | 1730 | Geno |
| 19. | H/Selam DeraseAdo | HSHSda | Sidama-H/selam-01 | 2750 | Derase Ado |
| 20. | H/Selam Gulumo | HSHSgu | Sidama-H/selam-01 | 2750 | Gulumo |
| 21. | H/Selam Gulumo Yell. | HsHsgu 2 | Sidama-H/selam-01 | 2750 | Gulumo |
| 22. | Limo Bededet | LIHSbe | Hadya-Limo-Bilalo | 2700 | Bededet |
| 23. | Limo Dimbuluk | LIHSdi | Hadya-Limo-Bilalo | 2700 | Dimbuluk |
| 24. | Limo shewanat | LIHSsw | Hadya-Limo-Bilalo | 2700 | Shewanat |
| 25. | Mareka Loma Ame | MLHSam | Dauro-MarekaLoma-Gozabam | 2400 | Ame |
| 26. | Mareka loma Shedo | MLHTsh | Dauro-MarekaLoma-Gozabam | 2400 | Shedodinya |
| 27. | Mareka loma Tela | MLHSte | Dauro-MarekaLoma-Gozabam | 2400 | Tela |
| 28. | Seka Bedede | SEMSbe | Jimma-Seka-Shebe | 1850 | Bedede |
| 29. | Seka Geno | SEMSgn | Jimma-Seka-Shebe | 1850 | Geno |
| 30. | Seka Mengie | SEMTmg | Jimma-Seka-Shebe | 1850 | Mengie |
| 31. | Y/chefe Genticha A | YCMTge | Gedeo-Y/Chefe-Chita | 1900 | Genticha |
| 32. | Y/Chefe Astara A | YCMSas | Gedeo-Y/Chefe-Chita | 1900 | Astara |
| 33. | Corm rot | | Areka | 1800 | Hala |
| 34. | Sheath rot | | Mareka Loma | 2400 | Ame |
| 35. | Dead heart leaf rot | | Seka & Goma | | |

*N.B. The first two letters of each code are abbreviated wereda names.
The letters M and H indicate medium and high altitudes (M<&=to 2300 and H>&=to 2400).
The letters S and T indicate susceptible and tolerant clones
The last two letters are abbreviated clone names

FIG 1. MAP OF THE STUDY AREA




* Disease samples were collected from Wereilai in Circle

## 3.2. Isolation and Preservation

From each of the 32 bacterial samples collected, a loopful of the suspension was streaked on YPSA plates (Yeast extract, 5g; Peptone, 10g; Sucrose, 20g; Agar, 12-15g in 1liter distilled water with pH 7.4 and autoclaved at 121 $^{o}$C for 15 minutes). The plates were incubated at 28 $^{o}$C for 48-72 hours according to Schaad and Stall (1988). Bacterial colonies from each plate were further sub cultured and pure bacterial colonies were transferred to YPSA slants incubated at 28 $^{o}$C for 48-72 hours and preserved at 4 $^{o}$C for further work.

## 3.3. Morphological and Biochemical Tests

### a. Gram staining reaction

The Gram-reaction of each isolate was determined following the staining procedure in Schaad (1988). First, thinly spread bacterial smear was prepared on a clean slide, dried in air and fixed by heating. The dried smear was flooded with crystal violet solution for one minute and washed in tap water for few seconds. It was again flooded with iodine solution for one minute and washed and blot-dried. It was then decolorized with 95% ethyl alcohol by applying drop by drop until no more color flows from the smear and washed and blot dried. Finally slides were counter stained for about 10 seconds with safranin, washed and examined under microscope using oil immersion objective. Isolates that appeared pink, Gram negative bacteria were subjected for further tests.

**b. Growth on Asparagine medium**

All Gram-negative isolates were allowed to grow on Asparagine medium (Asparagine, 0.5g; $KH_2PO_4$, 0.1g; $MgSO_4.7H_2O$, 0.2g; $KNO_3$, 0.5g; $CaCl_2$, 0.1g; NaCl, 0.1g and agar, 12-15g (for plates) in 1 liter distilled water with PH 7 and autoclaved at 121 $^oC$ for 15 minutes) at 28 $^oC$ for 48-72 hours with out any other carbon and nitrogen sources (Dye 1962 cited in Bradbury, 1984). This is used as a diagnostic test for *Xanthomonas* because they are not able to grow on it while others like yellow *Enterobacteriaceae* and many *Pseudomonads* can grow on it. The growth of the bacteria on Asparagine agar plates and broth was recorded and those isolates that were unable to grow on the medium were taken for further tests. In all cases, uninoculated medium was taken as negative control. Isolates that grew on Asparagine but which formerly showed yellowish mucoid growth and found grown alone on YPSA medium initially were included in further tests.

**c. Growth on Nutrient agar with 5% Glucose**

Each isolate was streaked on nutrient agar with 5% glucose (Nutrient agar, 23g; 5% Glucose in 1 liter distilled water with PH 7 and autoclaved at 121$^oC$ for 15 minutes) and incubated at 28 $^oC$ for 48-72 hours. Mucoid and yellow colony growth on this medium is one of the characteristics that differentiate *Xanthomonas campestris* from other *Xanthomonas* species (Bradbury, 1984). Therefore the growth and colony color of each isolate was recorded.

**d. Presence of Xanthomonadin pigment**

Each isolate was streaked on nutrient agar and incubated at 28 $^{o}$C for 48 hours. About 2-3 loopful colonies of each bacterial isolate were transferred to 3 ml of spectrophotometry grade methanol in test tubes and were placed in boiling water bath until the pigment was removed. The suspension was then centrifuged at 13,000 rpm for 15 minutes to remove cell debris. The supernatant was decanted and the methanol was allowed to evaporate by keeping the methanol extract in 50-60°C-water bath until the optical density of the pigment extract reaches 0.4 at 443 nm. 5nl of each extract was spotted on a precoated, silica gel plates and a total 25nl was spotted. The silica gel plates were developed in methanol solvent. The solvent was allowed to move approximately 10cm. and the yellow spots were marked with a pencil while wet. A yellow spot with an average Rf value of 0.45 was taken as positive for the presence of the pigment (Schaad and Stall, 1988).

**e. Salt Tolerance**

Isolates were inoculated to nutrient broth with 0%, 1%, 2%, 3%, 4% and 5% NaCl concentration to evaluate their salt tolerance (Hayward, 1964). Inoculated salt free (0%) nutrient broth was used as positive control and uninoculated broth of each salt concentration was used as negative control and the presence or absence of growth was recorded.

**f. Gelatin liquefaction**

Gelatin medium containing beef extract, 3g; Peptone, 5g and Gelatin, 120g in 1 liter distilled water was prepared, poured in to test tubes and autoclaved at 121 $^{o}$C for 15 minutes and

cooled without slanting. The media were stab inoculated with each isolate grown for 48 hours on YPSA medium and incubated at 28$^{o}$C. After 3, 7,and 21 days of incubation, each isolate was evaluated for gelatin liquefaction. The isolates in test tubes were kept at 4 $^{o}$C for 30 minutes and gently tipped immediately. A medium that flows readily as the tube is gently tipped was taken as positive for gelatin liquefaction (Dickey and Kelman, 1988).

**g. Starch hydrolysis**

The isolates were streaked on starch agar medium (starch soluble, 20g; Peptone, 5g; Beef extract, 3g; agar, 15g in 1 liter distilled water with PH 7 and autoclaved at 121 $^{o}$C for 15 minutes) to evaluate their ability to hydrolyze starch (amylase production). The plates were incubated at 28$^{o}$C and for 2-3 days starch hydrolysis was observed by flooding the plates with Gram's iodine solution for 30 seconds. The appearance of clear zone around the line of growth of each isolate indicated starch hydrolysis (Aneja, 1996).

**h. Casein Hydrolysis**

The ability of the isolates to degrade the protein casein by producing proteolytic exo-enzymes was tested by growing the isolates on milk agar plates (Skim milk powder, 100g; Peptone, 5g; Agar, 15g in 1 liter distilled water with PH 7.2 autoclaved at 121 $^{o}$C for 15 minutes). Clear zone around the growth of the isolates was recorded as positive for casein hydrolysis (Aneja, 1996).

### i. Nitrate Reduction

The ability of the isolates to reduce nitrate to nitrite was evaluated in a test medium that contains $KNO_3$, 1g; Peptone, 5g; Yeast extract, 3g and Agar, 3g in 1 liter distilled water, sterilized at 121$^{o}$C for 15 minutes in tubes. Each isolate was inoculated by stabbing and sealed with 3ml-sterilized molten agar to avoid false positives and incubated at 28$^{o}$C. Observation was made after 3, 5 and 7days of inoculation. Bubble formation beneath the upper agar layer was taken as positive result for nitrate reduction (Dickey and Kelman, 1988).

### j. Hydrogen sulfide production

The isolates were evaluated for $H_2S$ production using Sulphide Indole Motility (SIM) agar medium (Peptone, 30g; Beef extract, 3g; Ferrous ammonium sulfate, 0.2g; Sodium thiosulphate, 0.025g and Agar, 3g in 1 liter distilled water autoclaved at 121$^{o}$C for 15 minutes). The isolates were inoculated by stabbing and incubated at 28$^{o}$C for 48-72 hours. The presence of black coloration along the line of stab inoculation was recorded as positive for $H_2S$ production (Aneja, 1996).

### k. Indole production

Indole production of isolates was tested with a medium composed of Tryptone, 10g; $CaCl_2$, 0.03M; NaCl, 5g in 1 liter of distilled water and autoclaved at 121$^{o}$C for 15 minutes. Each isolate was inoculated into the tryptone broth and incubated at 28$^{o}$C. After 2 and 5 days of incubation, 0.5 ml of Kovak's Reagent (P-dimethyl amino benzaldehyde 5g, Butanol 75ml,

and concentrated HCl 25ml) was added into each tube and shook gently. A dark red color in the surface layer was taken as positive for indole production (Aneja, 1996).

**l. Oxidase Test**

A small loopful of each bacterial isolate was rubbed on a filter paper with drops of 1X (W/V) aqueous N, N, N, N- tetra methyl -p-Phenylenediamine dihydrochloride solution. Isolates which developed purple color within 10 seconds were taken as positive, purple color in 10-60 seconds were taken as slow positive and those with no color for more than 60 seconds taken as negative to oxidase test (Dickey and Kelman, 1988).

**m. Catalase Test**

Few drops of 3% hydrogen peroxide was added on the surface of 48 hours old culture of each isolate on YPSA medium and bubble formation was recorded as positive for catalase activity (Dickey and Kelman, 1988).

**n. Citrate Utilization**

Citrate utilization of the isolates was tested using Simon's citrate agar slants ($NH_4H_2PO_4$,, 1g; $K_2HPO_4$, 1g; NaCl, 5g; $MgSO_4 \cdot 7H_2O$, 0.2g; sodium citrate, 2g; Agar, 15g; Bromothymol blue, 0.08g in 1 liter distilled water (pH 6.9) and autoclaved at 121 $^oC$ for 15 minutes). A loopful from each isolate was streaked on the slant and incubated at 28 $^oC$ for 48-72 hours. A change of color from green to blue was taken as positive for citrate utilization and uninoculated tubes were used as negative control (Aneja, 1996).

**o. Malate Utilization**

Malate utilization of the isolates was tested using a broth medium containing malic acid, 2g; $NH_4H_2PO_4$, 1g $K_2HPO_4$, 1g; $MgSO_4.7H_2O$, 0.2g; Yeast extract, 1g; Bromothymol blue, 12.5 ml of 0.2% solution in 1 liter distilled water (PH 6.9) and autoclaved at 121 $^oC$ for 15 minutes. Forty-eight hours old bacterial isolates were inoculated and incubated at 28 $^oC$ for 48-72 hours. A color change from green to blue was taken as positive for malate utilization and uninoculated tubes used as negative control (Aneja, 1996).

## 3.4. Carbohydrate Utilization

Arabinose, cellobiose and fructose were selected for this test because utilization of these sugars can be used to differentiate *Xanthomonas campestris* from other pathovars (Bradbury, 1984). The Medium (Dye's medium C) was prepared with the composition of $NH_4H_2PO_4$, 0.5g; $K_2HPO_4$, 0.5g; $MgSO_4.7H_2O$, 0.2g; NaCl, 5g; Yeast extract, 10g; carbon source, 5g; bromocresol purple (1.5% in alcohol solution), 0.7ml and agar 12g in 1 liter-distilled water (Dye, 1962 cited in Bradbury, 1984). The medium was poured to test tubes and autoclaved at 121 $^oC$ for 15 minutes. The isolates were stab inoculated and incubated at 28 $^oC$. After 2, 4, 7, 14 and 21 days of incubation, a change in color from blue to yellow was recorded as positive for utilization of the carbohydrate. Uninoculated tubes were taken as negative control.

## 3.5. Hypersensitivity test

Forty-eight hours old cultures of each isolate were suspended in sterilized distilled water and adjusted to 0.3 O.D at 460 nm ($10^7$-$10^8$ cfu/ml bacterial cell concentration) using

spectrophotometer. An aliquot of 2ml of each bacterial culture suspension was injected using a sterilized hypodermic syringe into the intercellular spaces of expanded leaves of a one-month tobacco plant (*Nicotiana tabacum* var. white burley). Injection of sterilized distilled water was used as negative control. All the tobacco plants were kept in Green house at 25-30 $^{o}$C and 15-18 $^{o}$C day and night temperature until symptom developed and a complete collapse of tissues occurred with yellow chlorosis to brown necrosis around the injection point was taken as positive for the test (Quimio, 1992).

## 3.6. Pathogenicity Test

A susceptible enset clone 'Arkiya' from Areka experimental field was used for this test (Awasa Research Center Progress report, 1999). Three months old suckers were planted in pots with sun-dried soil, sand and manure mixture with the ratio of 3:1:1 (Quimio, 1992). The suckers were kept in Green house at 25-30 $^{o}$C day and 15-18 $^{o}$C night temperature. Isolates that were preserved at 4 $^{o}$C for this purpose were grown on YPSA medium at 28 $^{o}$C for 48 hours, harvested, suspended in sterilized water and adjusted to 0.3 O.D at 460nm ($10^{7}$-$10^{8}$ cfu/ml bacterial cell concentration) using spectrophotometer. An aliquot of 3ml of the bacterial suspension were inoculated using a hypodermic sterile syringe to the second innermost leaf petiole with 3 replication. Sterilized distilled water was also inoculated as negative control. The reaction of the plants was observed in every week for 3 months.

## 3.7. Genetic characterization of the isolates using Repetitive PCR

### 3.7.1. DNA extraction

The DNA extraction was done following the protocol in Gabriel and De Feyter *et al*., (1992), for all isolates including those not able to grow and those grown on asparagine medium and two reference strains DSMZ 1050 (*Xanthomonas campestris* Pv. *campestris*) and DSMZ 1350 (*Xanthomonas campestris*) from DSMZ Culture Collection Center, Germany. In addition, bacterial isolates taken from corm rot, sheath rot and dead heart leaf rot were included to see whether these causal agents have any similarity with the enset bacterial wilt causal agent.

A loopful of each isolate was suspended in 500-µl phosphate buffered saline (PBS) in eppendorf tube and mixed by vortexing and centrifuged in 12000 rpm for 15minutes. The supernatant and the viscous material were discarded and the pellet was washed with 1ml NE buffer (50mM Ethylene diamine tetra acetate (EDTA), 0.15M NaCl, pH 8.0) and centrifuged repeatedly. Then, 1mg/ml lysozyme was added to the pellet in 600µl NE buffer and incubated at 37 $^{o}$C for 30 minutes (modified).

Proteinase K to final concentration of 150µg/ml and 30 µl of Sodium Dodecyl Sulphate (SDS) were added to the suspension and incubated at 50 $^{o}$C for 1 hour. Equal volume of Chloroform-Phenol-Isoamyl alcohol (24:25:1) buffered with 10mM Tris-HCl pH 8.0 was added for extraction. The suspension was mixed by vortexing and centrifuged at 12000 rpm for 5 minutes to separate the layers. The upper layer was transferred to a fresh eppendorf tube and 0.1 vol. 3M NaAc and 1 vol. Iso-propanol were added and mixed by vortexing. The precipitate spooled out and rinsed with 1ml of 70% Ethanol. It was then centrifuged for 2

minutes at 12000 rpm and the ethanol was removed and the pellet was dissolved with sterilized distilled water.

Each extracted DNA was purified with column chromatography using mini spin S-300 (modified). The purified DNA was treated with 100µg/ml RNAse by incubating it at 37 $^{o}$C over night (modified). The purity of the extracted DNA was evaluated by running on 1% agarose gel. The gel (50ml 1 x TBE (Tris base 10.8g, 5.5g Boric acid, 4ml EDTA in 996ml distilled water) and 0.5g agarose) was heated in microwave for 60-70 seconds until melting completely and 2.5 µl Ethidium bromide was added to the gel before casting. The running buffer was 100ml 1 x TBE with 5µl Ethidium Bromide. After complete polymerization of the agarose gel, 1µl of each DNA with 3µl loading buffer was loaded into the wells on the gel and run at 80 volt and 37 ampere for 45 minutes and photographed using UVP transeluminator.

### 3.7.2. Repetitive Polymerase Chain Reaction (rep-PCR) Analysis

The primers used for PCR amplification were ERIC 1R and ERIC 2 with sequences of 5'-ATGTTAAGCTCCTGGGGATTCAC-3' and 5'- AAGTAAGTGACTGGGGTGAGCG-3' respectively (Veracruz *et al*., 1996 and Restepo, 2000) synthesized in TIB MOL-BIOL Company, Germany.

A sample of 1µl genomic DNA of each isolate, 5µl ERIC 1, 5µl ERIC 2 and 14µl sterilized distilled water was added to ready to go bids (dNTPs, Taq polymerase and buffer) and labeled. The PCR program was edited according to Veracruz *et.al*., (1996) as initial denaturation at 95 $^{o}$C for 7 minutes followed by 35 cycles that included denaturation at 94 $^{o}$C

for 1 minute, annealing at 52 $^{o}$C for 1 minute, extension at 65 $^{o}$C for 8 minutes and the final extension was at 65 $^{o}$C for 15 minutes followed by final incubation at 4 $^{o}$C. 10µl from each amplified PCR product was taken, loaded and run on a 0.75% agarose gel in 1 x TBE buffer stained with Ethidium Bromide at 80 volt and 37 ampere for 2 hours and photographed with a UVP transeluminator. A 1-kb ladder (Molecular marker) was used for molecular size reference of the bands of each isolate yielded from rep-PCR.

### 3.7.3. Data Analysis

The molecular weight of the bands in each lane was taken for analysis. In the regions where no bands were observed, 0 was used to indicate the absence. The analysis was done using SPSS statistical software. Discriminant Factor Analysis, Cluster Analysis and Principal Component Analyses were done to group isolates based on the available measurement yielded from repetitive PCR (Manly 1992).

## 3.8. Antibacterial test of *Pychnostachis abyssinica* extracts against *Xanthomonas campestris* Pv. *musacearum*

### 3.8.1. Extraction of the plant material

The leaf, bract, stem and root of *Pychnostacis abyssinica*, locally called ‘Fanfo’ were collected from Hadya zone, Limo wereda in large amount for extraction purpose.

**a. Hot and Cold Extraction of the Bract and the Leaf**

The bract was chopped and allowed to dry for some time. The dried bract and the wilted leaf were taken separately for hot extraction in Soxhlet apparatus. After 5 hours of extraction with methanol, the extracts were cooled, concentrated in rotary evaporator and dried in a dessicator. For cold extraction, the chopped dried bract and the wilted leaf were transferred to different columns and washed continuously with methanol until a light green color was observed. The extracts were concentrated in a rotary evaporator and left in a dessicator over night to dry. The dried extracts were weighed and kept at 4 $^{o}$C.

**b. Hot Extraction of the stem and Root**

The chopped stem and root were taken for extraction with methanol in Soxhlet apparatus separately. The extracts obtained were concentrated in rotary evaporator. The dried extract was weighed, labeled and kept at 4 $^{o}$C.

### 3.8.2. In-vitro evaluation of the crude extract

From each crude extract, a suspension was prepared with a concentration of 0.01g/ml using methanol as solvent. The same concentration of streptomycin and oxytetracycline were also prepared using water and methanol as solvent respectively.

Antibiotic assay discs with a diameter of 1/4 inch were used for the test. The discs were impregnated with10µl, 25µl, 50µl and 75µl of each suspension with 3 replication and allowed d to dry over night. Simultaneously, each isolate was grown on nutrient broth for 24 hours at 28 $^{o}$C. Using a sterilized swab each isolate was swabbed on YPSA (Yeast Peptone Sucrose agar) plates and immediately the impregnated discs were placed on the plates and then incubated at 28 $^{o}$C for 48-72 hours and the inhibition zone was measured.

# 4. RESULTS

## 4.1. Morphological and Biochemical Characteristics

Forty-eight bacterial colonies were picked from the 32 bacterial samples collected from infected enset plants and grown on YPSA plates. Most of the isolates showed mucoid growth with light to deep yellow and creamy (honey) color of colonies (Appendix1).

All the isolates were tested for their reaction to Gram staining and for their growth ability on asparagine agar and broth. All isolates were found to be gram negative except one isolate $ANHTmz_2$ (24) (Appendix 1). Among the gram negatives, 26 isolates that did not grow on asparagine medium and other three isolates that grew on asparagine medium were taken for further tests (Appendix 1). These 3 isolates were GIMB (38) from banana, YCMTge (22) and $GEMSbe_2$ (47). The first two are yellow and highly mucoid on YPSA plates and no other bacterial colonies were found grown together. The third isolate was taken because it has deep yellow colony color.

The growth of the isolates on nutrient agar with 5% glucose medium showed variation in color and growth character of colonies. There were isolates, which showed highly mucoid type of growth with yellow colony color as compared with their growth on YPSA and there were also isolates with less mucoid growth and yellow to creamy (honey) colony color (Table 2). Among the 29 isolates tested for Xanthomonadin pigment production, 13 isolates produced the pigment (Table 2) where most of these isolates exhibited light to deep yellow colony color on YPSA medium.

Table 2. Growth on nutrient agar with5% glucose and pigment production

| Isolates | Characteristics of growth on Nutrient agar with 5% Glucose | Xanthomonadin Production |
|---|---|---|
| 1, 2, 12, 13, 14,19, 20,22,47 | Light yellow to deep yellow colony color and mucoid growth | + |
| 38 | Yellow colony color and mucoid growth | - |
| 11, 15, 16, 17 | Light yellow colony color with less mucoid growth | + |
| 3, 4, 7, 8, 10, 23 | Light yellow colony color with less mucoid (tiny) colonies | - |
| 5, 9, 18, 29,30, 33, 37, 42 | Creamy (honey) colony color and highly mucoid growth | - |
| 43 | Creamy (honey) and tiny colonies (less mucoid) | - |

+ = Positive
-= Negative

Variation among isolates was observed in all biochemical tests. For NaCl tolerance test, all isolates tolerated 1% and 2% NaCl except one isolate $HSHSgu_1$ (3), which did not grow on 2% NaCl. 10 isolates were found tolerant to 5%, 4 isolates to 4% and 6 isolates were found tolerant to 3% NaCl (Table 3).

Table 3. NaCl tolerance of isolates

| NaCl Concentration (%) | NaCl tolerant isolates |
|---|---|
| 5 | 4,7,20,22,30,33,37,38,42,43 |
| 4 | 5,18,23,29 |
| 3 | 8,9,13,16,17,19 |
| 2 | 1,2,10,11,12,14,15,47 |
| 1 | 3 |

On observation made for gelatin liquefaction from 3 to 21 days, 23 isolates liquefied gelatin where 9 of them liquefied within 3 days (Table 4).

Table 4. Gelatin liquefaction ability of isolates

| Isolates able to liquefy in 3 days | Isolates able to liquefy in 7 days | Isolates able to liquefy in 21 days | Isolates not able to liquefy |
|---|---|---|---|
| 1,2, 4, 9, 11, 13, 19, 33, 47 | 3, 8, 10, 15, 16, 18, 20, 22, 37, 38, 43 | 23, 29,42 | 5, 7,12,14,17,30 |

Only 3 isolates were able to reduce nitrate, 13 isolates produced $H_2S$ gas and 3 isolates found positive to indole production (Table 5). Oxidase reaction test was negative to 16 isolates, weak positive to 2 isolates and the rest were positive and Catalase reaction was positive to all (Table 5). With regard to citrate and malate utilization of the isolates, only 1 isolate did not utilize malate and 8 isolates did not utilize citrate. All the other isolates utilized both organic acids (Table 5). In casein and starch hydrolysis ability, 4 isolates were found positive for both casein and starch. 5 isolates hydrolyzed casein but not starch and another 5 isolates hydrolyzed starch but not casein. 15 isolates hydrolyzed neither casein nor starch (Table 5).

## 4.2. Carbohydrate Utilization

All isolates except isolate GEMSbe$_2$ (47) utilized and produced acid from fructose and 5 of them produced acid with gas. Twenty isolates utilized cellobiose in which 10 of them produced acid with gas and 9 isolates did not utilize cellobiose at all. Arabinose was utilized by 16 isolates among which 2 isolates produced acid with gas and the rest 13 isolates did not utilize the sugar. In all cases, the degree and the time needed for utilization found varied (Table 6).

Table 5. Reaction of isolates to different Biochemical tests

| Biochemical Test | Isolates | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 22 | 23 | 29 | 30 | 33 | 37 | 38 | 42 | 43 | 47 |
| Casein hydrolysis | +3 | +3 | NG | +2 | - | NG | - | +3 | - | +3 | - | +3 | - | - | - | - | - | +3 | - | - | - | - | - | +3 | - | - | - | - | +3 |
| Starch hydrolysis | +3 | - | +1 | - | - | +1 | +2 | - | - | +2 | - | - | +2 | +2 | - | - | - | +3 | - | - | - | - | - | - | - | - | - | - | +3 |
| Nitrate reduction | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| $H_2S$ production | - | - | + | - | + | - | - | - | - | + | - | + | - | - | + | - | - | + | - | + | + | + | + | - | + | - | - | + | + |
| Indole production | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | + | - | - | - | - | - | - | - | - | - | - |
| Oxidase reaction | + | + | - | + | - | - | - | + | + w | + | - | + | + | + w | + | + | - | + | - | - | - | - | - | + | - | - | - | - | - |
| Catalase activity | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Citrate utilization | - | - | + | + | - | - | - | + | + | + | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Malate utilization | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |

+3= 3-4cm clear zone
+2= 1-2 cm clear zone
+1= < 1cm clear zone

+= Positive
-= Negative
NG= no growth

+w= weak positive

Table 6. Carbohydrate utilization

| Carbon source | Isolates which utilized nitrite Within 3 days | | Isolates which utilized nitrite Within 7 days | | Isolates which utilized nitrite Within 21days | | Isolates not able to utilize |
|---|---|---|---|---|---|---|---|
| | Acid production. | Acid+gas Production | Acid Production. | Acid+gas Production | Acid production. | Acid+gas | |
| Arabinose | 10 | -- | 14, 16, 23, 29, 43 | -- | 9, 12, 15, 17,18, 22, 30, 33 | 20,38 | 1,2,3,4,5,7,8,11, 13,19,37,42,47 |
| Cellobiose | 3,8 | 5,11,18,23, 29,30,37,38, 16, 43 | 12,15,20, | -- | 7,10,14,17,22 | -- | 1,2,4,9,13,19,33, 42,47 |
| Fructose | 16,18,20,22, 8,29,37 | 23,30,38,43, 5 | 3,4,9,12,15, 19 | -- | 1,2,7,10,11,13, 14,17,33,42 | -- | 47 |

### 4.3.1. Hypersensitivity and Pathogenicity Reaction

The injected leaves of tobacco showed positive reaction to all isolates within 48-72 hours where chlorosis type reaction to brown necrosis was observed around the injection point (Fig.2). Among the isolates FGMSto (7) showed aggressive reaction with very deep brown necrosis earlier than the other isolates. Those leaf parts inoculated with sterilized distilled water remained green.

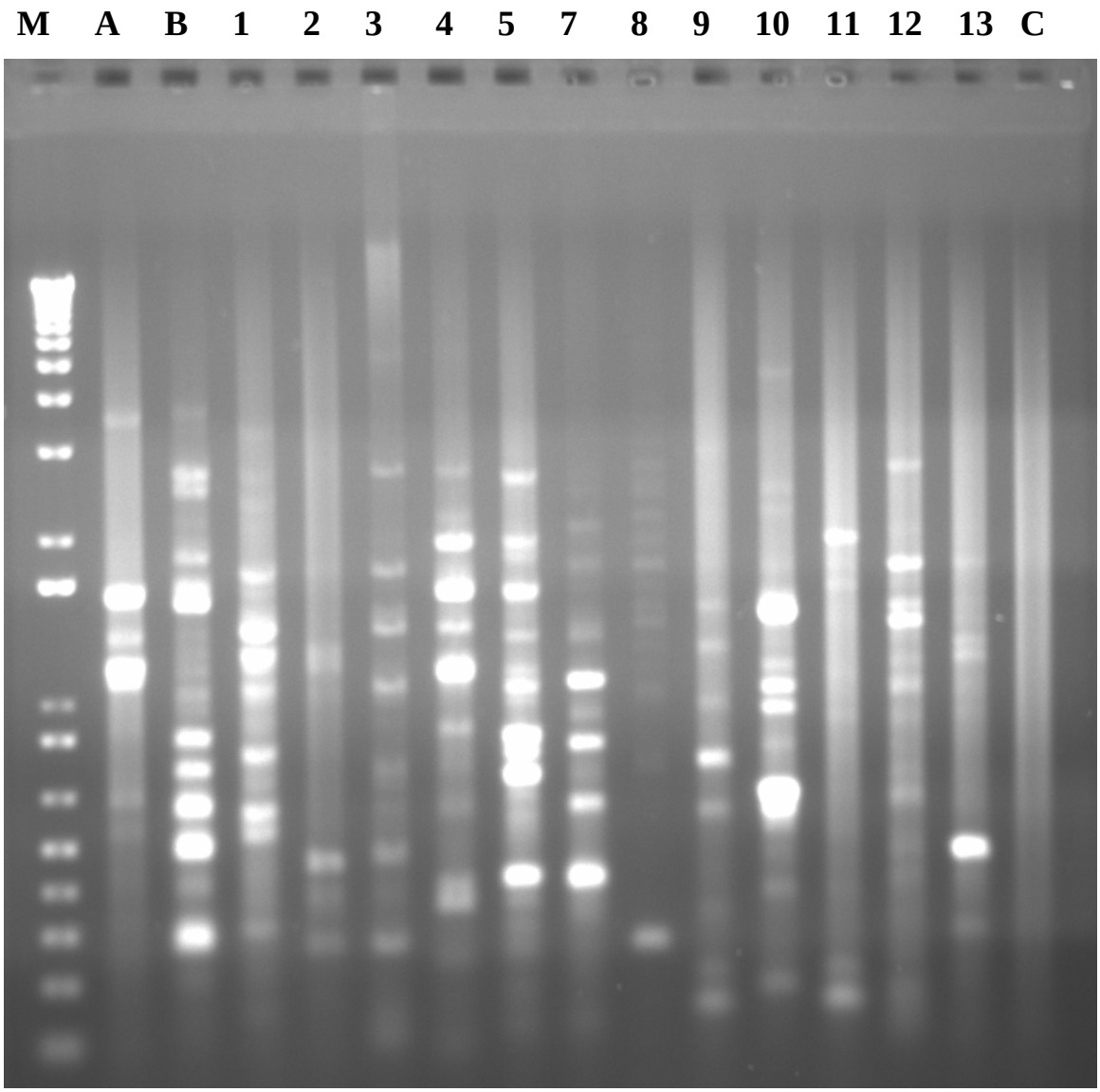
Regarding the pathogenicity test, the inoculated leaves of the enset suckers showed light yellow to dark brown necrosis around the inoculated area of the leaves within 7 days and no necrosis was observed in control suckers inoculated with sterilized water. However, no complete wilting of the suckers was observed in 12 weeks observation period.

Fig 2. Hypersensitivity test on Tobacco

## 4.4. Repetitive-PCR (rep-PCR) Products

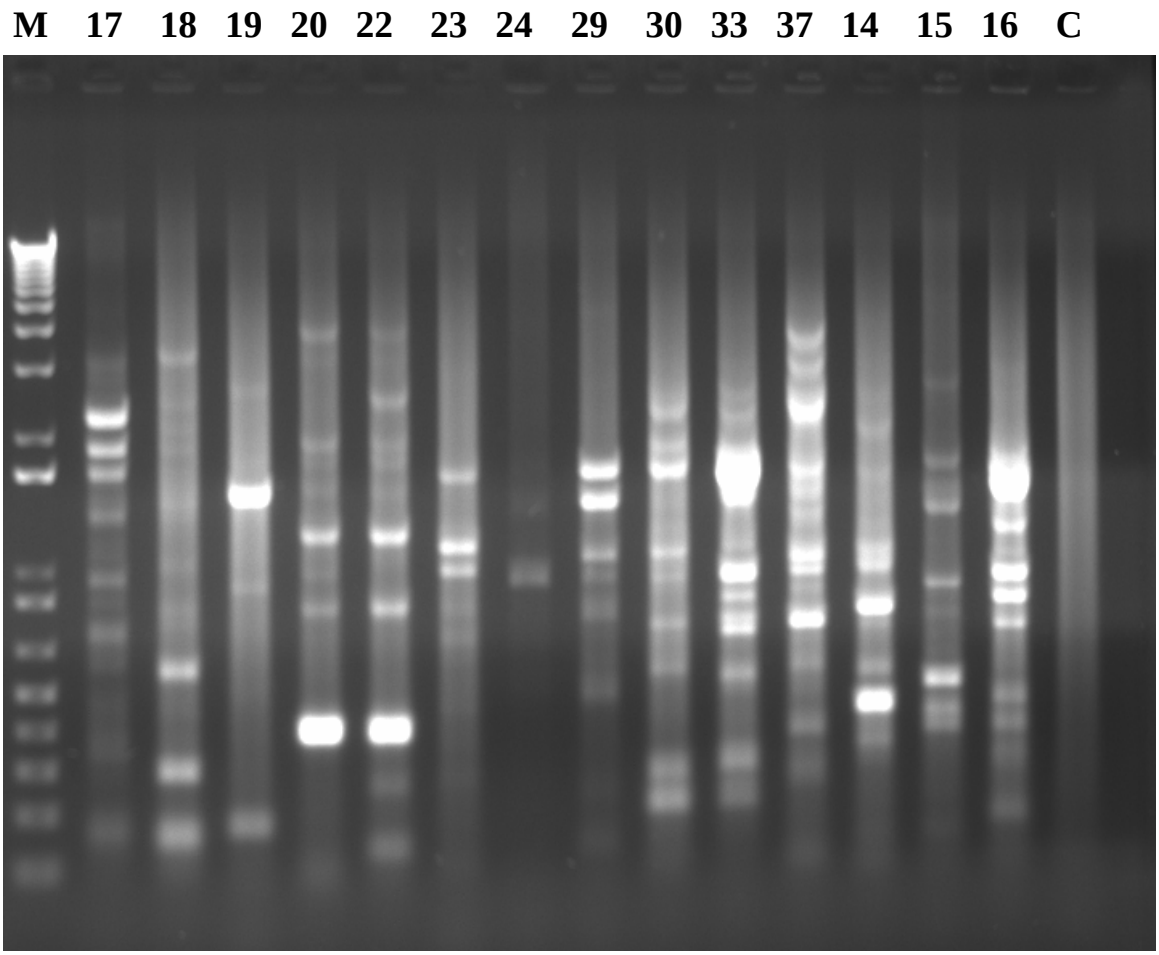
### 4.4.1. Band patterns of the isolates

In this analysis other than the isolates, which were subjected to the above biochemical tests, the two reference strains (DSMZ 1050 and DSMZ 1350) and those that were grown on asparagine medium were subjected to rep-PCR analysis. In addition, three isolates from corm rot, sheath rot and dead heartleaf rot of enset disease samples were included for comparison. The ERIC primers yielded different band patterns in all isolates where 1kb Plus DNA Ladder was used as molecular marker (Fig 3 A, B, C) and Fig 4 (A, B).




**A**

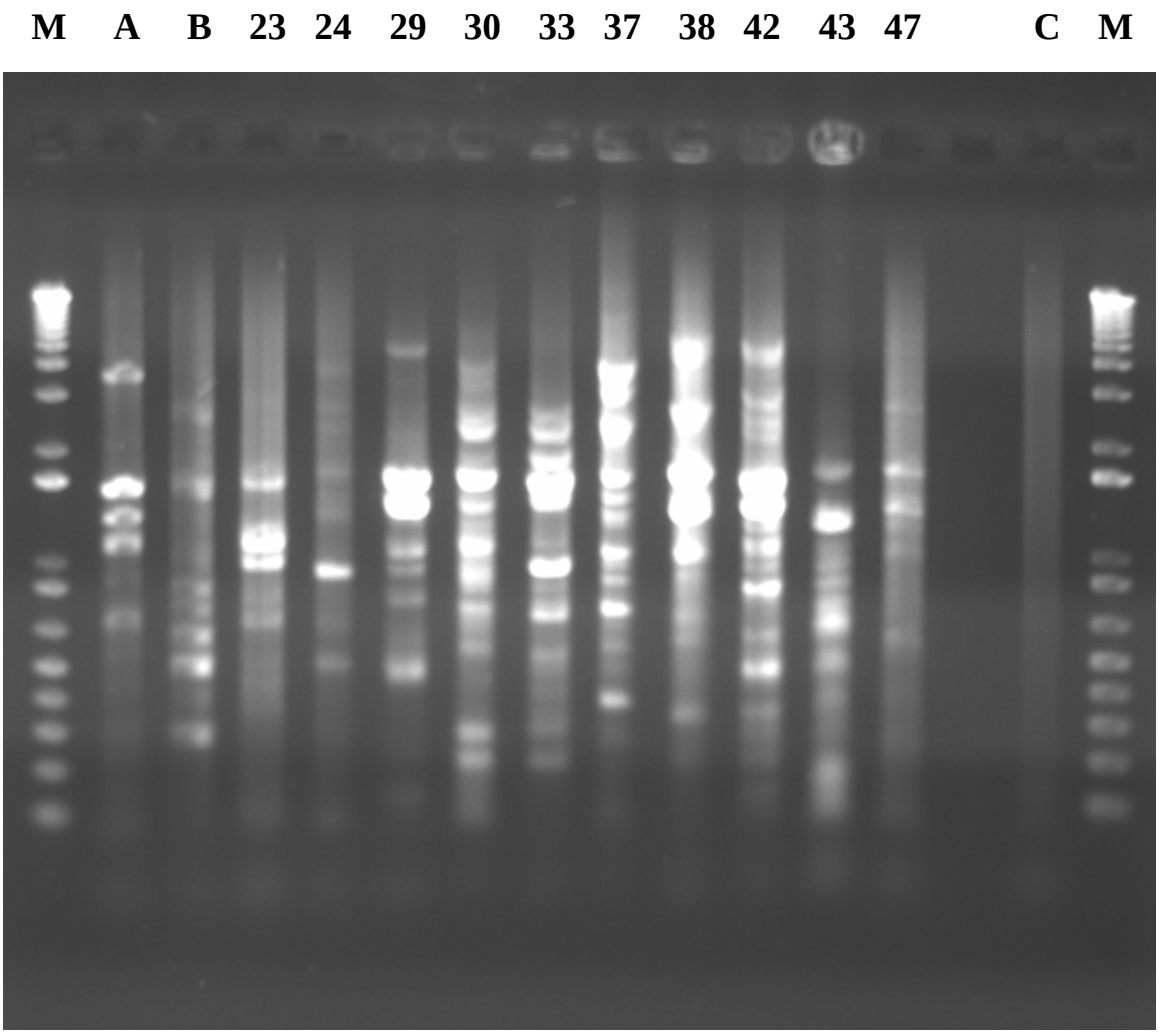
N.B.
M: Molecular marker
A: DSMZ 1050
B: DSMZ 1350
1. FGMTge
2. YCMSas
3. HSHSgu1
4. HSHSda1
5. HSHSda2
7. FGMSto
8. CHMTye1
9. ANHSgi1
10. CHMSne1
11. BOMSgn1
12. MLHSam1
13. GOMSgn1
C. Control (Sterilized water)




**B**

N.B.

| | |
|---|---|
| M. Molecular Marker | 29. SEMSgn |
| 17. ANHTmz$_1$ | 30. GOMSad |
| 18. LIHSbe | 33. GEMSbe |
| 19.SEMSbe$_1$ | 37.BOMTtu |
| 20.LIHSsw$_2$ | 14.SEMTmg |
| 22.YCMTge | 15. LIHSsw1 |
| 23.ARMSnk | 16.ANHSsi$_1$ |
| 24. ANHTmz$_2$ | C. Control (sterilized water) |



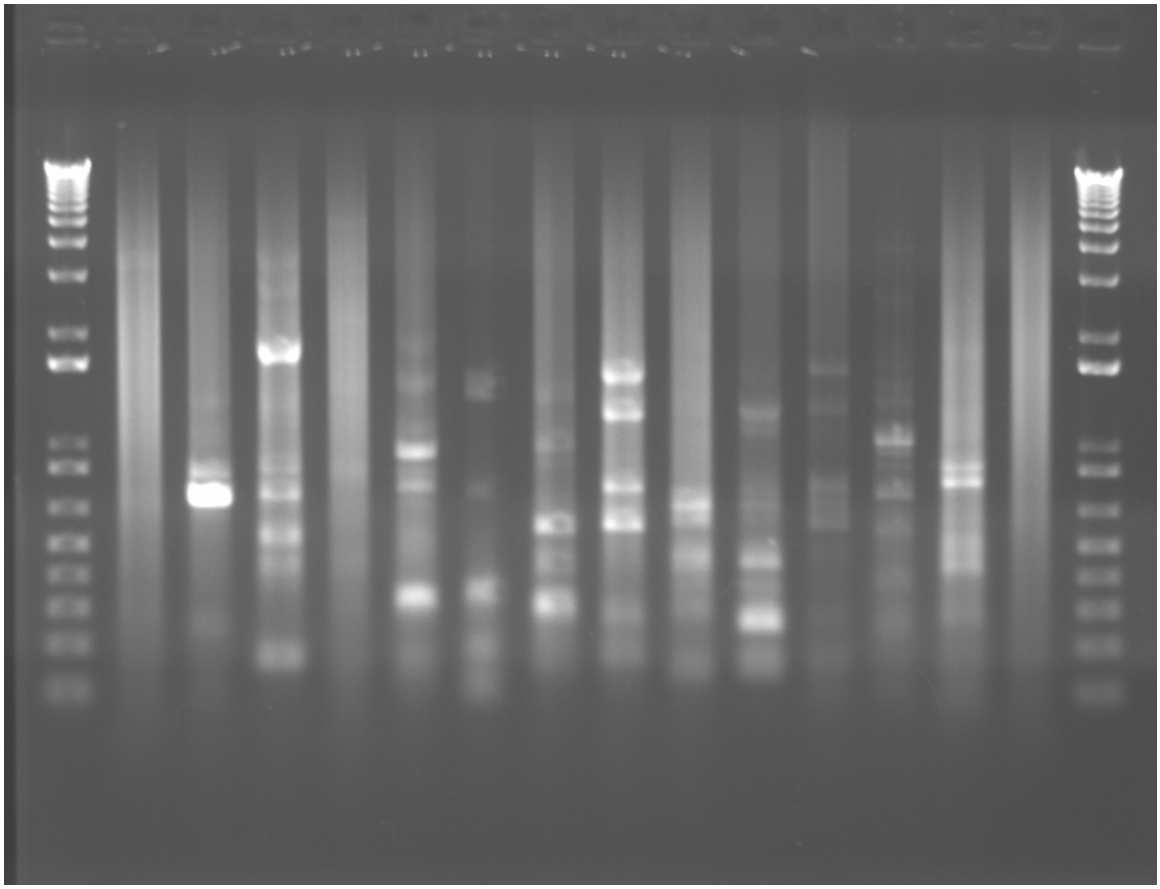

**C**

N.B

| | | | | |
|---|---|---|---|---|
| M. Molecularmarker | 23. ARMSnk | 33. GEMSbe$_1$ | 42. SEMSbe $_2$ | M. molecular marker |
| A. DSMZ 1050 | 29. SEMSgn2 | 37. BOMTtu$_2$ | 43. LIHSdi | C. Control |
| B. DSMZ1350 | 30. GOMSad | 38. GIMB | 47.GEMSbe$_2$ | |

Fig 3. (A, B, C) Agarose gel pictures showing rep-PCR band patterns of *Xanthomonas campestris* Pv. *musacearum* strains generated with ERIC1 and ERIC 2 primers

**M 44 35 46 31 25 26 28 8B 48 34 39 37B MT C M**



A

M - Molecular marker

44- MLHSam2

35- MLHSt

46- SEMSbe3

31- GOMSgn$_2$

25- ANHSgi$_2$

26- BOMSsi

28- SEMSbe4

8B- CHMTye

48- GEMTsh

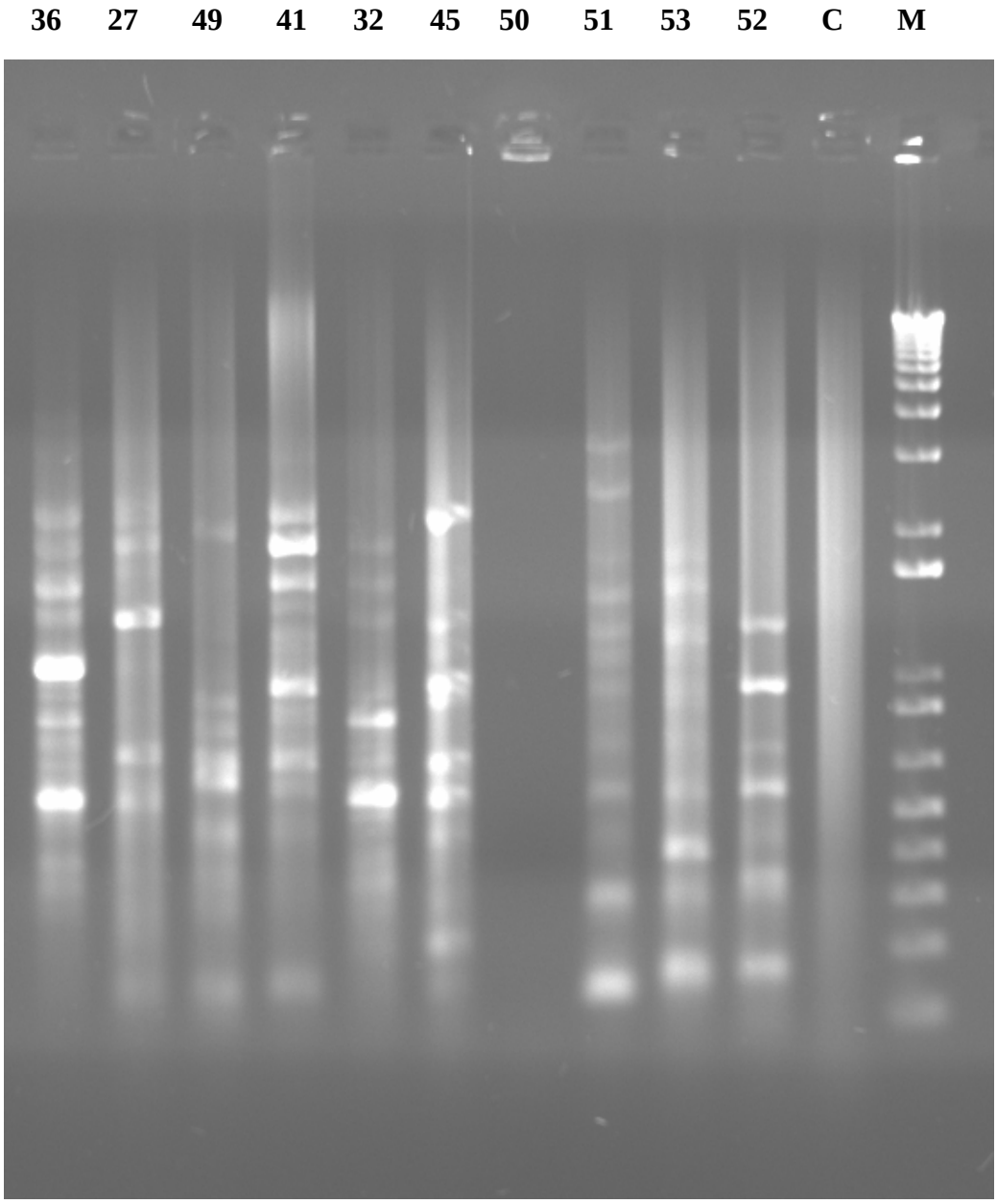
34- MLHTsh

39- CHMSne$_2$

37(B)- BOMTtu

C- Negative Control

M- Molecular marker




**B**

| | | | |
|---|---|---|---|
| 36- BOMTtu2 | 41- SEMSbe4 | 50- HSHSgu3 | 52- Dead heart leaf rot |
| 27- SEMSgn2 | 32- GEMSbu | 51- Corm rot | C- Negative control |
| 49- HSHSgu2 | 45- BOMSgn2 | 53- Sheath rot | M- Molecular marker |

Fig. 4 (A, B) Agarose gel pictures showing rep-PCR band patterns of isolates suspected not to be *Xanthomonas campestris* Pv. *musacearum* strains generated with ERIC1 and ERIC 2 primers

## 4.5. Discriminant Function Analysis

Discriminant function analysis is used to separate two or more groups of individuals based on given measurements for these individuals on several variables (Manly, 1992). The bands from row 1 (R1) to row 25 (R25) with different molecular weight per isolate yielded from rep-PCR were taken as variables and subjected to discriminant function analysis to separate isolates in to different groups.

### 4.5.1. Canonical Discriminant Function in grouping isolates

According to Manly (1992) "The canonical discriminant functions F1, F2,…,Fz are linear combinations of the original variables chosen in such a way that F1 reflects group differences as much as possible; F2 captures as much as possible of the group differences not displayed by F1; and F3 captures group differences not displayed by F1 and F2; etc. The hope is that the first few functions are sufficient to account for almost all of the important group differences."

Function1 corresponding to the largest eigenvalue 54.208 accounted for greatest amount of variation that is 92.5% and function2 with 4.396 eigenvalue contributed for 7.5 % variation (Table 7). R1 and R2 contributed for the variation obtained in function1 and function2 (Table 8) and the other variables failed the tolerance test (minimum tolerance level is 0.001).

Table 7. Eigenvalue of the first two functions used in the analysis for grouping isolates

**Eigenvalues**

| Function | Eigenvalue | % of Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 54.208[a] | 92.5 | 92.5 | .991 |
| 2 | 4.396[a] | 7.5 | 100.0 | .903 |

a. First 2 canonical discriminant functions were used in the analysis.

Table 8.

**Standardized Canonical Discriminant Function Coefficients**

| | Function | |
|---|---|---|
| | 1 | 2 |
| R1 | .005 | 1.000 |
| R2 | 1.000 | -.005 |

R2 is the variable that contributed to the largest variation among isolates.

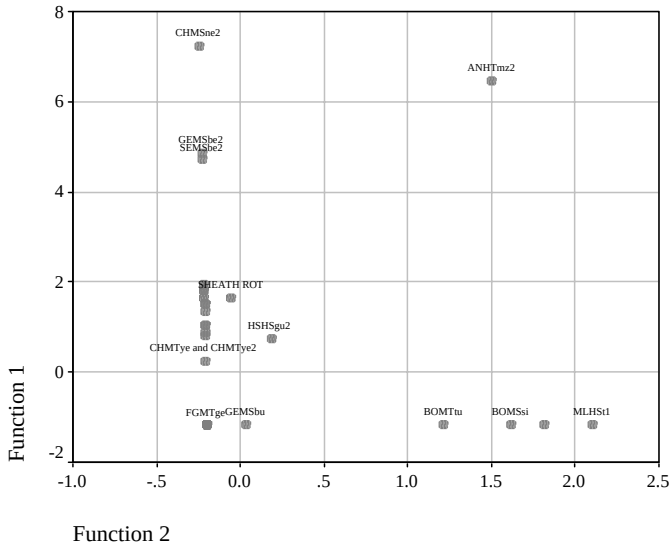



Fig 5. Plots of all isolates against their values for two canonical discriminant functions

1. MLHSam
2. CHMSne
3. ANHTmz2
4.SEMSbe2,GeMSbe2
5. Sheath rot
6. CHMTye
7.FGMTge,YCMSas,HSHSgu,HSHSda2,ANHSgi,CHMSne,SEMTmg,LIHssw1,ANHSsi,ANHTmz1,LIHSbe, SEMSbe,LIHSsw2,YCMTgeARMSnk,ANHSgi2,SEMSbe3,SEMSgn,GOMSgn2,GEMSbe,MLHTsh,GIMB, LIHSdi, GOMSad,SEMTmg2, GEMTsh,HSHSgu3, DHL, Xcc ...
8. BOMTtu

Among the isolates with largest value for F1, CHMSne2 and ANHTmz2 can be mentioned which scored 7.232 and 6.476 respectively. These two isolates are among the isolates, which grew on Asparagine medium. Most isolates, which were able to grow on Asparagine medium, had similar value for F1 that is -1.176. Refer Appendix 2 for F1 and F2 values of all isolates.

### 4.5.2. Canonical Discriminant Function in grouping regions

The regions from where the isolates collected were subjected to this analysis based on the banding patterns of their isolates yielded from rep-PCR.

Table 9. Eigenvalues of the first twelve functions used in the analysis for grouping regions

**Eigenvalues**

| Function | Eigenvalue | % of Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 6.506[a] | 30.9 | 30.9 | .931 |
| 2 | 4.533[a] | 21.5 | 52.4 | .905 |
| 3 | 2.736[a] | 13.0 | 65.3 | .856 |
| 4 | 2.355[a] | 11.2 | 76.5 | .838 |
| 5 | 1.676[a] | 8.0 | 84.5 | .791 |
| 6 | .949[a] | 4.5 | 89.0 | .698 |
| 7 | .760[a] | 3.6 | 92.6 | .657 |
| 8 | .596[a] | 2.8 | 95.4 | .611 |
| 9 | .458[a] | 2.2 | 97.6 | .560 |
| 10 | .271[a] | 1.3 | 98.9 | .462 |
| 11 | .132[a] | .6 | 99.5 | .341 |
| 12 | .109[a] | .5 | 100.0 | .313 |

a. First 12 canonical discriminant functions were used in the analysis.

The first three functions with cumulative variance of 65.5% were used to choose the important variables contributing for the largest variation among regions from where the

isolates were collected. R16 and R18 are the variables that contributed for highest variations among regions (Fig 6). Refer Appendix 3 for the values of each variable for the three functions.

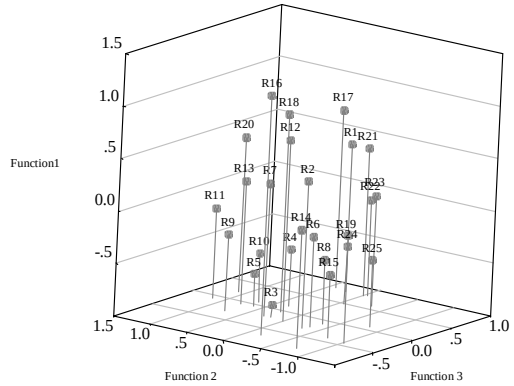



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Fig 6. Values of each variable for the three functions, F1, F2 and F3

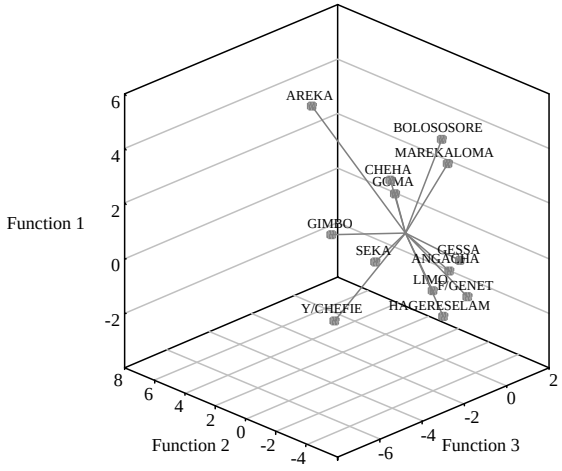



Fig 7. Plots of 12 regions against their values for 3 canonical discriminant functions

In Figure 7, regions were plotted on the three - D graph to see the similarity or difference among them based on the variability of the isolates. The distance and direction of each region

from the centroid where the value for F1, F2 and F3 is 0 indicate the similarity or difference. In the above case, AREKA is the region with the highest variability with respect to F1 with a value of 3.741 (Appendix 4). This variability is contributed by R16.

### 4.5.3. Canonical Discriminant Function in grouping altitudes

The altitudes of the regions from where the isolates were collected were grouped again based on the values of each isolates yielded from rep-PCR.

Table 10. Eigenvalues of the first twelve functions used in the analysis for grouping altitudes

**Eigenvalues**

| Function | Eigenvalue | % of Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 4.814[a] | 27.8 | 27.8 | .910 |
| 2 | 3.484[a] | 20.1 | 47.9 | .881 |
| 3 | 2.834[a] | 16.4 | 64.3 | .860 |
| 4 | 1.709[a] | 9.9 | 74.1 | .794 |
| 5 | 1.167[a] | 6.7 | 80.8 | .734 |
| 6 | .921[a] | 5.3 | 86.2 | .692 |
| 7 | .894[a] | 5.2 | 91.3 | .687 |
| 8 | .592[a] | 3.4 | 94.7 | .610 |
| 9 | .471[a] | 2.7 | 97.5 | .566 |
| 10 | .332[a] | 1.9 | 99.4 | .499 |
| 11 | .109[a] | .6 | 100.0 | .313 |

a. First 11 canonical discriminant functions were used in the analysis.

The first three functions with 64.3% cumulative variation were used to choose the variables with large contribution for variation among altitudes. R16 and R18 were again found to contribute the highest (Fig 8). Refer Appendix 5 for the values of each variable for the three functions.

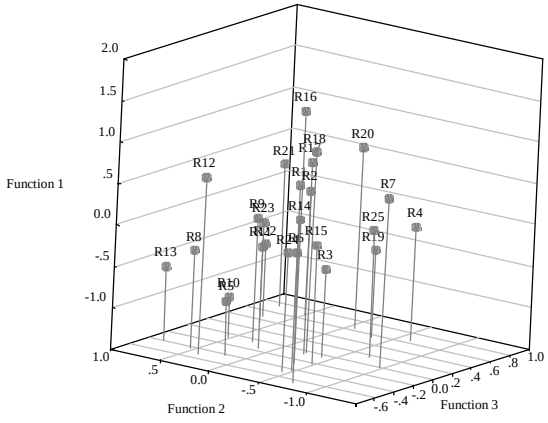



Fig 8. Values of each variable for the three functions, F1, F2 and F3

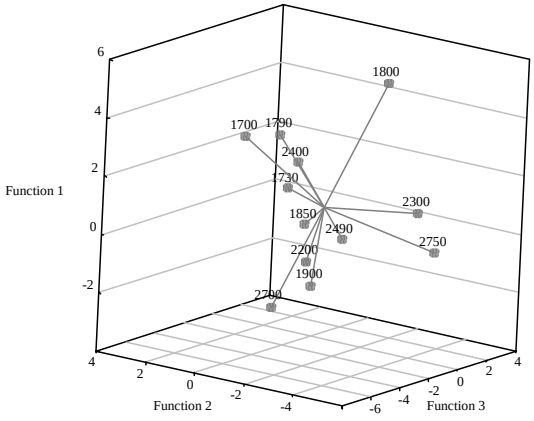



Fig 9. Plots of 12 altitudes against their values for the canonical discriminant functions

The altitude 1800 has the highest variability with respect to function1 with a value of 4.686 (Appendix 6). This altitude represents the region AREKA.

## 4.6. Cluster Analysis

### 4.6.1. Clustering of Isolates

Cluster analysis is used for grouping 'objects', which have scores on different variables into classes so those 'similar' ones are in the same class. Clustering of isolates was done based on the values of clustering variable(s) and isolates are grouped as follows based on the percentage of similarity (Fig 10).

I- 100-95% similarity

G1. Xcc (DSMZ1050) and Xc (DSMZ 1350)
G2. LIHSsw2 and YCMTge
G3. FGMTge and G1
G4. HSHSgu3 and MLHSam2
G5. BOMSgn and MLHSam
G6. CHMTye2 and CHMSne2
G7. Corm rot and Sheath rot

II- 94-90%

G1. LIHSbe and GOMSad
G2. HSHSgu1 and ANHSgi2
G3. CHMSne and BOMTtu2
G4. MLHSt1and G4(I)
G5. CHMTye1 and ANHSgi1
G6. DHLR & GEMTsh
G7. LIHSdi and G5(I)
G8. SEMSbe3 and BOMSgn2
G9. GOMSgn and BOMTtu
G10. GEMSbe and G1(II)
G11. HSHSda1 and ANHTmz2
G12. SEMSgn and LIHSsw1
G13. FGMSto and G2(II)

III. 89-85%

G1. YCMSas and ARMSnk
G2. SEMSgn2 and GEMSbu
G3. ANHSsi1 and G7(I)
G4. SEMSbe 4 and Mtb
G5. BOMSsi and G13(II)
G6. SEMTmg1 and SEMSbe2
G7. HSHSda2 and G3(I)
G8. SEMSbe1 and G1(III)
G9. GEMSbe and G12(II)
G10. SEMTmg2 and G2(I)
G11. SEMSgn2 and G4(II)
G12. G4(III) and G9(II)
G13. ANHTmz1 and G7 (III)
G14. SEMSbe3 and G7(II)
G15. GOMSgn2 and G10 (III)

IV. 84-80%

G1. G11(III) and G12(III)
G2. BOMTtu1 and G10(II)
G3. G9(III) and G5(II)
G4. GIMB and G6(II)
G5. G1(IV) and G3(II)
G6. G3(III) and G6(I)
G7. G3(IV) and G11(II)
G8. MLHTsh and G6(II)
G9. G12(III) and G8(III)
G10. G2(IV) and G7(IV)
G11. G15(III) and G5(III)
G12. HSHSgu2 and G4(IV)
G13. G5(IV)andG6(IV)

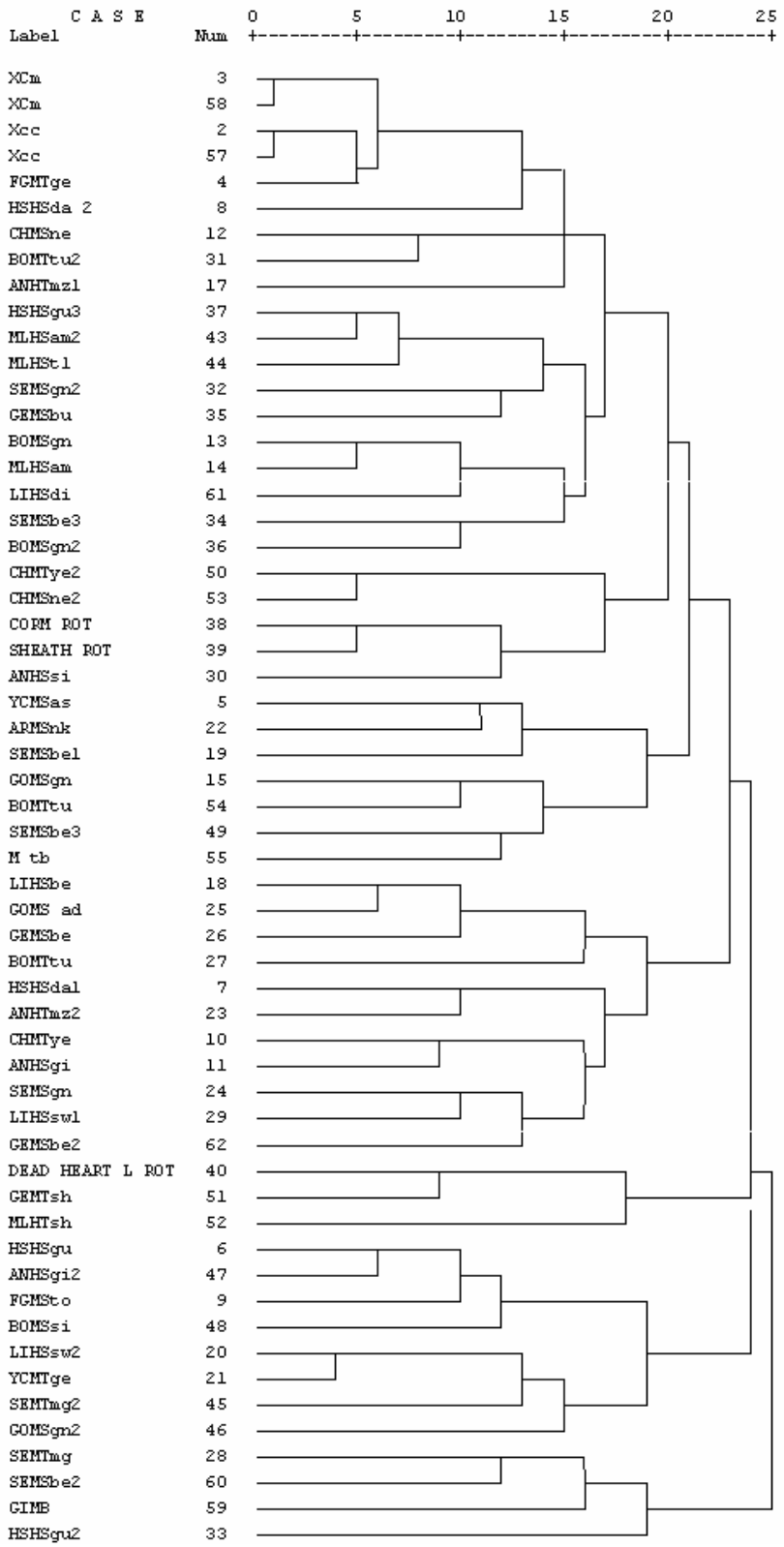
V. 79-75%

G1. G13(IV) and G9(IV)
G2. G1(V) and G10(V)
G3. G2(V), G11(IV) and G8(IV)
G4. G3(V) and G12(IV)

* * * * * * H I E R A R C H I C A L  C L U S T E R  A N A L Y S I S * * * * * *

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine



### 4.6.2. Clustering of Regions

The regions where the isolates originated were grouped into 4 major groups based % of similarity (Fig 11).

I- 100- 95% similarity

- G1. Gessa and Seka
- G2. Gessa and Angacha
- G3. Hagereselam, G1 and G2
- G4. Bolososore and Mareka

II- 94-90%

- G1. Limo and Goma
- G2. Bolososore and G1(II)
- G3. Yirgachefie, Cheha and G2(II)

III- 89-85%

- G1. G3(II) and Fissehagenet

IV- 79-75%

- G1. Areka and G1(III)
- G2. Gimbo and G1(IV)

### 4.6.3. Clustering of Altitudes

The 12 different altitudes ranging from 1700 to 2750 were grouped into 4 major groups based % of similarity (Fig 12).

I- 100-95% Similarity

- G1. 2200 and 2700
- G2. 1850 and 2490
- G3. G1 and G2
- G4. 1790, 2400 and G3
- G5. 1900 and 2750

II- 94-90%

- G1. 1800 and G4(I )
- G2. 1730 and G5(I)
- G3. G2(II) and G1(II)

III- 80%

- G1. 2300 and G2(II)

IV- 75%

- G1. 1700 and G1(III)

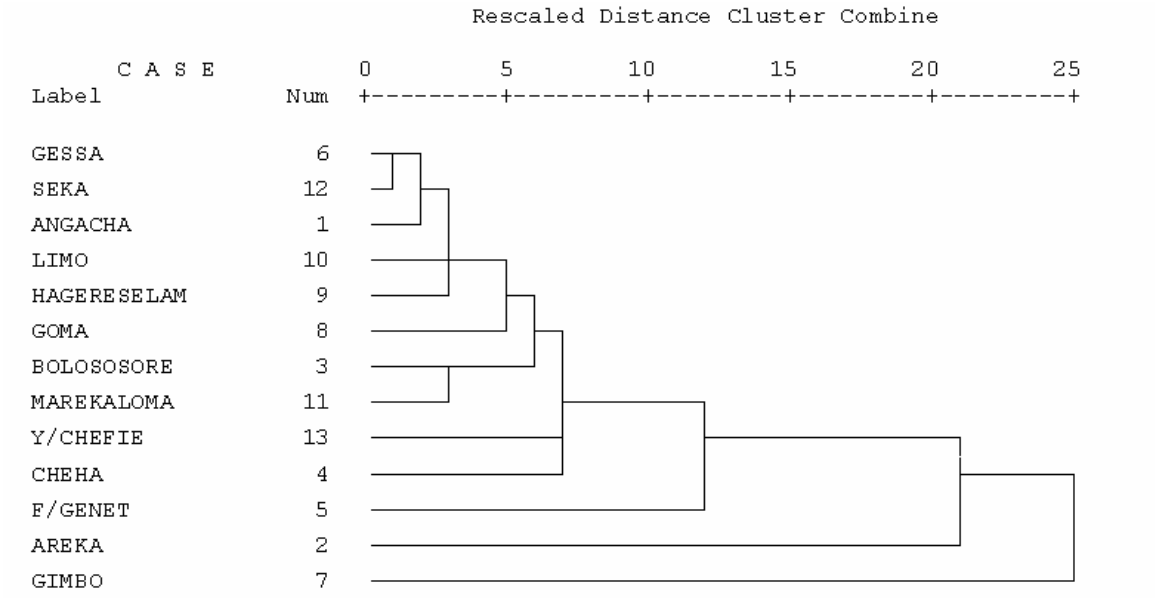



Fig 11. Dendrogram using average linkage among regions

* * * * * * H I E R A R C H I C A L  C L U S T E R  A N A L Y S I S * * * * * *

Dendrogram using Average Linkage (Between Groups)

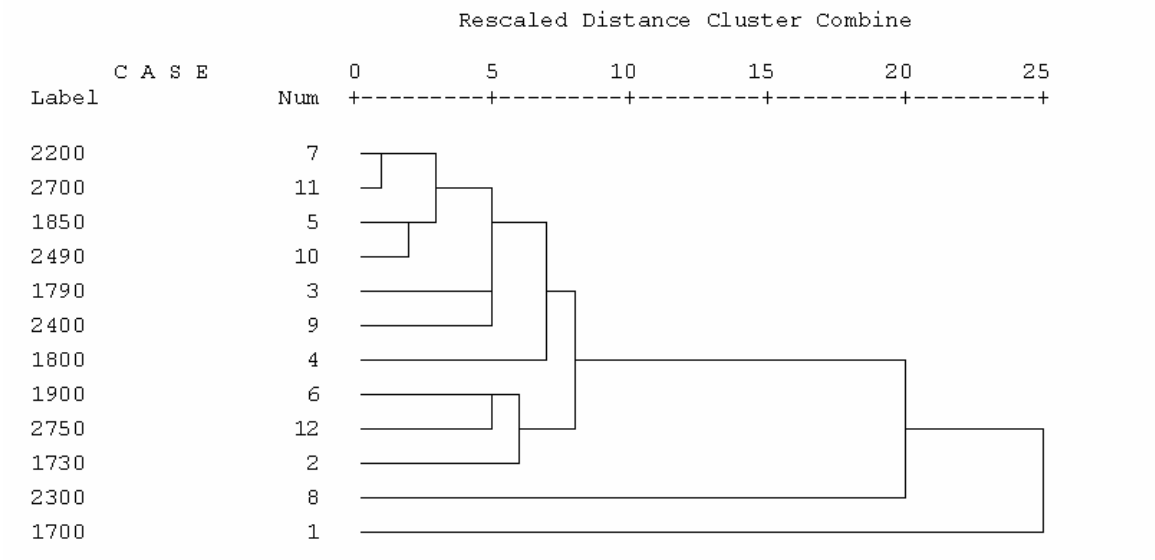



Fig12. Dendrogram using average linkages among altitude

## 4.7. Principal component analysis

This analysis is the simplest of the multivariate methods. The objective of the analysis is to take P variables X1, X2,…,Xp and find combination of these to produce indices Z1, Z2…Zp that are uncorrelated. The lack of correlation is useful property as it means the indices are measuring different 'dimensions' in the data (Manly, 1992).

From the discriminant factor analysis, variables that contributed for largest variation in isolates, regions and altitude grouping were identified. These are R1 and R2 for variation among isolates and R16 and R18 for variation in both regions and altitudes. Therefore, R2 was taken for extraction of principal components to do the analysis while taking isolates as variables and R16 to extract principal components to do the analysis while regions and altitudes are taken as variables based on their values for F1.

In Discriminant analysis, the variability of isolates was done for all bands (rows) and tried to see which of the bands is the highest contributor for the variability however, in principal component analysis, the variability of isolates was done only for the selected band. Therefore, principal component analysis can be taken as a data reduction process.

### 4.7.1. Comparison among Isolates based on R2

For this analysis, isolates were taken as variables. The first 12 components were found to account for nearly 92% of the total variance (Appendix 7). Although these 12 components are

important in indicating variation, only three principal components were taken for simplicity (Fig 13).

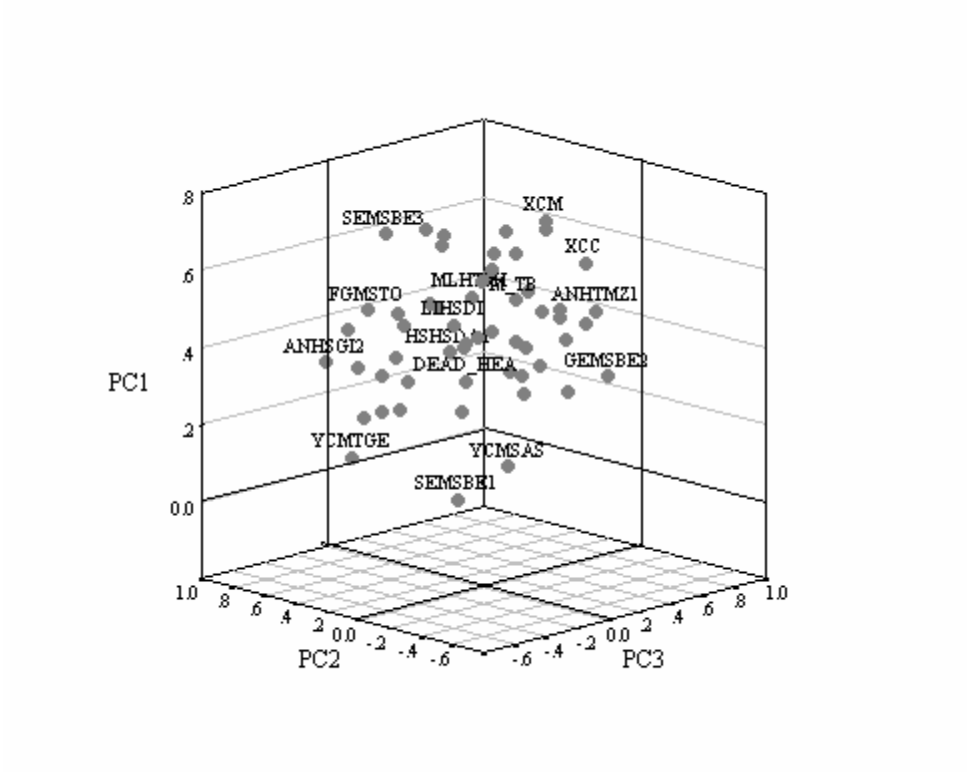



Fig 13. Isolates plotted based on their values for the three principal components

In the above figure, the first principal component (PC1) indicates which of the isolates have the highest variability for R2 **alone**. The isolates Sheath rot, SEMSbe3, BOMTtu2 and ANHSsi with values of 0.748, 0.736, 0.725 and 0.724 respectively for PC1 can be mentioned as isolates which have the highest varibility for R2 and are similar. Refer Appendix 8 for the values of isolates for PC1,PC2 and PC3.

### 4.7.2. Comparison among Regions based on R16

For this analysis, regions were taken as variables. The first 12 components were found to account for nearly 92% of the total variance (Appendix 9). Although these 12 components are important in indicating variation, only three principal components were taken for simplicity (Fig 14).

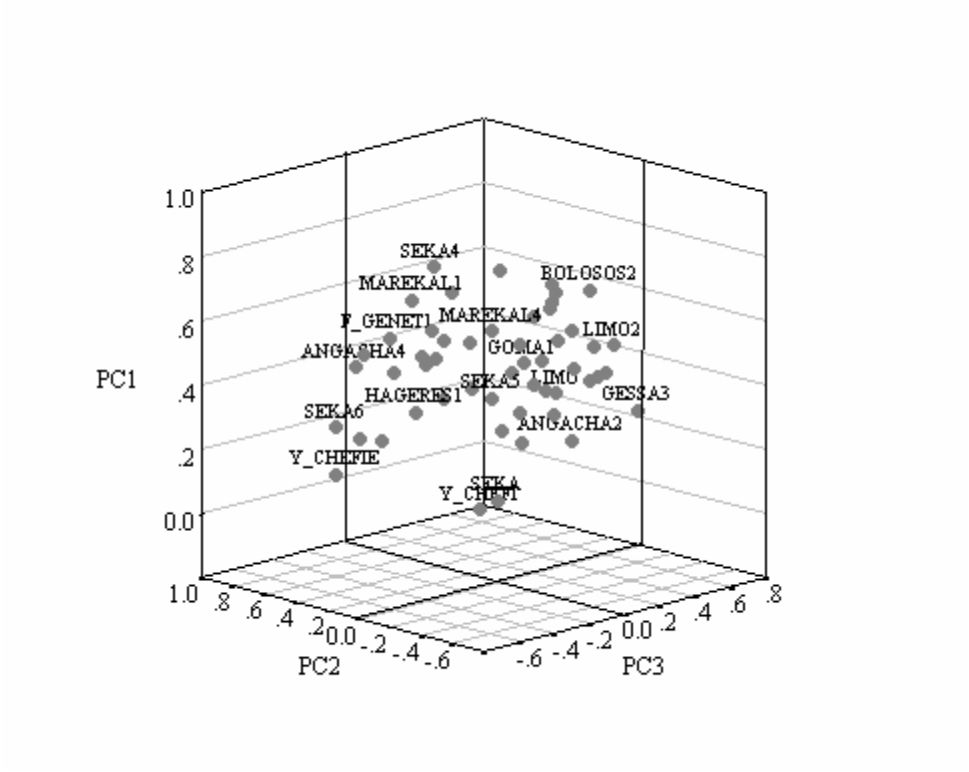



Fig 14. Regions plotted based on their values for the three principal components

The values of the regions for PC1, PC2 and PC3 are based on the variability of isolates for R16 **alone**. Bolososore, Seka and Angacha are among the regions that showed the highest variability for R16 . This indicates that there is high variability within isolates collected from each region. For example, there are about 6 isolates collected from Bolososore and there is

high variability among these isolates for R16 than other bands. Refer Appendix 10 for the values of the regions for PC1, PC2 and PC3.

### 4.7.3 Comparison among Altitudes based on R16

For this analysis, altitudes were taken as variables. The first 12 components were found to account for nearly 92% of the total variance (Appendix 11). Although these 12 components are important in indicating variation, only three principal components were taken for simplicity (Fig 15).

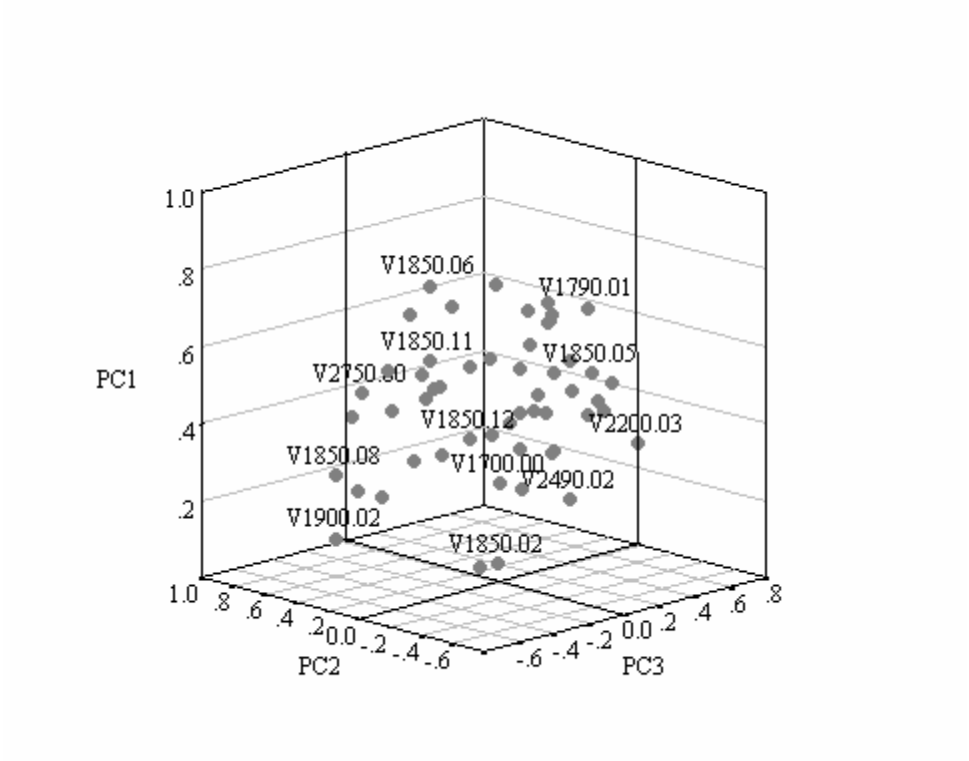



Fig 15. Altitudes plotted based on their values for the three principal components

The altitudes 2490 and 1850 showed the highest variability. 2490 represents the region Angacha and 1850 represents the regions Seka and Cheha. In the former results of principal component analysis, Angacha had shown highest variability for R16. Similar result is obtained in altitudes. From the two regions represented by 1850, Seka had also shown highest variability for R16 in PC analysis for regions. Refer Appendix 11 for the values of altitudes for PC1, PC2 and PC3.

## 4.8. Inhibitory Effect of crude extracts from *Pychnostacis abyssinica*

The crude extracts of the leaf, bract, stem and root of *Pychnostacis abyssinica* and antibiotics oxyteracycline and streptomycin were evaluated for their inhibitory effect on isolates that were not able to grow on asparagine medium and suspected to be *Xanthomonas campestris* Pv. *musacearum* isolates following the agar disc diffusion method. Extracts from leaf and bract through hot and cold extraction with methanol showed some inhibitory effect on some isolates. For example, 10-11mm inhibition zone by the leaf hot extract and 12-13mm by bract hot extract was observed on some of the isolates. The root and stem extracts shown no inhibition except some indications on some isolates. Table 11 indicates the inhibition zone of every extract and the two antibiotics on the isolates.

Table 11. Inhibitory effect of the Crude extracts from *Pychnostachis abyssinica* on different isolates

| I/N | Oxytetracycline | | | | Streptomycin | | | | Leaf Hot | | | | Leaf Cold | | | | Bract Hot | | | | Bract Cold | | | | Root | | | | Stem | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 25 | 50 | 75 | 10 | 25 | 50 | 75 | 10 | 25 | 50 | 75 | 10 | 25 | 50 | 75 | 10 | 25 | 50 | 75 | 10 | 25 | 50 | 75 | 10 | 25 | 50 | 75 | 10 | 25 | 50 | 75 |
| 1 | 24 | 24 | 25 | 25 | 2 | 26 | 3 | 31 | - | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 2 | 17 | 17 | 2 | 2 | 2 | 21 | 23 | 25 | _ | _ | _ | _ | _ | _ | _ | _ | 6 | 6 | 6 | 6 | _ | _ | _ | __ | _ | _ | _ | _ | _ | _ | _ | _ |
| 3* | 32 | 32 | 34 | 35 | 27 | 31 | 33 | 35 | 5 | 10 | 10 | 11 | _ | _ | 8 | 12 | 7 | 8 | 12 | 13 | _ | 5 | 10 | 15 | _ | _ | 7 | 9 | _ | _ | _ | 6 |
| 4 | 25 | 25 | 25 | 27 | 2 | 22 | 23 | 24 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 5* | 3 | 3 | 3 | 3 | 17 | 18 | 20 | 20 | _ | _ | _ | _ | _ | _ | _ | _ | 11 | 8 | 9 | 9 | _ | 6 | 7 | 17 | _ | _ | _ | _ | _ | _ | _ | _ |
| 7 | 23 | 23 | 24 | 24 | 19 | 20 | 23 | 25 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 8 | 14 | 14 | 14 | 14 | 11 | 14 | 17 | 20 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 9 | 19 | 19 | 19 | 20 | 25 | 30 | 35 | 37 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 10 | 22 | 23 | 23 | 23 | 0 | 0 | 06 | 06 | _ | _ | 7 | 7 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 11 | 18 | 19 | 20 | 20 | 1 | 15 | 15 | 19 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 12* | 27 | 27 | 27 | 27 | 23 | 23 | 26 | 26 | _ | _ | _ | 8 | _ | _ | 7 | 7 | _ | _ | 6 | 8 | _ | _ | _ | 7 | _ | _ | 7 | 7 | _ | _ | + | + |
| 13 | 18 | 18 | 18 | 18 | 2 | 21 | 25 | 25 | _ | _ | _ | + | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 14* | 15 | 17 | 18 | 19 | 21 | 22 | 25 | 27 | _ | _ | 7 | 9 | _ | _ | 7 | 7 | _ | _ | 7 | 8 | _ | _ | 6 | 7 | _ | _ | _ | _ | _ | _ | _ | _ |
| 15* | 35 | 4 | 4 | 4 | 32 | 33 | 36 | 4 | _ | 7 | 8 | 11 | _ | _ | 7 | 7 | _ | _ | 1o | 13 | _ | 7 | 7 | 12 | _ | _ | _ | _ | _ | _ | _ | _ |
| 16* | 33 | 33 | 34 | 34 | 2 | 21 | 22 | 25 | _ | _ | 9 | _ | 7 | 8 | _ | _ | _ | _ | _ | _ | 9 | 9 | 9 | 9 | _ | _ | _ | _ | _ | _ | _ | _ |
| 17 | 2 | 17 | 19 | 19 | 25 | 3 | 33 | 33 | _ | _ | 8 | 6 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 18 | 25 | 25 | 25 | 25 | 22 | 22 | 25 | 25 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 19 | 7 | 9 | 10 | 11 | 13 | 13 | 17 | 15 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 20* | 28 | 28 | 29 | 3 | 19 | 2 | 2 | 25 | _ | _ | _ | _ | 6 | 8 | 8 | 7 | _ | 7 | 7 | 7 | _ | + | _ | + | _ | _ | _ | + | _ | _ | _ | _ |
| 22 | 32 | 32 | 31 | 32 | 16 | 2 | 21 | 23 | _ | _ | _ | _ | _ | + | + | + | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 23 | 15 | 15 | 14 | 16 | 27 | 28 | 3 | 32 | _ | _ | _ | _ | _ | _ | 7 | 9 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 24 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 29* | 25 | 25 | 24 | 25 | 18 | 2 | 21 | 22 | _ | _ | _ | _ | _ | 10 | 10 | 10 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 30 | 25 | 25 | 27 | 27 | 19 | 2 | 21 | 21 | _ | _ | _ | _ | _ | _ | _ | _ | + | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 33 | 19 | 19 | 2 | 18 | 22 | 26 | 27 | 27 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | + | + | + | + | _ | _ | _ | + |
| 37 | 23 | 23 | 24 | 24 | 18 | 2 | 22 | 23 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 38* | 3 | 3 | 3 | 3 | 2 | 22 | 23 | 23 | _ | _ | _ | _ | 7 | 7 | 7 | 7 | 7 | + | + | + | 7 | + | + | + | _ | _ | _ | + | _ | _ | _ | + |
| 42 | 24 | 24 | 25 | 24 | 18 | 2 | 21 | 23 | _ | _ | _ | _ | _ | + | + | + | _ | _ | + | _ | _ | + | + | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 43 | 2 | 2 | 24 | 24 | 19 | 21 | 25 | 25 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| 47 | 2 | 21 | 22 | 22 | 25 | 3 | 3 | 34 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | + | _ | _ | _ | _ | _ | _ | _ | _ |

Key

+ = Extracts with some indication of inhibition

* = Isolates which were inhibited better than the others

N.B. The inhibition zones are measured in millimeter

The extracts are in µl

# 5. DISCUSSION

During sample collection, it was observed that enset bacterial wilt is the main production constraint in all zones regardless of altitudes as reported by enset farmers and agricultural experts. Although the yield loss is not clearly known, the presence of such wide distribution of enset bacterial wilt disease in high, mid and low altitudes of enset growing weredas was reported earlier by Spring *et al.*, (1996) and Dereje (1985). Next to enset bacterial wilt, mealy bugs in Gedio and corm rot in Wolaita zones were reported as important constraints in enset production.

Variation of enset bacterial wilt disease had been observed right at the fields of collection. There were fields with few infected plants and fields with many small and big (near to harvest) enset plants infected with the disease. Especially in Gurage mid lands (Cheha), enset fields were found with severe infection due to bacterial wilt. As it was reported by Spring *et al.*, (1996), Gurage zone was found with high incidence and distribution of the disease among the three zones (Sidama, Hadya and Gurage) surveyed. Such variability in severity of the disease on enset fields could lead to different speculations.

The bacterial exudates collected from newly infected petiole of enset were different in color where light to deep yellow and creamy (honey) color of ooze (exudate) was observed. Even from the same enset field but from different enset clones, different color of exudate was observed where isolates from Limo and Hagereselam could be good examples.

During isolation process, there were isolates that grew faster and very aggressive such as: GIMB (38), SEMTmg (29), LIHSsw2 (20) and isolates with very slow growth like HSHSgu1 (3) even to the extent of no growth in one of the samples of FGMSto (7). Growth might be inhibited due to various reasons. For example, the ability of the bacterial isolates to survive in sterilized water could be poor or the presence of some antagonistic microorganisms, which might enter during collection, could suppress their survival. Regarding survival Wasnikar *et al*., (1991) studied the survival ability of *Xanthomonas campestris* Pv. *beticola* and found that the bacterium survived in sterilized water for more than 240 days and survived in unsterilized samples to about 30 days when stored at room or lower temperature. Therefore, study on survival of *Xanthomonas campestris* Pv. *musacearum* in different media could be important to minimize the factors that inhibit its survival during sample collection, isolation and preservation.

Asparagine medium with out any alternative carbon and nitrogen source was used as a diagnostic test to isolate *Xanthomonas* from other yellow *enterobacteriaceae* and many *Pseudomonads* as stated by Dye (1962) cited in Bradbury (1984). However, there were isolates, which grew alone without any other contaminants or mutants in YPSA medium and grown well in asparagine medium. Therefore, it could be difficult to take asparagine medium alone as diagnostic test for *Xanthomonas* and there is a need for development of selective or semiselective medium (media) for causal agent of enset bacterial wilt, *Xanthomonas campestris* Pv. *musacearum.*

Detection of variation is the main objective of this work and any observation even out of the recognized characteristics of *Xanthomonas campestris* such as positive reaction to oxidase were recorded. Variations were observed in most biochemical tests, which gave clue to the presence of variation within *Xanthomonas campestris* Pv. *musacearum* population in Ethiopia. Similar variations in biochemical tests such as gelatin liquefaction, starch hydrolysis, carbohydrate utilization, etc. were also observed in *Xanthomonas campestris* Pv. *magniferaeindicae* population (Dayakar and Gnanamanickam, 1996). Among isolates which were subjected to different biochemical tests, about 70% of them showed light yellow to deep yellow colony color that is one character of *Xanthomonas campestris* in general. Similar characterization work by Eshetu (1981) and Gizachew (2000) also showed the same results. However, there were isolates with creamy (honey) colony color.

All isolates except HSHSgu1 (3) were found tolerant to NaCl 2-5% where about 70 % were found tolerant to 3-5% NaCl but in the previous work by Eshetu (1981), 4% NaCl suppressed the growth of all *Xanthomonas campestris* Pv. *musacearum* isolates.

The reaction of most of the isolates to indole production, nitrate reduction, gelatin liquefaction and catalase tests are found similar with Eshetu (1981) and Gizachew (2000) studies and are also similar with general characteristic of *Xanthomonas campestris* described in Bradbury (1984). With regard to starch hydrolysis, differences in reaction among isolates were observed among isolates in this work and are in agreement with the previous work by Eshetu (1981) but different from Gizachew (2000) where all the isolates used in his work did not hydrolyze starch. Bradbury (1984) described the characteristics of *Xanthomonas*

*campestris*, like starch hydrolysis with neither positive nor negative but 'd ' (11-89% strains are Positive). This indicates the possibility of finding pathovars and strains as positive or negative for that specific test within the species. This ensures the presence of variation among isolates of given population.

Citrate and malate utilization of most of the isolates was found to be positive which agree with description by Bradbury (1984) however not found in agreement with Gizachew (2000) work. Regarding carbohydrate utilization, fructose was utilized by all isolates except one isolate GEMSbe (47) and about 50% of the isolates could not utilize arabinose different with the results in Gizachew (2000) work where all isolates used in his study utilize arabinose and fructose.

Among the isolates tested in this work FGMTge (1) and YCMSas (2) showed consistently similar reactions to various biochemical tests. These isolates showed deep yellow colony color, they were tolerant to 2% NaCl concentration, liquefied gelatin within 3 days of inoculation, hydrolyzed casein and were also similar in carbohydrate utilization. However, in cluster analysis these two isolates were not grouped together and another two isolates, LIHSsw (20) and YCMTge (22) showed many similar reactions in biochemical tests and were also grouped together with 95% similarity.

The presence of xanthomonadin pigment is usually taken as distinct character of the genus *Xanthomonas*. However, as stated by Bradbury (1984), the absence of the pigment does not exclude the isolate from the genus. There could also be possibility of losing the pigment

during culturing (Bradbury, 1984) or the isolates might not carry that character like the white pathovars of *Xanthomonas campestris* reported by Sugimori and Oliveira (1994).

Positive reaction to the non-host, tobacco (*Nicotiana tabacum*) is a confirmation for the isolates to be pathogen. It is commonly used as preliminary test before testing for their pathogenicity in the host of origin. This is also true for *Xanthomonas campestris* Pv. *musacearum* (Quimio, 1992; Gizachew, 2002). The isolates infiltrated to tobacco leaves had shown variation in time for symptom development (48-72 hours) although all were positive to this test. For example, FGMSto reacted fast and showed very deep brown necrosis around the area of injection. There were also isolates with slow reaction and light yellow necrosis on the tobacco leaves.

Enset is the origin of all isolates except GIMB, which is from banana. The collection was done carefully from newly infected leaf petioles by picking the bacterial ooze from the pockets to avoid contamination and in addition, isolates that were tested for their pathogenicity, were those, that did not grow on asparagine medium. Having such low probability of inoculating non- *Xanthomonas campestris* Pv. *musacearum* isolates, the reaction to pathogenicity test on a susceptible clone was found to be negative to all isolates. They did not show complete wilt symptom except necrosis around the injected point of the petioles. Even if there was some probability of isolating non-pathogen bacteria, the isolates like FGMTge, YCMSas, YCMTge, LIHSsw, ARMSnk should have showed the symptom because they were grown alone on YPSA medium during isolation.

One possible reason for failure in pathogenicity could be the effect of the growth media (YPSA). Although YPSA was recommended for all *Xanthomonas campestris* pathovars, there could be mutation or loss of virulence of the bacterial isolates while growing on this medium. The same problem was faced while screening enset clones for resistance to enset bacterial wilt disease in Awasa Agricultural Research Center, Pathology section (Awasa Agricultural Research Center Progress Report, 1997). While conducting this experiment, the field samples collected were grown on YPSA and inoculated to enset clones and all showed negative reaction but when the samples were inoculated directly without growing them in artificial medium (YPSA), all clones showed wilt symptom and died within 1 to 2 months time except few enset clones which are tolerant. This implies that there is a need to develop or evaluate the available semi selective or selective media of other pathovars for *Xanthomonas campestris* Pv. *musacearum* that could provide a growing condition similar to the natural environment.

Other than the medium, there might be some technical problems, which could lead to shifting or mutation of virulent strains to avirulent forms and failed to produce wilting. The bacterial isolates might also lose some factors like extracellular polysaccharides, enzymes, toxins, growth regulators or mutation of hrp genes might happen that enable them to induce infection due to various reasons. In most cases strains found mutant for hrp genes were found non pathogenic to compatible hosts in different plant pathogenic bacteria.

As the electrophoresis gel pictures indicated, there was variation in band patterns among isolates detected visually and from the different analysis done. There were also isolates that

showed similar band patterns like YCMTge and LIHSsw2. This was further confirmed in the cluster analysis where they showed more than 95% similarity.

In the discriminant function analysis the isolates, which scored the highest values for F1 and F2 were mainly those excluded from the biochemical tests because of the reason that they grew on asparagine medium. Most of the isolates, which did not grow on asparagine medium and taken for further tests, were found grouped together with some exceptions. There were also isolates grown on asparagine medium and grouped with non-growers and isolates that did not grow on asparagine and grouped outside. These results some how seem supportive to the use of asparagine medium as diagnostic test for *Xanthomonads* (Bradbury, 1984).

Areka with an altitude of 1800 was found to score the highest value for function 1 in discriminant function analysis for regions and altitudes. Isolates collected from Areka were ARMSnk (23) and corm rot where corm rot is a bacterial disease of enset with unidentified causal agent (Quimio and Mesfin, 1996). ARMSnk (23) was grouped with the other isolates, which did not grow on Asparagine medium. Therefore, the reason for large values of Areka for function 1 in this analysis could be the presence of corm rot in the analysis. This might indicate that the causal agent of corm rot might be a different species.

In cluster Analysis, not only isolates collected from different enset-growing regions showed variation, but also isolates collected from the same region showed variation. This was indicated in the groups formed based on the analysis of the banding patterns yielded from rep-PCR. Regarding better similarity among isolates, FGMTge was found to have more than

95% similarity followed by ANHTmz1 and HSHSda2 which showed about 85% similarity with the reference strains DSMZ 1050 and DSMZ 1350. Isolates YCMTge and LIHSsw2 were also found to have about 95% similarity where the locations of these isolates that is Yirgachefie and Limo are very far from each other; their altitudes are also different that is 1900 for Y/Chefie and 2700 for Limo. This could indicate that the variation and the similarity might not follow any trend and that it could be random.

In cluster analysis for regions; Gessa, Seka, Angacha and Hagereselam showed more than 95% similarity, which implies that isolates from these regions are more related. Isolates from Bolososore and Mareka loma were also found to have more than 95% similarity. This could be due to the short distance between these two regions where there could be possibility of finding similar isolates.

Isolates from Fisseha Genet, Areka and Gimbo showed less similarity with other regions. This might be due to corm rot from Areka and GIMB isolated from infected banana. *Xanthomonas campestris* Pv. *musacearum* is known to attack banana; however, there could be possibility of having a different strain of *Xanthomonas campestris* Pv. *musacearum* that could attack banana naturally.

In principal component analysis variability of isolates, regions and altitudes was analysed for specific bands selected for this purpose not for all bands. The results from this analysis revealed that isolates which showed high variability for R2 and R16 were from Seka, Angacha, Mareka loma and Bolososore. Among the regions , Bolososore and Angacha share

same border. The distance between Mareka loma and Bolososore is also short . Therefore, such similarities in variabilty for specific bands could be due to the nearness of the regions. However, variations in values for variability were observed in isolates collected from the same region. Bolososore and Seka isolates could be good examples. This indicates that there is high probability of variation among isolates collected from the same region which further strengthen the results obtained from cluster analysis.

The idea of evaluating *pychnostacis abyssinica* against *Xanthomonas campestris* Pv. *musacearum* was based on the farmers' traditional practice. In addition, there has been many studies conducted on evaluation of different plant extracts against *Xanthomonas campestris* pathovars in many countries and most of the studies were successful. For example, diffusates from various parts of *Phyllanthus emblica, Acacia nilotica, Sapindus mukorossis* and *Terminalia chebula* exhibited an inhibition zone 4.83-6.00mm at 50 g/liter against *Xanthomonas campestris* Pv. *citri* appeared to be the most effective (Akhtar et al., 1995) and extracts from *Acacia arabica, Achras zapota,* and from other 6 higher plants were also found inhibitory to various pathovars of *Xanthomonas campestris* (Satish *et al*., 1999). Therefore, the crude extracts from different parts of *Pychnostachis abyssinica* were evaluated against the isolates, which did not grow on asparagine medium.

The crude extracts from the bract and the leaf showed better inhibition than the stem and root which indicated that the active ingredient could be accumulated in these parts which are highly aromatic. The inhibition zones by the hot bract and leaf extracts were promising so

that further isolation and evaluation of the active ingredient from these parts of the plant could give better inhibitory effect.

Among the 29 isolates tested, about 13 were inhibited by the crude extracts. The amount of the extracts used were high because of the fact that the amount of active ingredient in such crude extracts could be very little. Therefore, based on such indicative results, isolation and evaluation of the active compound against *Xanthomonas campestris* Pv. *musacearum* could contribute in controlling the disease.

## Conclusion and Recommendation

In general from this research work, the presence of variation among different isolates was observed in their reaction to biochemical tests and in the PCR analysis. This is valuable information to further study the cause for their variation and for devising various control measures. The molecular techniques applied from DNA extraction to PCR could also open the way for further molecular level research on enset bacterial wilt disease causal agent so that the basic information that can be generated from further studies could contribute in devising control strategies against the disease.

The inhibitory effects of the crude extracts from the indigenous plant *Pychnostachis abyssinica* were also found promising where further isolation and evaluation of the active ingredient is important. In addition, other medicinal plants that were found effective against

other *Xanthomonas campestris* pathovars should be evaluated against enset bacterial wilt causal agent. Further study on the in vivo application is also important.

The results from this work have also raised many questions regarding diagnostic tests and inoculation technique and a more important question on the bacterium it self that is about its virulence, how it loses and what factors could contribute for that. Therefore, for further research on the causal agent of enset bacterial wilt disease and in devising control strategies, the following points could be important.

1. Evaluation of the available semi selective and selective media for *Xanthomonas campestris* Pv. *musacearum*
2. Survival studies of Xanthomonas campestris Pv. musacearum on different media like in sterilized water, in soil and in plant debris
3. Evaluation of the inoculation technique for pathogenicity test of bacterial wilt disease causal agent
4. Generating basic information about the bacterial virulence character especially developing PCR protocols to detect the hypersensitivity and pathogenicity gene (hrpx gene), which is important for diagnostic purpose.
5. Evaluation of extracts from medicinal plants which were evaluated on other *Xanthomonas campestris* pathovars in other countries and extracts from indigenous medicinal plants against the causal agent of enset bacterial wilt
6. Generating basic information on the mechanisms of disease development whether toxic, enzymatic or physical blockage by extracellular polysaccharides

Simultaneously with conducting basic studies on the causal agent, it is important to encourage and support the enset farmer in application of sanitary measures in organized way to minimize the loss incurred by the disease. Besides, since enset is a major crop in Ethiopia used as staple and co staple food for many people, attention for research and development of technologies that improve the production system of enset could contribute for better food security in Ethiopia.

# 6. References


Admasu, T., Asnaketch, W/T., Assefa, A., Bakalu, M., Bizuayehu, H., Brandt, A.S., Michael, P.D., Endale, T., Gizachew, W/M., Clifton, K.H., Kelsa, K., Kidist, B., McCabe, T.J., Mulugeta, D., Ousman, S., Seble, S., Senait, B., Shiferaw, T., Shigeta, m., Spring, A., Tamirat,L., Tariku, M., Tesfaye, H., Tesfaye, T. and Yewelsew Abebe.1997. Phase II Final Report of the GTZ funded Enset Needs Assessment Project.

Agrios, G.N. 1988. *Plant Pathology*. 3rded. Academic Press, London.

Akhtar, M.A., Rahber-Bhatti, M.H. and Aslam, M. 1997. Antibacterial activity of plant diffusate against *Xanthomonas campestris* Pv. *citri*. *International Journal of Pest Management.* **43(2)**: 149-153.

Aneja, K.R. 1996. *Experiments in Microbiology, Plant pathology, Tissue Culture and mushroom cultivation.* Wishwa Prakashan New Age International (P) Limited NewDelhi.

Araujo, A.E. and Siqueri, F.V. 1999. Evaluation of the efficiency of the use of copper oxychloride and a mixture of oxytetracycline + streptomycin sulfate to control angular leaf spot in cotton. *Comunicado Tecnico Embrapa Algodao*. **105**:3.

Asnaketch, W.1997. The ecology and production of *Ensete ventricosum* in Ethiopia: Doctoral Thesis, Swedish University of Agricultural Sciences, Uppsala.

Awasa Agricultural Research Center. 2000. Progress Report 1999-2000

Awasa Agricultural Research Center. 1999. Progress Report 1998-1999

Awasa Agricultural Research Center. 1997. Progress Report 1996-1997

Becker, A., Katzen, F., Puhler, A. and Ielpi, L. 1998. Xanthan gum biosynthesis and application:biochemical/geneticperspective. *Applied microbiology and Biotechnology*. **50(2)**:145-152.

Bizuayehu, H., Mulugeta, D. and Endale, T. 1996. Agronomy Research on Enset. In: *Enset-based Sustainable agriculture in Ethiopia* (Tsedeke Abate, Clifton Hiebsch and Steve Brandt eds.). Proceedings of the First international workshop on Enset. Dec 13-21 1993. IAR, Addis Ababa, Ethiopia. Pp.235-241

Bradbury, J.F. 1984. *Xanthomonas* Dowson. 1939. In: *Bergey's manual of systematic Bacteriology*, Vol.1. (Krieg, N. R. and Holt, J.G. eds). Williams and Wilkins, Balimore. Pp.199-210

Bragard, C., Verdier, V. and Maraite, H. 1995. Genetic diversity among *Xanthomonas campestris* strains pathogenic for small grains. *Applied and Environmental Microbiology*. **61(3)**: 1020-1026.

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. 56pp.

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

Cheesman, E.E. 1947. Classification of the Banana. I. The genus *Ensete. Horan. Kew Bulletin.* **2**: 97-106.

Csizinszky, A.A., Civerolo, E.L. and Jones, J.B. 1993. Inactivation of *Xanthomonas campestris* pvs. in vitro with plant extracts. First world congress on medicinal and

aromatic plants for human welfare (WOCMAP), Maastricht, Netherlands, 19-25 July 1992. *Acta Horticulturae*. **331**: 301-305

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

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

Dayakar, B.V and Gnanamanickam, S.S. 1996. Biochemical and pathogenic variation in strains of *Xanthomonas campestris* Pv. *mangiferaeindicae* from Southern India. *Indian Phytopathology*: **49(3)**: 227-233.

Dereje A. 1981. Studies on bacterial wilt of enset. Paper presented at the 13th National Crop Improvement Conference. Addis Ababa, Ethiopia. 23-25 March 1981. IAR, Ethiopia.

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

Dickey, R. S. and Kelman, A. 1988. 'Caratovora' or soft rot group. In: *Laboratory guide for identification of plant pathogenic bacteria 2nd ed.* (ed. N.W. Schaad.). APS Press St. paul, Minnesota. Pp.81-84.

Dixon, G.R. 1984. *Plant Pathogens and their control in Horticulture*. Macmillan Publishers LTD. London and Basingstoke Companies and representatives throughout the world.

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

Endale, T. 1990. Enset in Ethiopian Agriculture. Institute of Agricultural Research News. Areka Research Center

Eshetu, W. 1981. The role of Poecilocarda nigrinervis, pentanolia nigronervosa, and Plantococcus ficus in the transmission of enset wilt pathogen *Xanthomonas musacearum* sp. n. in Wolaita, Ethiopia. M.Sc. Thesis, Addis Ababa, Ethiopia.

Eyasu, E. 1998. Is soil fertility declining? Perspectives on environmental change in Southern Ethiopia. Managing Africa's soils Drylands Programme, IIED. No.2:38 p.

Fekadu, D., and Ledin, I. 1997. Weight and chemical composition of the plant parts of enset (*Ensete ventricosum*) and the intake and degradability of enset by cattle. *Livestock Production Science*. **49(3)**: 249-257.

Gabriel, D.W. and De Feyter, R. 1992. RFLP analyses and gene tagging for bacterial identification and taxonomy. In: *Molecular Plant Pathology. Vol.1, A Practical Approach*. (S.J. Gurr, M.J.McPherson and D.J. Bowles,eds). IRL Press, Oxford. Pp. 51-66.

Gizachew, W/M. 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.

Hayward, A.C. 1964. Characteristics of *Pseudomonads solanacearum. Journal of applied bacteriology*. **27**:265-277.

Hugouvieux, V., Barber, C.E. and Daniels, M.J. 1998. Entry of *Xanthomonas campestris* Pv. *campestris* into hydathodes of Arabidopsis thaliana leaves : a system for studying early infection events in bacterial pathogenesis. *Molecular Plant Microbe Interactions*. **11(6)**: 537-543.

Hwang, I. and. Lim, S.M. 1998. Pathogenic variability in isolates of *Xanthomonas campestris* Pv. *glycines*. *Korean Journal of Plant Pathology*.**14 (1)**:19-22.

Jung, W., Heu, S.G. and Cho, Y.S. 1998. Influence of growth conditions for the production of bacteriocin, glycinecin, produced by *Xanthomonas campestris* Pv. *glycines* 8 ra. *Korean Journal of Plant Pathology*. **14(5)**: 376-381.

Kefale,A. and Sandford, S. 1991. Enset in North Omo region. Farmer's research technical Pamphlet, No.1. Addis Ababa, Ethiopia.

Khare, U.K. and Khare, M.N. 1995. Studies on Survival of *Xanthomonas campestris* Pv. *glycines*. *Indian Phytopathology*. **48(2)**: 180-181.

Kocks, C.G., Ruissen, M.A., Zadoks, J.C. and Duijkers, M.G. 1998. Survival and extinction of *Xanthomonas campestris* Pv. *campestris* in soil. *European Journal of Plant pathology*. **104(9)**:911-923.

Lee, R. and Zawdie, G. 1997. Population growth, environment stress and innovation in Ethiopian peasant agriculture. *Science , Technology and Development.*15(1):104-126.

Lenka, S., and Ram, S. 1997. A note on the efficacy in vivo of various antibiotics and fungicide chemicals against *Xanthomonas campestris* Pv. *campestris* causing black rot of cauliflower. *Orissa Journal of Horticulture*. **25(1)**:90-92

Louws, F.J., Fulbright, D.W., Stephens, C.T. and De-Bruijn, F.J. 1995. Differentiation of genomic structure by rep-PCR finger printing to rapidly classify *Xanthomonas campestris* Pv. *vesicatoria*. *Phytopathology*. **85(5)**: 528-536.

Lye, K.A. and Edwards, S. 1997. Musaceae: Flora of Ethiopia and Eritrea. Vol.6. *Hydrocharitaceae* to *Arecaceae.* (Edwards,S., Sebsebe, D. and Hedberg, I., eds). Addis Ababa, Ethiopia and Uppsala, Sweden. Pp. 317-321.

Manly, B.J. 1992. *Multivariate Statistical Methods. Ä Primer*. Chapman and Hall. London. Glasgow. New York.. Tokyo. Melbourne. Madras.

Manners, J.G. 1993. *Principles of Plant Pathology*. 2nd ed. Cambridge University Press.

Mesfin, T. 1989. Enset (*Ensete ventricosum*) wilt syndrome: monopathogenic origin or complex? Proceeding of the Ethiopian Phytopathological Committee 14th Annual Meeting. 30-31 March, 1989. Ambo, Ethiopia.

Million, T., Kidist, B. and Mulugeta, D. 1999. Working with farmers to control enset bacterial wilt disease using sanitary measure. Summary of data on farmers' interview conducted before application of sanitary measures in Gurage Zone.

Norman, D.J., Chase, A.R., Stall, R.E. and Jones, J.B. 1999. Heterogenity of *Xanthomonas campestris* Pv. *hederae* strains from araliaceous hosts. *Phytopathology*. **89(8)**: 646-652.

Olmstead, J.1974. The Versatile enset plant: its use in the Gamu highland. *Journal of Ethiopian studies* **12 (2)**: 147-153.

Patil, M.R. and Ghoderao, B.N. 1997. Evaluation of Some medicinal and aromatic plants against cotton bacterial blight infection. *PKV Research Journal*. **21 (2)**: 179.

Pergrine, W.T.H., and Bridge, J. 1992. The lesion nematode *Pratylenchus goodeyi*, an important pest of ensete in Ethiopia. *Tropical Pest Management*. **38(3)** : 325-326.

Pijls, L.T.J., Timmer, A.M., Wolde-Gebriel, Z. and West, C.E. 1995.Cultivation, preparation and consumption of ensete (*Ensete ventricosum*) in Ethiopia. *Journal of the Science of food and Agriculture*.**67(1)**:1-11

Pruvost, O., Couteau, A., Perrier, X. and Luisetti, J. 1998. Phenotypic diversity of *Xanthomonas* sp. *mangiferaeindicae. Journal of Applied Microbiology*.**84(1)**:115-124.

Pursglove, J.W. 1972. *Tropical Crops.* Monocotyledons. Holsted Press, A division of John Wiley and Sons, Inc. New York.pp.243-244

Quimio, J.A. and Mesfin,T. 1996. Diseases of Enset. In *Enset-based Sustainable agriculture in Ethiopia* (Tsedeke Abate, Clifton Hiebsch and Steve Brandt eds.). Proceedings of the First international workshop on Enset. Dec 13-21 1993. IAR, Addis Ababa, Ethiopia. Pp.188-203

Quimio, J.A. 1991. First quarter report of the plant pathologist: July 1 to September 30,1991. Enset Team Support Project Sidamo GamoGofa. Peasants Agricultural Development Program-PADEPIII. Awasa Research Center (IAR). Awasa, Ethiopia.

Quimio, J.A. 1992. Annual report of the plant pathologist: July 17,1991 to July 16,1992. EnsetTeam Support Project Sidamo GamoGofa. Peasants Agricultural Development Program-PADEPIII. Awasa Research Center (IAR). Awasa, Ethiopia.

Restrepo, S., Velez, C.M. and Verdier, V. 2000. Measuring the genetic diversity of *Xanthomonas axonopodis* Pv.*manihotis* within different fields in Colombia. *Phytopathology*. **90(7)** : 683-690.

Satish, S., Raveesha, K.A. and Janardhana, G.R. 1999. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. *Letters in Applied Microbiology*.**28 (2)**: 145-147.

Schaad, N.W. 1988. Initial Identification of Common Genera. In: Laboratory guide for identification of plant pathogenic bacteria 2nd ed. (ed. N.W. Schaad.). APS Press St. paul, Minnesota. Pp.81-84.

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

Schiegel, H.G. 1995. *General Microbiology*. 7th ed. Cambridge University Press.

Shigeta, M. 1990. 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.

Simmonds, N.W. 1962. *The Evolution of the Bananas*. Tropical Science Series, Butler and Tanner, Ltd. Frome and London. 165pp.

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

Sugimori, M.H. and De-Oliveira, A.R. 1994. Serological characterization of white pathovars of *Xanthomonas campestris*. *Summa Phytopathologica*. **20(3-4)**: 168-170.

Swart, A., Mesfin, B. and Tiedt, L.R. 2000. Description of *Aphelenchoides ensete* sp.n. (Nematode: *Aphelenchoididae)* from Ethiopia. *Journal of Nematode Morphology and Sytematics.* **3(1)**:69-76.

Talwar, M., Kumar, P. and Saxena, S.C. 1996. Evaluation of chemicals including antibiotics against *Xanthomonas campestris* Pv. *mangiferaeindicae*, the bacterial canker pathogen of mango. *Advances in Plant Sciences*. **9(2)**: 235-237.

Taye, B. 1980. Morphology of *Ensete* embryo in vitro culture. Ethiopian Journal of *Agricultural Science* **2**: 23-28.

Taye, B. 1984. Evaluation of some *Ensete ventricosum* clones for food yield with emphasis on the effect of length of fermentation on carbohydrate and calcium content. *Tropical Agriculture***. 61(2)**: 111-116.

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

Taye, B. 1996. An Over view of enset research and the future technological needs for enhancing its production and utilization. In *Enset-based Sustainable agriculture in Ethiopia* (Tsedeke Abate, Clifton Hiebsch and Steve Brandt eds.). Proceedings of the First international workshop on Enset. Dec 13-21 1993. IAR, Addis Ababa, Ethiopia. Pp.1-14.

Terefe, B and Endale, T.1989. A review of the available research recommendations and future strategies on enset. Proceedings of the 19th National Crop Improvement conference. April 1987. Addis Ababa. Institute of Agricultural Research.

Terefe, B. 1991. The position of enset (*Ensete ventricosum*) Research in Ethiopia. In. *INIBAP* ( *International Network for the improvement of Banana and Plantain*) Regional Network for Eastern Africa, proceedings of the Regional Advisory Committee Meeting. Kampala, Uganda 23-25 Sept 1991.pp. 29-32.

Thammaiah, N., Khan, A. and John, E. 1995. Effect of extract from *Adhatoda zeylanica* on *Xanthomonas campestris* Pv. *viginicola* causing bacterial blight of cowpea. *Advances in Agricultural research in India*. **4**: 109-117.

Thaveechai, N., Leksomboon, C., Kositratana, W., Paradornutwat, A. and Rojanaridpiched, C. 1993. Survival of *Xanthomonas campestris* Pv. *manihotis* under natural field conditions. *Kasetsart Journal of Natural Sciences*. **27(1)**: 25-32.

Tigist, D. 1986. Is Ethiopia's *Ensete ventricosum* crop her greatest potential food? *Agriculture International*. **38(12)**: 362-365.

Veracruz, C.M., Ardales, E.Y., Skinner, D.Z., Talag, J., Nelson, R.J., Louws, F.J., Leung, H., Mew, T.W. and Leach, J.E. 1996. Measurement of haplotypic variation in *Xanthomonas oryzae* Pv. *oryzae* within a single field by rep-PCR and RFLP analyses. *Phytopathology*, **86(12)**: 1352-1359.

Wasnikar, A.R., Nayak, M.L. and Bhale, M.S. 1991. Survival of Xanthomonas campestris Pv. beticola in different water samples. *International Journal of Plant diseases*. **9(1)**: 83-86.

Wells, J.M., Liao, C. and Hotchkiss, A.T. 1998. In-vitro inhibition of soft-rotting bacteria by EDTA and nisin and in vivo response on inoculated fresh cut carrots. *Plant Disease.* **82 (5)**: 491-495.

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

Yohannes, U. and Mengel, K. 1994. Response of enset (*Ensete ventricosum* (Welw.) Cheesman) to mineral fertilizers in southwest Ethiopia. *Fertilizer Research* **37**:107-113

Zeweldu, T and Ludders, P. 1998. Prelimenary tissue culture investigation in ensete (Ensete spp.). *Angewandte-Botanik*. **72(1-2)**: 25-27.57.

# APPENDIX

Appendix 1. Isolates and their growth on YPSA and Asparagine media and their Gram reaction

| Isolate No. | Isolate name | Growth on YPSA medium | Gram reaction | Growth on Asparagine |
|---|---|---|---|---|
| *1 | FGMTge | Deep yellow small colonies, less mucoid | - | - |
| *2 | YCMSas | Deep yellow small colonies, less mucoid | - | - |
| *3 | $HSHSgu_1$ | Deep yellow very small colonies, less mucoid | - | - |
| *4 | $HSHSda_1$ | Creamy (honey) small colonies less mucoid | - | - |
| *5 | $HSHSda_2$ | Creamy (honey) medium colonies highly mucoid | - | - |
| *7 | FGMSto | Light yellow small colonies, less mucoid | - | - |
| *8 | CHMTye | Deep yellow small dried colonies | - | - |
| *9 | $ANHSgi_1$ | Light yellow small colonies mucoid | - | - |
| *10 | $CHMSne_1$ | Light yellow small colonies mucoid | - | - |
| *11 | BOMSgn | Deep yellow medium colonies, mucoid | - | - |
| *12 | $MLHSam_1$ | Yellow medium colonies, mucoid | - | - |
| *13 | $GOMSgn_1$ | Deep yellow medium colonies, mucoid | - | - |
| *14 | $SEMTmg_1$ | Light yellow small colonies, less mucoid | - | - |
| *15 | $LIHSsw_1$ | Light yellow medium colonies, mucoid | - | - |
| *16 | $ANHSsi_1$ | Light yellow small colonies, less mucoid | - | - |
| *17 | $ANHTmz_1$ | Light yellow small colonies, mucoid | - | - |
| *18 | LIHSbe | Creamy (honey) big colonies mucoid | - | - |
| *19 | $SEMSbe_1$ | Deep yellow medium colonies, mucoid | - | - |
| *20 | $LIHSsw_2$ | Yellow big colonies, highly mucoid | - | - |
| *22 | YCMTge | Yellow big colonies, highly mucoid | - | -(+b) |
| *23 | ARMSnk | Creamy (honey) small, less mucoid | - | - |
| 24 | $ANHTmz_2$ | White small, dried colonies | + | - |
| 25 | $ANHSgi_2$ | Creamy medium, dried colonies | - | + |
| 26 | BOMSsi | Creamy (honey) big, highly mucoid | - | + |
| 27 | $SEMSgn_1$ | Creamy small colonies, less mucoid | - | + |
| 28 | $SEMSbe_3$ | Creamy small colonies, dried | - | + |
| *29 | $SEMSgn_2$ | Yellowish big colonies, highly mucoid | - | - |
| *30 | GOMSad | Creamy (honey) big colonies, highly mucoid | - | - |
| 31 | $GOMSgn_2$ | Creamy very small colonies, less mucoid | - | + |
| 32 | GEMSbu | Creamy big colonies, highly mucoid | - | + |
| *33 | $GEMSbe_1$ | Creamy big colonies, highly mucoid | - | - |
| 34 | MLHTsh | Creamy medium colonies, dried | - | + |
| 35 | MLHSt | Creamy big colonies, highly mucoid | - | + |
| 36 | $BOMTtu_2$ | Creamy (Shiny) big colonies, highly mucoid | - | + |
| *37 | $BOMTtu_1$ | Creamy medium colonies, less mucoid | - | - |
| *38 | GIMB | Light yellow big colonies, very highly mucoid | - | + |
| 39 | $CHMSne_2$ | Creamy very small colonies, less mucoid | - | + |
| 40 | $ANHSsi_2$ | Creamy big colonies, highly mucoid | - | + |
| 41 | $SEMSbe_4$ | Light yellow medium colonies, mucoid | - | +Green |
| *42 | $SEMSbe_2$ | Creamy big colonies, mucoid | - | - |
| *43 | LIHSdi | Creamy (Shiny) medium colonies, mucoid | - | - |
| 44 | MLHSam2 | Shiny light yellow very small colonies, dried | - | + |
| 45 | BOMSge | Creamy medium colonies, dried | - | + |
| 46 | $SEMTmg_2$ | Creamy (shiny) medium colonies, less mucoid | - | + |
| *47 | $GEMSbe_2$ | Deep yellow medium colonies, mucoid | - | + |
| 48 | GEMTsh | Creamy big colonies, mucoid | - | +Green |
| 49 | $HSHSgu_2$ | Light yellow (Shiny), dried | - | +Green |
| 50 | $HSHSgu_3$ | Creamy (shiny), dried | - | +Green |

*= Isolates suspected to be *xanthomonas campestris* Pv. *musacearum* and subjected for further biochemical tests
+green= green coloration in asparagine broth- (+b)= not grown on Asparagine agar plates but grown on broth

Appendix 2. Isolates and their values for F1 and F2

**Functions at Group Centroids**

| | Function | |
|---|---|---|
| ISOLATEN | 1 | 2 |
| 1.00 | -1.176 | -.200 |
| 2.00 | -1.176 | -.200 |
| 3.00 | -1.176 | -.200 |
| 4.00 | 1.805 | -.214 |
| 5.00 | -1.176 | -.200 |
| 7.00 | 1.346 | -.212 |
| 8.00 | .238 | -.207 |
| 9.00 | -1.176 | -.200 |
| 10.00 | -1.176 | -.200 |
| 11.00 | 1.805 | -.214 |
| 12.00 | 1.958 | -.215 |
| 13.00 | 1.499 | -.213 |
| 14.00 | -1.176 | -.200 |
| 15.00 | -1.176 | -.200 |
| 16.00 | -1.176 | -.200 |
| 17.00 | -1.176 | -.200 |
| 18.00 | -1.176 | -.200 |
| 19.00 | -1.176 | -.200 |
| 20.00 | -1.176 | -.200 |
| 22.00 | -1.176 | -.200 |
| 23.00 | -1.176 | -.200 |
| 24.00 | 6.476 | 1.501 |
| 25.00 | -1.176 | -.200 |
| 26.00 | -1.167 | 1.619 |
| 27.00 | .888 | -.210 |
| 28.00 | -1.176 | -.200 |
| 29.00 | -1.176 | -.200 |
| 30.00 | -1.176 | -.200 |
| 31.00 | -1.176 | -.200 |
| 32.00 | -1.175 | 3.876E-02 |
| 33.00 | -1.176 | -.200 |
| 34.00 | -1.176 | -.200 |
| 35.00 | -1.165 | 2.103 |
| 36.00 | 1.041 | -.210 |
| 37.00 | -1.169 | 1.215 |
| 38.00 | -1.176 | -.200 |
| 39.00 | 7.232 | -.241 |
| 41.00 | 1.041 | -.210 |
| 42.00 | 4.710 | -.228 |
| 43.00 | -1.176 | -.200 |
| 44.00 | -1.166 | 1.821 |
| 45.00 | 1.499 | -.213 |
| 46.00 | -1.176 | -.200 |
| 47.00 | 4.863 | -.229 |
| 48.00 | -1.176 | -.200 |
| 49.00 | .737 | .187 |
| 50.00 | -1.176 | -.200 |
| 51.00 | 1.652 | -.213 |
| 52.00 | -1.176 | -.200 |
| 53.00 | 1.653 | -5.58E-02 |
| 54.00 | -1.176 | -.200 |
| 55.00 | .811 | -.209 |

Unstandardized canonical discriminant functions evaluated at group means

Appendix 3. R-values for Function 1 to 12 contributed fro variability among regions

**Standardized Canonical Discriminant Function Coefficients**

| | Function | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| R1 | .397 | .268 | .803 | -.118 | .220 | .267 | .279 | .054 | .352 | -.116 | -.039 | .199 |
| R2 | .407 | -.324 | -.311 | -.042 | .238 | -.616 | .064 | -.003 | .198 | -.221 | .202 | .702 |
| R3 | -.881 | .195 | -.209 | -.125 | -.005 | -.068 | .409 | .312 | -.252 | -.476 | .034 | .250 |
| R4 | -.451 | .326 | .136 | -.457 | .368 | .246 | -.101 | -.016 | -.029 | -.129 | -.228 | .306 |
| R5 | -.691 | .617 | -.052 | .677 | -.174 | .669 | .372 | .141 | .586 | .418 | .199 | -.360 |
| R6 | -.144 | -.335 | -.212 | .298 | -.347 | .748 | .125 | -.001 | -.007 | .841 | -.042 | .179 |
| R7 | .269 | .277 | -.223 | .527 | .459 | .255 | -.267 | .181 | .335 | .249 | -.121 | .393 |
| R8 | -.379 | -.389 | -.104 | -.137 | -.993 | -.088 | .620 | .162 | -.333 | -.043 | .289 | .253 |
| R9 | -.277 | .749 | -.282 | -.551 | -.313 | -.237 | .020 | .031 | .065 | -.183 | .215 | .173 |
| R10 | -.536 | .687 | .086 | .508 | .439 | .094 | .184 | -.267 | .879 | .480 | .512 | .634 |
| R11 | -.138 | 1.142 | -.079 | .639 | .275 | .346 | .090 | .680 | -.237 | .145 | .172 | .009 |
| R12 | .734 | -.011 | -.252 | .487 | -.292 | -.655 | .027 | .068 | .106 | -.138 | .202 | -.378 |
| R13 | .180 | .775 | -.061 | .571 | .375 | .452 | .359 | -.261 | .021 | .721 | -.370 | .067 |
| R14 | .128 | -.770 | -.794 | .103 | -.204 | .385 | -.260 | .240 | -.064 | -.566 | -.298 | -.059 |
| R15 | -.398 | -.758 | -.388 | -.068 | -.313 | -.083 | .211 | -.089 | -.284 | -.434 | -.470 | .243 |
| R16 | 1.291 | -.176 | -.692 | -.567 | .124 | -.285 | .047 | .150 | -.323 | -.370 | .061 | -.455 |
| R17 | .689 | .443 | .852 | .002 | -.250 | .776 | .033 | -.339 | .180 | .377 | .437 | -.103 |
| R18 | .885 | .247 | -.039 | .220 | -.153 | .717 | -.400 | -.113 | .420 | -.056 | .352 | .151 |
| R19 | -.355 | -.035 | .512 | -.076 | .572 | .121 | -.077 | -.002 | -.649 | .332 | -.084 | -.346 |
| R20 | .465 | 1.123 | .243 | -.702 | .316 | -.194 | -.097 | .216 | -.100 | .443 | -.256 | .037 |
| R21 | .405 | .009 | .786 | -.085 | -.359 | -.722 | -.334 | -.040 | .031 | -.017 | -.290 | .167 |
| R22 | -.094 | -.001 | .829 | .541 | .542 | -.552 | .388 | -.474 | -.189 | -.217 | -.482 | .309 |
| R23 | .050 | -.312 | .588 | .125 | -.041 | -.135 | .155 | .218 | .616 | -.057 | .524 | .034 |
| R24 | -.077 | -1.042 | -.443 | .134 | -.044 | -.030 | -.073 | -.117 | -.024 | -.085 | .126 | -.093 |
| R25 | -.366 | -.833 | .088 | -.445 | -.229 | .606 | .173 | .661 | .221 | .224 | .062 | -.246 |

Appendix 4. Regions and their values for function 1 to 12

**Functions at Group Centroids**

| | Function | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REGIONN | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| ANGACHA | -.812 | -1.913 | .177 | -1.187 | .209 | .271 | .833 | -.464 | -.842 | -.504 | .469 | 2.401E-03 |
| AREKA | 3.741 | 5.708 | -.800 | -5.023 | .481 | 2.322 | -1.639 | -1.461 | -.159 | .647 | .141 | -.598 |
| BOLOSOSORE | 3.378 | -.361 | .960 | .438 | -.965 | .413 | -.283 | .933 | .407 | -.284 | .264 | -8.27E-02 |
| CHEHA | 2.462 | 8.908E-02 | -1.154 | 2.246 | 2.135 | .549 | -.152 | -.129 | -.466 | .456 | .121 | .329 |
| F/GENET | -.470 | -5.349 | -1.359 | -.626 | 1.057 | -.916 | -.305 | .289 | .498 | .961 | 3.209E-02 | -.795 |
| GESSA | -1.189 | -.715 | 1.567 | .970 | -.196 | -.197 | -.634 | -1.536 | 1.053 | -9.17E-02 | .149 | .147 |
| GIMBO | 2.581 | -2.483 | -5.749 | -1.230 | -4.940 | -.553 | -.267 | -.720 | -.165 | .606 | -.116 | .794 |
| GOMA | .722 | 2.618 | .862 | -.456 | 8.043E-02 | -2.816 | -.637 | .130 | -.660 | .126 | .217 | 2.361E-02 |
| HAGERESELAM | -2.901 | -.928 | .637 | -1.403 | .546 | .426 | -1.063 | .794 | -6.80E-02 | -9.88E-04 | -.223 | .373 |
| LIMO | -2.654 | .951 | 1.459 | 1.655 | -1.727 | .689 | .265 | 3.669E-02 | -.747 | .672 | -8.53E-02 | -.225 |
| MAREKALOMA | 2.259 | -.131 | 1.392 | -1.063 | .310 | -.217 | 1.307 | -.163 | .205 | .185 | -.553 | .120 |
| SEKA | -.610 | .715 | -1.391 | .708 | 7.678E-02 | -6.06E-02 | -.125 | -7.07E-02 | -3.25E-02 | -.583 | -.317 | -.219 |
| Y/CHEFIE | -3.178 | 2.970 | -1.693 | -.448 | .344 | 2.614E-03 | 1.317 | .645 | 1.108 | .286 | .443 | .104 |

Unstandardized canonical discriminant functions evaluated at group means

Appendix 5. R-values for Function 1 to 12 contributed for variability among altitudes

**Standardized Canonical Discriminant Function Coefficients**

| | Function | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| R1 | .022 | .614 | .712 | .405 | .195 | .549 | -.066 | .360 | .429 | -.113 | .323 |
| R2 | .464 | -.229 | -.054 | -.440 | -.386 | .242 | .015 | -.286 | .472 | -.249 | .226 |
| R3 | -.442 | -.401 | -.036 | -.079 | -.335 | .204 | -.027 | .179 | -.282 | -.275 | .054 |
| R4 | -.124 | -.636 | .646 | -.297 | -.159 | -.040 | -.008 | .291 | .166 | -.037 | .436 |
| R5 | -.833 | .193 | -.440 | .465 | .547 | .232 | .019 | .428 | .218 | .724 | -.293 |
| R6 | -.070 | -.493 | -.441 | .823 | .247 | -.355 | -.003 | -.114 | -.352 | .534 | .166 |
| R7 | .543 | -.967 | .001 | -.338 | .643 | -.146 | -.189 | -.165 | -.006 | .481 | .277 |
| R8 | -.324 | .545 | -.439 | .676 | -.768 | -.150 | .328 | .023 | -.305 | -.177 | -.197 |
| R9 | .002 | .275 | -.069 | -.311 | -.486 | .074 | .442 | .027 | .107 | -.023 | -.215 |
| R10 | -1.006 | .516 | -.083 | -.100 | .233 | .541 | .124 | .127 | 1.022 | .324 | .059 |
| R11 | -.275 | .144 | -.148 | -.365 | .547 | .405 | .232 | .664 | -.531 | .140 | -.153 |
| R12 | .632 | .389 | -.514 | -.186 | .227 | .264 | .042 | -.493 | -.047 | -.102 | -.319 |
| R13 | -.596 | .842 | -.423 | -.041 | .214 | .245 | -.209 | .496 | .094 | .318 | .530 |
| R14 | .452 | -.718 | -.653 | .295 | .198 | -.174 | .074 | .343 | .000 | -.291 | .159 |
| R15 | -.060 | -.491 | -.229 | -.065 | -.592 | -.399 | -.081 | .160 | .078 | -.287 | .542 |
| R16 | 1.541 | -.368 | -.279 | -.377 | -.451 | .133 | -.208 | .030 | -.260 | -.253 | -.330 |
| R17 | .336 | .499 | .703 | .514 | .256 | -.379 | -.035 | .270 | .305 | .230 | -.310 |
| R18 | .910 | -.224 | -.008 | .257 | .623 | -.427 | .075 | .204 | .415 | .155 | -.282 |
| R19 | -.450 | -.335 | .546 | .072 | .259 | .161 | -.287 | -.139 | -.701 | -.208 | .043 |
| R20 | .676 | -.062 | .657 | -.397 | -.004 | .378 | .136 | -.262 | -.469 | .352 | .298 |
| R21 | .210 | .807 | .744 | -.270 | -.047 | .053 | .499 | -.387 | .237 | -.207 | .485 |
| R22 | -.629 | .705 | .474 | -.388 | .053 | .109 | -.697 | -.308 | .051 | -.372 | .667 |
| R23 | -.291 | .600 | .343 | .534 | .190 | .734 | .268 | .062 | .634 | -.114 | -.244 |
| R24 | -.065 | -.462 | -.501 | .378 | -.024 | .082 | -.002 | -.117 | .232 | -.353 | -.015 |
| R25 | .022 | -.688 | .164 | 1.008 | -.080 | .012 | .067 | .261 | -.376 | .613 | -.388 |

Appendix 6. Altitudes and their values for function 1 to 12

**Functions at Group Centroids**

| | Function | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ALTNUM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| 1700 | 3.881 | -.441 | -5.927 | 1.758 | -2.610 | -1.713 | 2.594 | -1.139 | .458 | -.139 | .532 |
| 1730 | .535 | 1.874 | 1.172 | -2.634 | -.441 | .766 | 1.011 | -1.435 | -.619 | -.428 | -.130 |
| 1790 | 2.548 | 1.780 | .310 | 1.533 | 1.129 | .722 | .826 | .489 | .135 | -.128 | -.266 |
| 1800 | 4.686 | -1.538 | 1.959 | -1.702 | -.506 | -2.298 | -.165 | .595 | -1.92E-02 | .879 | -.470 |
| 1850 | .118 | -.372 | -1.409 | -.908 | .669 | 2.866E-02 | -.451 | .351 | -4.67E-02 | -.209 | .117 |
| 1900 | -2.049 | -.462 | -.937 | -.548 | -1.267 | 1.324 | .365 | .449 | .716 | .836 | -.304 |
| 2200 | -1.713 | .747 | .777 | .285 | .894 | -.949 | -.361 | -1.043 | 1.463 | -2.78E-02 | -2.55E-02 |
| 2300 | 1.446 | -5.093 | -1.718 | 2.299 | 1.578 | 1.136 | -1.379 | -2.636 | -1.393 | 1.143 | -.437 |
| 2400 | 1.467 | 1.568 | 1.263 | .596 | -.907 | .486 | -1.115 | -7.26E-02 | -8.28E-02 | .419 | .512 |
| 2490 | -.603 | -.820 | .458 | 1.002 | -1.403 | -.152 | -.793 | .105 | -.153 | -.963 | -.334 |
| 2700 | -3.284 | 1.600 | -8.15E-02 | .673 | .179 | -1.050 | .443 | .218 | -.965 | .400 | -1.90E-02 |
| 2750 | -.860 | -3.491 | 2.357 | .272 | .294 | .242 | 1.281 | .249 | 1.390E-02 | -.157 | .394 |

Unstandardized canonical discriminant functions evaluated at group means

Appendix 7. Eigenvalues for the 12 principal components contributed for variability among Isolates to R2

**Total Variance Explained[a]**

| | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| Component | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 14.028 | 24.611 | 24.611 | 14.028 | 24.611 | 24.611 |
| 2 | 6.575 | 11.535 | 36.146 | 6.575 | 11.535 | 36.146 |
| 3 | 5.568 | 9.768 | 45.914 | 5.568 | 9.768 | 45.914 |
| 4 | 4.772 | 8.372 | 54.286 | 4.772 | 8.372 | 54.286 |
| 5 | 4.546 | 7.975 | 62.261 | 4.546 | 7.975 | 62.261 |
| 6 | 3.708 | 6.506 | 68.767 | 3.708 | 6.506 | 68.767 |
| 7 | 3.235 | 5.676 | 74.444 | 3.235 | 5.676 | 74.444 |
| 8 | 2.791 | 4.896 | 79.340 | 2.791 | 4.896 | 79.340 |
| 9 | 2.569 | 4.507 | 83.847 | 2.569 | 4.507 | 83.847 |
| 10 | 1.798 | 3.155 | 87.002 | 1.798 | 3.155 | 87.002 |
| 11 | 1.699 | 2.980 | 89.983 | 1.699 | 2.980 | 89.983 |
| 12 | 1.156 | 2.027 | 92.010 | 1.156 | 2.027 | 92.010 |
| 13 | .990 | 1.737 | 93.747 | | | |
| 14 | .848 | 1.488 | 95.235 | | | |
| 15 | .737 | 1.292 | 96.527 | | | |
| 16 | .628 | 1.103 | 97.630 | | | |
| 17 | .378 | .663 | 98.293 | | | |
| 18 | .330 | .578 | 98.871 | | | |
| 19 | .271 | .476 | 99.347 | | | |
| 20 | .173 | .304 | 99.651 | | | |
| 21 | 9.529E-02 | .167 | 99.818 | | | |
| 22 | 6.546E-02 | .115 | 99.933 | | | |
| 23 | 3.059E-02 | 5.367E-02 | 99.986 | | | |
| 24 | 7.831E-03 | 1.374E-02 | 100.000 | | | |
| 25 | 1.263E-15 | 2.216E-15 | 100.000 | | | |
| 26 | 9.219E-16 | 1.617E-15 | 100.000 | | | |
| 27 | 8.893E-16 | 1.560E-15 | 100.000 | | | |
| 28 | 6.265E-16 | 1.099E-15 | 100.000 | | | |
| 29 | 6.004E-16 | 1.053E-15 | 100.000 | | | |
| 30 | 5.823E-16 | 1.022E-15 | 100.000 | | | |
| 31 | 4.847E-16 | 8.503E-16 | 100.000 | | | |
| 32 | 4.561E-16 | 8.002E-16 | 100.000 | | | |
| 33 | 3.399E-16 | 5.963E-16 | 100.000 | | | |
| 34 | 3.019E-16 | 5.296E-16 | 100.000 | | | |
| 35 | 2.384E-16 | 4.182E-16 | 100.000 | | | |
| 36 | 1.902E-16 | 3.336E-16 | 100.000 | | | |
| 37 | 1.561E-16 | 2.739E-16 | 100.000 | | | |
| 38 | 1.440E-16 | 2.527E-16 | 100.000 | | | |
| 39 | 7.418E-17 | 1.301E-16 | 100.000 | | | |
| 40 | 4.934E-18 | 8.656E-18 | 100.000 | | | |
| 41 | -9.34E-18 | -1.638E-17 | 100.000 | | | |
| 42 | -4.59E-17 | -8.058E-17 | 100.000 | | | |
| 43 | -6.42E-17 | -1.127E-16 | 100.000 | | | |
| 44 | -8.43E-17 | -1.479E-16 | 100.000 | | | |
| 45 | -9.61E-17 | -1.686E-16 | 100.000 | | | |
| 46 | -1.41E-16 | -2.475E-16 | 100.000 | | | |
| 47 | -1.87E-16 | -3.274E-16 | 100.000 | | | |
| 48 | -2.51E-16 | -4.409E-16 | 100.000 | | | |
| 49 | -2.79E-16 | -4.891E-16 | 100.000 | | | |
| 50 | -4.06E-16 | -7.114E-16 | 100.000 | | | |
| 51 | -4.81E-16 | -8.432E-16 | 100.000 | | | |
| 52 | -5.86E-16 | -1.029E-15 | 100.000 | | | |
| 53 | -8.15E-16 | -1.429E-15 | 100.000 | | | |
| 54 | -9.00E-16 | -1.579E-15 | 100.000 | | | |
| 55 | -1.12E-15 | -1.958E-15 | 100.000 | | | |
| 56 | -1.33E-15 | -2.341E-15 | 100.000 | | | |
| 57 | -1.89E-15 | -3.308E-15 | 100.000 | | | |

Extraction Method: Principal Component Analysis.

a. Only cases for which VAR00001 = 1 are used in the analysis phase.

# Appendix 8. Isolates and their values for the 12 principal components

**Component Matrix[a,b]**

| | Component | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| XCC | .590 | -9.24E-02 | .556 | .305 | -.311 | -.148 | .211 | -1.54E-02 | -6.50E-02 | -1.93E-02 | -1.38E-02 | -1.64E-03 |
| XCM | .721 | -6.93E-02 | .324 | 1.855E-02 | -.148 | -.449 | 7.685E-02 | .247 | -8.66E-02 | 6.726E-02 | -.118 | -5.16E-02 |
| FGMTGE | .587 | 1.421E-02 | 4.317E-04 | .375 | -.142 | -.148 | -.533 | -1.51E-03 | -7.82E-02 | -.307 | .203 | -6.50E-02 |
| YCMSAS | .110 | -6.30E-02 | 9.370E-02 | .592 | .232 | -.365 | .126 | -.539 | -1.80E-02 | 4.096E-02 | .193 | -8.55E-02 |
| HSHSGU | .437 | .565 | -.304 | -.178 | -.339 | -.226 | -6.86E-02 | -.318 | -8.97E-02 | -.141 | -.130 | -7.44E-02 |
| HSHSDA1 | .314 | .549 | .338 | .428 | -.158 | 6.621E-02 | 1.354E-02 | .149 | .308 | -2.64E-03 | -.269 | -.159 |
| HSHSDA_2 | .464 | .426 | .129 | 5.853E-02 | -.218 | -.507 | -.346 | .137 | 9.474E-02 | .103 | -.126 | 6.616E-02 |
| FGMSTO | .498 | .461 | -.282 | -9.93E-02 | -.142 | -.304 | -.393 | -3.09E-02 | -.311 | 7.874E-02 | 2.693E-03 | 7.467E-02 |
| CHMTYE | .537 | -7.15E-03 | .275 | 2.529E-02 | .374 | .339 | -.150 | 9.456E-02 | -.416 | 8.445E-02 | -.214 | 3.390E-02 |
| ANHSGI | .400 | -6.11E-02 | .462 | 7.384E-02 | 9.761E-02 | .259 | -.421 | -.229 | -.439 | 5.803E-02 | -4.47E-02 | .241 |
| CHMSNE | .737 | -.145 | -9.28E-03 | -.109 | .301 | -.304 | -.227 | 2.265E-02 | -.168 | -.172 | .184 | -5.74E-02 |
| BOMSGN | .667 | -.229 | -.178 | -.379 | -2.96E-02 | -1.60E-02 | .130 | -.258 | 6.439E-02 | .153 | -.394 | -2.97E-02 |
| MLHSAM | .695 | -.247 | -4.49E-02 | -8.60E-02 | .105 | -.256 | .185 | -.349 | .172 | .120 | -.349 | -7.01E-02 |
| GOMSGN | .587 | 6.022E-02 | 7.047E-02 | 6.799E-03 | .497 | 1.234E-02 | .184 | -.367 | -6.77E-03 | -.237 | -5.16E-02 | -1.91E-02 |
| ANHTMZ1 | .544 | -.500 | .215 | .213 | -5.66E-02 | -.216 | -.382 | -1.88E-02 | -4.61E-02 | .157 | .219 | 3.055E-02 |
| LIHSBE | .253 | .421 | .584 | -.502 | 5.778E-02 | -1.19E-02 | 6.329E-02 | -6.55E-02 | .198 | -8.13E-02 | .178 | -1.24E-02 |
| SEMSBE1 | 6.978E-02 | -.123 | -.285 | -8.57E-02 | .267 | .110 | .471 | .554 | -.152 | .243 | .355 | -5.79E-02 |
| LIHSSW2 | .184 | .631 | .101 | .128 | -.415 | .287 | .153 | .245 | -1.42E-02 | .185 | -8.64E-02 | .190 |
| YCMTGE | 8.766E-02 | .648 | -.189 | .173 | -.328 | .213 | .182 | .359 | -.110 | -7.50E-02 | 3.817E-02 | .293 |
| ARMSNK | .306 | -.159 | 9.695E-02 | .358 | .273 | -1.50E-02 | .378 | -5.52E-03 | -3.41E-02 | .639 | .104 | -.240 |
| ANHTMZ2 | .197 | .244 | .779 | -.113 | -.166 | .263 | -2.84E-03 | .150 | -4.09E-02 | -4.04E-03 | 4.813E-03 | -.308 |
| SEMSGN | .439 | -.105 | .539 | -1.38E-02 | -4.81E-02 | .410 | 6.272E-02 | -.167 | -9.47E-02 | -.206 | .297 | 9.236E-02 |
| GOMS_AD | .354 | .445 | .407 | -.418 | 5.552E-02 | -1.82E-02 | .135 | -.415 | -6.36E-02 | .109 | .196 | 6.421E-02 |
| GEMSBE | .351 | .434 | .320 | -.397 | .213 | -9.74E-02 | .394 | 9.119E-02 | -2.70E-02 | 6.021E-02 | -.158 | 3.278E-02 |
| BOMTTU | .408 | .578 | 6.967E-02 | -.138 | 9.606E-02 | .254 | .123 | -3.90E-02 | 7.500E-02 | .224 | .411 | -.183 |
| SEMTMG | .466 | -.155 | -.279 | -.258 | .603 | .169 | -.279 | -9.51E-02 | -.144 | .188 | -2.36E-02 | .188 |
| LIHSSW1 | .462 | 1.277E-02 | .499 | -.421 | 1.995E-02 | .271 | 3.234E-02 | 7.490E-02 | .288 | -.177 | .136 | .229 |
| ANHSSI | .724 | 5.208E-02 | -.204 | 9.619E-02 | -.338 | .306 | .117 | .101 | -.216 | .132 | -.186 | -.122 |
| BOMTTU2 | .725 | -.190 | .208 | -.227 | .166 | -.177 | -.211 | .394 | 5.081E-02 | 5.954E-02 | 2.043E-02 | -7.35E-02 |
| SEMSGN2 | .545 | -.344 | 2.971E-02 | -.503 | -.293 | 1.761E-02 | -3.40E-02 | .128 | .335 | 5.115E-02 | .143 | .111 |
| HSHSGU2 | .206 | .554 | -9.82E-02 | -.216 | .393 | .114 | -.440 | .330 | 4.877E-02 | -5.07E-02 | -2.82E-02 | -.215 |
| SEMSBE3 | .736 | .207 | -.411 | -.220 | -.114 | .125 | .127 | .141 | 2.742E-02 | -.203 | 4.315E-02 | -5.59E-02 |
| GEMSBU | .685 | -.125 | -5.46E-02 | -.425 | 3.168E-02 | -3.79E-02 | .103 | .210 | -3.18E-02 | -.341 | .221 | -.145 |
| BOMSGN2 | .719 | -4.24E-02 | -.313 | -.229 | -.106 | .255 | -.107 | -9.09E-02 | .281 | .216 | 2.006E-02 | -.280 |
| HSHSGU3 | .512 | -.571 | -.304 | -.111 | -.407 | 2.549E-02 | -4.69E-02 | .170 | -9.91E-02 | -8.85E-02 | 3.452E-02 | 9.813E-02 |
| CORM_ROT | .604 | -.166 | -.505 | .420 | -.141 | -9.30E-02 | .195 | 4.739E-02 | 4.230E-02 | -3.06E-02 | 4.408E-02 | -8.58E-03 |
| SHEATH_R | .748 | 7.943E-02 | -.291 | .392 | -.164 | -6.44E-02 | .185 | 6.516E-02 | -.142 | -.189 | 2.207E-02 | -8.43E-02 |
| DEAD_HEA | .346 | -1.49E-02 | -.124 | .425 | .439 | .169 | -.489 | .185 | .254 | -.144 | -.114 | -.144 |
| MLHSAM2 | .523 | -.520 | -.310 | -6.06E-02 | -.380 | 2.610E-03 | 4.348E-02 | -5.16E-02 | -.195 | -3.76E-02 | .312 | .183 |
| MLHST1 | .557 | -.518 | -3.54E-02 | -5.94E-02 | -.450 | .129 | -4.00E-02 | -.239 | -.145 | .247 | 2.176E-02 | 4.142E-02 |
| SEMTMG2 | .299 | .561 | -8.42E-02 | .657 | -.107 | -6.42E-02 | .143 | -.151 | -.158 | 3.543E-03 | 6.184E-02 | .117 |
| GOMSGN2 | .224 | .456 | -.308 | .218 | -1.64E-02 | -.263 | .164 | .202 | .474 | .165 | .119 | .396 |
| ANHSGI2 | .318 | .808 | -.193 | -6.93E-02 | 1.476E-02 | 3.280E-02 | -6.43E-03 | -.363 | 4.623E-03 | -.120 | -9.28E-02 | .105 |
| BOMSSI | .387 | .308 | -.490 | -.267 | -.303 | -.215 | -.172 | -.277 | .236 | 3.913E-02 | .184 | -.124 |
| SEMSBE31 | .398 | -.309 | 5.129E-02 | .323 | .381 | 6.377E-03 | .307 | .279 | .321 | -.309 | -.154 | 4.929E-02 |
| CHMTYE2 | .449 | 2.416E-02 | -.538 | .231 | .125 | .608 | 5.068E-03 | -.109 | 1.208E-02 | 8.762E-02 | 2.505E-02 | -3.16E-02 |
| GEMTSH | .335 | -6.31E-02 | .182 | .451 | 5.513E-02 | .209 | -.574 | .221 | .372 | .149 | -5.97E-02 | .120 |
| MLHTSH | .543 | 7.267E-02 | -8.16E-03 | .220 | 5.633E-02 | .235 | -.122 | -.240 | .544 | .241 | .180 | 7.740E-02 |
| CHMSNE2 | .517 | .232 | -.321 | .182 | 8.662E-02 | .624 | 3.232E-02 | -5.48E-02 | -.190 | -.109 | 1.192E-02 | -.119 |
| BOMTTU1 | .476 | -9.82E-02 | -4.50E-02 | 4.100E-02 | .576 | -.113 | .286 | -.256 | .331 | -.246 | -2.82E-02 | .120 |
| M_TB | .543 | -9.74E-02 | .102 | .422 | .338 | .134 | .365 | 5.717E-02 | -.219 | -.238 | 6.253E-03 | 5.170E-02 |
| XCC1 | .590 | -9.24E-02 | .556 | .305 | -.311 | -.148 | .211 | -1.54E-02 | -6.50E-02 | -1.93E-02 | -1.38E-02 | -1.64E-03 |
| XCM1 | .721 | -6.93E-02 | .324 | 1.855E-02 | -.148 | -.449 | 7.685E-02 | .247 | -8.66E-02 | 6.726E-02 | -.118 | -5.16E-02 |
| GIMB | .249 | 8.123E-02 | -5.93E-02 | 6.993E-02 | .697 | -.447 | -2.09E-02 | .142 | -7.66E-03 | .209 | .199 | .170 |
| SEMSBE2 | .305 | .359 | -.121 | -.269 | .509 | -9.10E-02 | -3.99E-02 | .126 | -.461 | .198 | -.149 | .166 |
| LIHSDI | .555 | -.275 | -.461 | -.426 | 2.224E-02 | -7.63E-02 | .183 | .140 | 5.951E-02 | -5.40E-02 | -.302 | 7.559E-02 |
| GEMSBE2 | .356 | -.451 | .341 | -2.92E-02 | -.127 | .508 | -5.37E-02 | -.125 | .220 | 9.688E-02 | -.253 | .217 |

Extraction Method: Principal Component Analysis.

a. 12 components extracted.

b. Only cases for which VAR00001 = 1 are used in the analysis phase.

Appendix 9. Eigenvalues for the 12 principal components contributed for variability among Regions to R16

**Total Variance Explained**[a]

| Component | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 12.345 | 23.741 | 23.741 | 12.345 | 23.741 | 23.741 |
| 2 | 6.553 | 12.603 | 36.343 | 6.553 | 12.603 | 36.343 |
| 3 | 5.128 | 9.861 | 46.204 | 5.128 | 9.861 | 46.204 |
| 4 | 4.423 | 8.506 | 54.710 | 4.423 | 8.506 | 54.710 |
| 5 | 4.113 | 7.910 | 62.619 | 4.113 | 7.910 | 62.619 |
| 6 | 3.219 | 6.191 | 68.810 | 3.219 | 6.191 | 68.810 |
| 7 | 2.888 | 5.553 | 74.363 | 2.888 | 5.553 | 74.363 |
| 8 | 2.613 | 5.025 | 79.388 | 2.613 | 5.025 | 79.388 |
| 9 | 2.215 | 4.259 | 83.647 | 2.215 | 4.259 | 83.647 |
| 10 | 1.726 | 3.320 | 86.967 | 1.726 | 3.320 | 86.967 |
| 11 | 1.627 | 3.129 | 90.096 | 1.627 | 3.129 | 90.096 |
| 12 | 1.145 | 2.201 | 92.297 | 1.145 | 2.201 | 92.297 |
| 13 | .796 | 1.531 | 93.828 | | | |
| 14 | .761 | 1.463 | 95.291 | | | |
| 15 | .723 | 1.390 | 96.680 | | | |
| 16 | .585 | 1.125 | 97.806 | | | |
| 17 | .306 | .588 | 98.394 | | | |
| 18 | .280 | .538 | 98.932 | | | |
| 19 | .214 | .411 | 99.343 | | | |
| 20 | .159 | .306 | 99.649 | | | |
| 21 | 8.954E-02 | .172 | 99.821 | | | |
| 22 | 6.289E-02 | .121 | 99.942 | | | |
| 23 | 2.363E-02 | 4.545E-02 | 99.988 | | | |
| 24 | 6.479E-03 | 1.246E-02 | 100.000 | | | |
| 25 | 1.436E-15 | 2.762E-15 | 100.000 | | | |
| 26 | 8.938E-16 | 1.719E-15 | 100.000 | | | |
| 27 | 8.262E-16 | 1.589E-15 | 100.000 | | | |
| 28 | 7.188E-16 | 1.382E-15 | 100.000 | | | |
| 29 | 6.325E-16 | 1.216E-15 | 100.000 | | | |
| 30 | 4.565E-16 | 8.779E-16 | 100.000 | | | |
| 31 | 4.036E-16 | 7.761E-16 | 100.000 | | | |
| 32 | 3.550E-16 | 6.827E-16 | 100.000 | | | |
| 33 | 3.161E-16 | 6.079E-16 | 100.000 | | | |
| 34 | 2.693E-16 | 5.178E-16 | 100.000 | | | |
| 35 | 2.507E-16 | 4.821E-16 | 100.000 | | | |
| 36 | 1.866E-16 | 3.588E-16 | 100.000 | | | |
| 37 | 1.010E-16 | 1.942E-16 | 100.000 | | | |
| 38 | 8.498E-17 | 1.634E-16 | 100.000 | | | |
| 39 | 1.726E-18 | 3.320E-18 | 100.000 | | | |
| 40 | -1.06E-17 | -2.040E-17 | 100.000 | | | |
| 41 | -8.87E-17 | -1.706E-16 | 100.000 | | | |
| 42 | -1.30E-16 | -2.501E-16 | 100.000 | | | |
| 43 | -1.97E-16 | -3.793E-16 | 100.000 | | | |
| 44 | -2.39E-16 | -4.599E-16 | 100.000 | | | |
| 45 | -2.73E-16 | -5.254E-16 | 100.000 | | | |
| 46 | -3.60E-16 | -6.930E-16 | 100.000 | | | |
| 47 | -4.18E-16 | -8.042E-16 | 100.000 | | | |
| 48 | -5.01E-16 | -9.629E-16 | 100.000 | | | |
| 49 | -5.30E-16 | -1.019E-15 | 100.000 | | | |
| 50 | -7.45E-16 | -1.434E-15 | 100.000 | | | |
| 51 | -1.15E-15 | -2.211E-15 | 100.000 | | | |
| 52 | -1.33E-15 | -2.559E-15 | 100.000 | | | |

Extraction Method: Principal Component Analysis.

a. Only cases for which VAR00001 = 1 are used in the analysis phase.

## Appendix 10. Regions and their values for the 12 principal components

**Component Matrix[a,b]**

| | Component | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| F_GENET | .562 | 9.782E-03 | -.157 | .318 | .327 | -.482 | -.227 | 7.087E-03 | 8.547E-02 | -.269 | .211 | -5.28E-02 |
| Y_CHEFI | 5.140E-02 | -3.56E-02 | -.144 | .605 | -7.17E-02 | -.301 | .558 | -.247 | 9.817E-02 | 3.002E-02 | .161 | -8.20E-02 |
| HAGERESE | .483 | .526 | -.301 | -.379 | -3.20E-02 | -.350 | .114 | -.198 | -.107 | -.119 | -9.96E-02 | -8.62E-02 |
| HAGERES1 | .249 | .582 | 3.970E-02 | .297 | .421 | 3.533E-02 | .138 | .330 | -4.48E-02 | -3.76E-02 | -.280 | -.178 |
| HAGERES2 | .425 | .429 | -3.19E-02 | 3.492E-02 | 8.907E-02 | -.588 | -6.55E-02 | .282 | .177 | .104 | -.253 | 2.711E-02 |
| F_GENET1 | .539 | .423 | -.247 | -.139 | -8.25E-02 | -.467 | -.288 | -.192 | .134 | .110 | -6.06E-02 | 4.924E-02 |
| CHEHA | .519 | -4.82E-04 | .412 | .302 | 5.122E-02 | .215 | -.319 | -.331 | .175 | 2.830E-03 | -.249 | 1.680E-02 |
| ANGACHA | .364 | -3.85E-02 | .473 | .264 | .346 | -.174 | -.223 | -.504 | .101 | 1.726E-02 | -7.26E-02 | .239 |
| CHEHA1 | .723 | -.163 | .146 | .155 | -.311 | -.323 | -.170 | -2.80E-02 | .182 | -.142 | .155 | -5.66E-02 |
| BOLOSOSO | .691 | -.268 | 5.285E-02 | -.312 | -.177 | 2.674E-03 | .210 | -.129 | -.195 | 9.734E-02 | -.383 | -4.82E-02 |
| MAREKALO | .676 | -.265 | 4.675E-02 | 2.159E-02 | -.216 | -.150 | .448 | -3.90E-02 | -.105 | 5.092E-02 | -.375 | -9.43E-02 |
| GOMA | .586 | 5.248E-02 | .237 | .290 | -.326 | 8.188E-02 | .284 | -.234 | -.126 | -.272 | 2.917E-02 | 2.996E-03 |
| ANGACHA1 | .481 | -.489 | .122 | .291 | .240 | -.441 | -5.38E-02 | 3.678E-02 | .226 | .176 | .105 | 1.491E-02 |
| LIMO | .227 | .444 | .717 | -.255 | -4.23E-02 | -9.02E-02 | .171 | .173 | -2.50E-03 | -3.63E-02 | .154 | -1.49E-02 |
| SEKA | 9.047E-02 | -.144 | -.137 | -3.94E-02 | -.364 | .604 | -.143 | .201 | .479 | .212 | .242 | -5.08E-02 |
| LIMO1 | .173 | .638 | -9.65E-02 | -.163 | .446 | .283 | -2.09E-02 | .111 | .172 | .136 | -.160 | .181 |
| Y_CHEFIE | .104 | .639 | -.355 | -.162 | .244 | .311 | -.162 | .125 | .216 | -8.40E-02 | 5.220E-03 | .298 |
| AREKA | .249 | -.141 | 6.290E-04 | .420 | -8.07E-02 | .295 | .319 | -4.23E-02 | .280 | .576 | -2.36E-02 | -.254 |
| ANGACHA2 | .106 | .299 | .673 | -6.83E-02 | .465 | 7.385E-02 | -1.28E-02 | 8.096E-02 | .178 | -8.11E-03 | -3.50E-02 | -.327 |
| SEKA1 | .385 | -7.48E-02 | .536 | 4.472E-02 | .432 | .146 | .163 | -.168 | .131 | -.200 | .302 | .114 |
| GOMA1 | .347 | .453 | .537 | -.230 | -8.86E-02 | -.109 | .332 | -.256 | 2.642E-02 | .116 | .170 | 7.506E-02 |
| GESSA | .326 | .443 | .464 | -.185 | -.288 | .173 | .234 | 8.867E-02 | .274 | -1.98E-02 | -.258 | 6.602E-03 |
| BOLOSOS1 | .450 | .563 | .187 | -4.90E-02 | 1.493E-02 | .233 | 9.479E-02 | -1.34E-03 | 4.351E-02 | .257 | .374 | -.176 |
| SEKA2 | .554 | -.205 | .146 | .178 | -.458 | 9.884E-02 | -.349 | -.291 | -.244 | .189 | 3.066E-02 | .205 |
| LIMO2 | .437 | 2.558E-02 | .674 | -.214 | .154 | .116 | 6.113E-02 | .268 | -9.41E-02 | -.124 | .150 | .236 |
| ANGACHA3 | .727 | 2.452E-02 | -.244 | -.163 | .350 | .261 | -4.43E-02 | -.144 | .171 | 5.828E-02 | -.226 | -.137 |
| BOLOSOS2 | .681 | -.194 | .344 | 3.496E-02 | -.130 | -.152 | -.289 | .333 | .193 | 7.581E-02 | -6.01E-02 | -9.14E-02 |
| SEKA3 | .549 | -.369 | .210 | -.500 | 7.191E-02 | -8.87E-02 | 1.199E-03 | .376 | -9.56E-02 | .142 | .105 | 9.546E-02 |
| HAGERES3 | .279 | .524 | .154 | .108 | -.263 | 2.232E-02 | -.605 | .140 | -.237 | -9.37E-03 | 5.554E-02 | -.202 |
| SEKA4 | .792 | .152 | -.238 | -.316 | -9.03E-02 | .186 | -8.15E-02 | 9.587E-02 | -1.40E-02 | -.185 | 8.375E-02 | -4.65E-02 |
| GESSA1 | .693 | -.153 | .182 | -.322 | -.199 | 2.316E-02 | -8.63E-02 | .180 | .210 | -.303 | .201 | -.150 |
| BOLOSOS3 | .793 | -9.87E-02 | -9.05E-02 | -.217 | 6.167E-02 | 9.883E-02 | -1.64E-02 | 7.244E-02 | -.337 | .249 | 6.476E-02 | -.280 |
| HAGERES4 | .510 | -.604 | -.272 | -.346 | .186 | -5.62E-02 | -.155 | 7.253E-02 | .142 | -5.50E-02 | -3.96E-03 | 8.249E-02 |
| DALE | .614 | -.202 | -.620 | .163 | 5.871E-02 | 7.379E-02 | .193 | 9.872E-02 | .194 | -7.46E-02 | -1.15E-02 | -1.41E-02 |
| MAREKAL1 | .730 | 5.816E-02 | -.444 | .145 | .142 | 5.075E-02 | .138 | -6.32E-03 | .324 | -.251 | -2.76E-02 | -8.69E-02 |
| SEKA5 | .384 | -3.17E-02 | -6.95E-02 | .675 | 2.716E-02 | -6.25E-05 | -.376 | .204 | -.296 | -.153 | -3.78E-02 | -.136 |
| MAREKAL2 | .524 | -.552 | -.293 | -.304 | .160 | -7.65E-02 | 2.478E-02 | -.119 | .220 | 3.762E-03 | .265 | .192 |
| MAREKAL3 | .535 | -.534 | -5.89E-02 | -.265 | .385 | -.104 | .128 | -.242 | 6.630E-02 | .242 | -3.47E-02 | 2.817E-02 |
| SEKA6 | .268 | .571 | -.415 | .383 | .236 | -2.43E-02 | .244 | -.161 | .225 | -1.93E-02 | 8.213E-03 | .107 |
| GOMA2 | .246 | .435 | -.406 | .101 | -.150 | -6.30E-03 | .190 | .531 | -3.08E-02 | .225 | 4.463E-02 | .381 |
| ANGACHA4 | .385 | .778 | -.126 | -9.08E-02 | -9.59E-02 | -6.65E-02 | .139 | -.243 | -.263 | -.110 | -1.03E-02 | .116 |
| BOLOSOS4 | .475 | .246 | -.378 | -.390 | -.108 | -.332 | 9.909E-02 | 2.511E-02 | -.299 | .105 | .244 | -9.79E-02 |
| SEKA7 | .349 | -.298 | 3.755E-02 | .458 | -.123 | .310 | .192 | .423 | 8.005E-02 | -.376 | -.150 | 5.097E-02 |
| CHEHA2 | .553 | -3.28E-02 | -.385 | .171 | .101 | .508 | -.126 | -.244 | -.317 | 6.363E-02 | .134 | 1.550E-03 |
| GESSA2 | .321 | -5.36E-02 | 6.600E-02 | .548 | .452 | -.125 | -.310 | .342 | -.256 | .182 | -6.24E-02 | .111 |
| MAREKAL4 | .570 | 5.619E-02 | 1.032E-02 | .279 | .231 | 3.305E-02 | .243 | .194 | -.429 | .312 | .223 | 8.751E-02 |
| CHEHA3 | .586 | .192 | -.211 | .120 | .176 | .508 | -.179 | -.338 | -.159 | -.154 | .136 | -7.68E-02 |
| BOLOSOS5 | .483 | -.111 | .137 | .350 | -.454 | .109 | .422 | .102 | -.195 | -.266 | 3.599E-02 | .138 |
| GIMBO | .246 | 7.560E-02 | 8.112E-02 | .474 | -.641 | -.141 | -4.42E-02 | .137 | .220 | .228 | 9.887E-02 | .157 |
| SEKA8 | .339 | .335 | .143 | 4.850E-02 | -.554 | 8.219E-02 | -.358 | -.306 | .170 | .165 | -.183 | .156 |
| LIMO3 | .603 | -.332 | -.174 | -.399 | -.385 | .129 | -5.69E-02 | .125 | -8.10E-02 | -6.90E-02 | -.289 | 5.792E-02 |
| GESSA3 | .334 | -.440 | .388 | 1.602E-02 | .487 | .222 | .109 | -5.18E-03 | -.246 | 4.565E-02 | -.232 | .215 |

Extraction Method: Principal Component Analysis.

a. 12 components extracted.

b. Only cases for which VAR00001 = 1 are used in the analysis phase.

Appendix 11. Eigenvalues for the 12 principal components contributed for Variability among Altitudes to R16

**Total Variance Explained**[a]

| Component | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 12.858 | 24.260 | 24.260 | 12.858 | 24.260 | 24.260 |
| 2 | 6.614 | 12.479 | 36.739 | 6.614 | 12.479 | 36.739 |
| 3 | 5.131 | 9.682 | 46.421 | 5.131 | 9.682 | 46.421 |
| 4 | 4.485 | 8.461 | 54.883 | 4.485 | 8.461 | 54.883 |
| 5 | 4.216 | 7.954 | 62.837 | 4.216 | 7.954 | 62.837 |
| 6 | 3.250 | 6.132 | 68.969 | 3.250 | 6.132 | 68.969 |
| 7 | 2.890 | 5.452 | 74.421 | 2.890 | 5.452 | 74.421 |
| 8 | 2.614 | 4.933 | 79.354 | 2.614 | 4.933 | 79.354 |
| 9 | 2.226 | 4.201 | 83.555 | 2.226 | 4.201 | 83.555 |
| 10 | 1.749 | 3.300 | 86.855 | 1.749 | 3.300 | 86.855 |
| 11 | 1.723 | 3.251 | 90.106 | 1.723 | 3.251 | 90.106 |
| 12 | 1.146 | 2.163 | 92.269 | 1.146 | 2.163 | 92.269 |
| 13 | .801 | 1.511 | 93.780 | | | |
| 14 | .761 | 1.436 | 95.217 | | | |
| 15 | .742 | 1.400 | 96.617 | | | |
| 16 | .587 | 1.107 | 97.724 | | | |
| 17 | .323 | .610 | 98.334 | | | |
| 18 | .303 | .571 | 98.905 | | | |
| 19 | .214 | .404 | 99.309 | | | |
| 20 | .159 | .300 | 99.610 | | | |
| 21 | .107 | .202 | 99.812 | | | |
| 22 | 6.418E-02 | .121 | 99.933 | | | |
| 23 | 2.873E-02 | 5.420E-02 | 99.987 | | | |
| 24 | 6.875E-03 | 1.297E-02 | 100.000 | | | |
| 25 | 1.789E-15 | 3.375E-15 | 100.000 | | | |
| 26 | 1.306E-15 | 2.464E-15 | 100.000 | | | |
| 27 | 9.268E-16 | 1.749E-15 | 100.000 | | | |
| 28 | 7.293E-16 | 1.376E-15 | 100.000 | | | |
| 29 | 5.537E-16 | 1.045E-15 | 100.000 | | | |
| 30 | 4.497E-16 | 8.486E-16 | 100.000 | | | |
| 31 | 3.878E-16 | 7.318E-16 | 100.000 | | | |
| 32 | 3.320E-16 | 6.264E-16 | 100.000 | | | |
| 33 | 2.287E-16 | 4.315E-16 | 100.000 | | | |
| 34 | 2.035E-16 | 3.839E-16 | 100.000 | | | |
| 35 | 1.725E-16 | 3.254E-16 | 100.000 | | | |
| 36 | 1.582E-16 | 2.985E-16 | 100.000 | | | |
| 37 | 8.417E-17 | 1.588E-16 | 100.000 | | | |
| 38 | 2.835E-17 | 5.348E-17 | 100.000 | | | |
| 39 | -2.56E-17 | -4.821E-17 | 100.000 | | | |
| 40 | -5.17E-17 | -9.748E-17 | 100.000 | | | |
| 41 | -1.46E-16 | -2.754E-16 | 100.000 | | | |
| 42 | -1.67E-16 | -3.142E-16 | 100.000 | | | |
| 43 | -1.78E-16 | -3.364E-16 | 100.000 | | | |
| 44 | -2.48E-16 | -4.685E-16 | 100.000 | | | |
| 45 | -2.75E-16 | -5.193E-16 | 100.000 | | | |
| 46 | -3.19E-16 | -6.020E-16 | 100.000 | | | |
| 47 | -3.64E-16 | -6.870E-16 | 100.000 | | | |
| 48 | -4.58E-16 | -8.633E-16 | 100.000 | | | |
| 49 | -5.21E-16 | -9.826E-16 | 100.000 | | | |
| 50 | -7.03E-16 | -1.327E-15 | 100.000 | | | |
| 51 | -8.82E-16 | -1.664E-15 | 100.000 | | | |
| 52 | -9.45E-16 | -1.783E-15 | 100.000 | | | |
| 53 | -1.18E-15 | -2.228E-15 | 100.000 | | | |

Extraction Method: Principal Component Analysis.

a. Only cases for which VAR00001 = 1 are used in the analysis phase.

Appendix 12. Altitudes and their values for the 12 principal components

**Component Matrix[a,b]**

| | Component | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| V2300.00 | .578 | 4.024E-03 | -.168 | .388 | -.224 | -.430 | -.248 | -5.51E-03 | 4.724E-02 | .350 | -.149 | -5.06E-02 |
| V1900.00 | 6.371E-02 | -4.96E-02 | -.155 | .565 | .227 | -.291 | .544 | -.258 | 7.293E-02 | .175 | .102 | -8.08E-02 |
| V2750.00 | .467 | .542 | -.298 | -.359 | -9.26E-02 | -.361 | .107 | -.202 | -.104 | -4.35E-02 | -.148 | -8.94E-02 |
| V2750.01 | .252 | .565 | 1.837E-02 | .420 | -.339 | 5.588E-02 | .137 | .327 | -4.50E-02 | -.197 | -.127 | -.193 |
| V2750.02 | .434 | .421 | -4.35E-02 | .102 | -.126 | -.595 | -7.41E-02 | .281 | .179 | -.240 | 9.219E-03 | 1.990E-02 |
| V1900.01 | .531 | .436 | -.246 | -.142 | 2.045E-02 | -.468 | -.298 | -.193 | .134 | -7.88E-02 | 8.392E-02 | 4.575E-02 |
| V1850.00 | .522 | 1.169E-02 | .409 | .294 | 4.856E-02 | .232 | -.316 | -.325 | .191 | -.203 | -8.44E-02 | 4.356E-03 |
| V2490.00 | .382 | -4.57E-02 | .461 | .359 | -.269 | -.137 | -.234 | -.506 | .102 | -5.99E-02 | -7.89E-03 | .237 |
| V1850.01 | .720 | -.133 | .156 | 5.033E-02 | .351 | -.319 | -.179 | -3.18E-02 | .163 | .230 | -6.09E-02 | -5.55E-02 |
| V1800.00 | .694 | -.238 | 6.800E-02 | -.361 | 9.380E-02 | -2.02E-02 | .214 | -.127 | -.169 | -.394 | -5.90E-02 | -5.98E-02 |
| V2400.00 | .689 | -.247 | 5.436E-02 | -3.93E-02 | .209 | -.174 | .447 | -3.99E-02 | -8.68E-02 | -.353 | -9.38E-02 | -.106 |
| V1730.00 | .577 | 7.816E-02 | .242 | .178 | .405 | 7.179E-02 | .284 | -.236 | -.125 | .113 | -.246 | 7.158E-03 |
| V.3 | .735 | -.251 | -6.66E-02 | .292 | -.284 | -.166 | 4.262E-02 | 3.933E-02 | .112 | -.311 | -.103 | 3.904E-02 |
| V2490.01 | .509 | -.491 | .117 | .334 | -.145 | -.400 | -7.17E-02 | 2.624E-02 | .194 | .105 | .228 | 8.754E-03 |
| V2700.00 | .203 | .465 | .719 | -.228 | -5.23E-02 | -.106 | .171 | .171 | -1.23E-02 | .150 | 2.416E-02 | -1.03E-02 |
| V1850.02 | 7.532E-02 | -.121 | -.122 | -.173 | .377 | .579 | -.123 | .214 | .482 | .129 | .285 | -4.00E-02 |
| V2700.01 | .166 | .629 | -.109 | -2.41E-02 | -.479 | .293 | -1.37E-02 | .116 | .182 | -.184 | 6.822E-02 | .173 |
| V1900.02 | 8.846E-02 | .636 | -.362 | -9.05E-02 | -.275 | .316 | -.154 | .132 | .221 | 3.791E-02 | -7.55E-02 | .296 |
| V1800.01 | .258 | -.140 | -1.95E-03 | .366 | .210 | .292 | .326 | -3.94E-02 | .282 | -.216 | .532 | -.257 |
| V2490.02 | .104 | .295 | .661 | 8.166E-02 | -.478 | 9.631E-02 | -1.42E-02 | 7.998E-02 | .170 | 1.510E-02 | -6.39E-03 | -.333 |
| V1850.03 | .390 | -6.80E-02 | .531 | .147 | -.383 | .185 | .157 | -.175 | .103 | .377 | -6.06E-02 | .121 |
| V1730.01 | .321 | .478 | .540 | -.225 | 6.364E-03 | -.123 | .328 | -.260 | 1.847E-02 | .107 | .172 | 7.978E-02 |
| V2200.00 | .309 | .464 | .469 | -.225 | .199 | .121 | .250 | .100 | .304 | -.247 | -.124 | 4.930E-03 |
| V1790.00 | .423 | .586 | .188 | -5.01E-02 | -1.41E-02 | .238 | 9.724E-02 | -2.98E-03 | 2.631E-02 | .231 | .377 | -.162 |
| V1850.04 | .541 | -.169 | .161 | 1.390E-02 | .522 | 9.822E-02 | -.348 | -.289 | -.234 | -7.56E-02 | .177 | .206 |
| V2700.02 | .429 | 4.824E-02 | .678 | -.164 | -.200 | .123 | 6.221E-02 | .265 | -.107 | .176 | -6.01E-02 | .242 |
| V2490.03 | .737 | 3.439E-02 | -.245 | -8.62E-02 | -.356 | .280 | -4.06E-02 | -.140 | .183 | -.205 | -2.50E-02 | -.145 |
| V1790.01 | .685 | -.169 | .351 | -7.26E-03 | .138 | -.152 | -.290 | .334 | .188 | -4.91E-02 | 5.828E-02 | -9.65E-02 |
| V1850.05 | .550 | -.340 | .226 | -.481 | -.189 | -8.56E-02 | -1.20E-03 | .371 | -.111 | 5.495E-02 | .176 | 9.453E-02 |
| V2750.03 | .254 | .543 | .155 | 3.849E-02 | .280 | 2.050E-02 | -.602 | .145 | -.230 | 1.446E-02 | -1.76E-04 | -.195 |
| V1850.06 | .772 | .191 | -.224 | -.365 | 3.534E-02 | .188 | -7.81E-02 | 9.626E-02 | -2.12E-02 | .157 | -.136 | -4.68E-02 |
| V2200.01 | .680 | -.113 | .200 | -.386 | .124 | 1.437E-02 | -8.39E-02 | .181 | .196 | .311 | -.199 | -.143 |
| V1790.02 | .786 | -6.71E-02 | -7.94E-02 | -.230 | -8.16E-02 | .117 | -1.93E-02 | 6.689E-02 | -.346 | -3.52E-02 | .255 | -.280 |
| V2700.03 | .530 | -.590 | -.261 | -.317 | -.245 | -3.94E-02 | -.158 | 7.145E-02 | .134 | 4.387E-02 | -4.38E-02 | 8.005E-02 |
| V1900.03 | .628 | -.196 | -.618 | .136 | 1.816E-02 | 8.608E-02 | .192 | 9.648E-02 | .183 | 5.413E-02 | -6.17E-02 | -1.64E-02 |
| V2400.01 | .738 | 6.602E-02 | -.447 | .153 | -7.54E-02 | 6.640E-02 | .137 | -7.54E-03 | .312 | .119 | -.227 | -9.01E-02 |
| V1850.07 | .396 | -3.56E-02 | -7.94E-02 | .638 | .178 | 3.643E-02 | -.382 | .200 | -.303 | 2.731E-02 | -.155 | -.137 |
| V2400.02 | .535 | -.533 | -.280 | -.293 | -.199 | -5.18E-02 | 1.648E-02 | -.125 | .193 | .280 | .118 | .195 |
| V2400.03 | .559 | -.527 | -5.42E-02 | -.176 | -.420 | -7.55E-02 | .119 | -.248 | 5.674E-02 | -8.67E-02 | .222 | 2.214E-02 |
| V1850.08 | .270 | .556 | -.433 | .438 | -.128 | -6.35E-03 | .239 | -.164 | .215 | 4.874E-02 | -3.86E-03 | .104 |
| V1730.02 | .236 | .439 | -.409 | 6.061E-02 | .165 | -2.37E-02 | .194 | .530 | -3.56E-02 | -5.38E-02 | .222 | .383 |
| V2490.04 | .359 | .792 | -.128 | -9.96E-02 | 5.463E-02 | -7.64E-02 | .137 | -.245 | -.256 | 1.710E-03 | -.116 | .118 |
| V1790.03 | .456 | .271 | -.368 | -.416 | 1.864E-03 | -.332 | 8.839E-02 | 1.530E-02 | -.320 | .176 | .189 | -9.14E-02 |
| V1850.09 | .360 | -.291 | 3.806E-02 | .381 | .271 | .310 | .200 | .425 | 7.830E-02 | 3.579E-02 | -.396 | 4.294E-02 |
| V1850.10 | .547 | -1.50E-02 | -.382 | .133 | 1.151E-02 | .542 | -.123 | -.245 | -.320 | 7.289E-02 | .102 | 6.429E-03 |
| V2200.02 | .348 | -7.45E-02 | 4.678E-02 | .647 | -.280 | -7.18E-02 | -.320 | .334 | -.269 | -.109 | .150 | .107 |
| V2400.04 | .572 | 6.460E-02 | 4.899E-03 | .306 | -.119 | 7.012E-02 | .233 | .181 | -.456 | 8.704E-02 | .375 | 9.098E-02 |
| V1850.11 | .575 | .211 | -.211 | .116 | -8.35E-02 | .542 | -.176 | -.337 | -.160 | .166 | -9.53E-02 | -7.11E-02 |
| V1790.04 | .475 | -8.43E-02 | .146 | .193 | .551 | 9.144E-02 | .424 | 9.870E-02 | -.200 | .127 | -.234 | .138 |
| V1700.00 | .235 | 9.270E-02 | 8.620E-02 | .286 | .739 | -.169 | -4.16E-02 | .140 | .217 | 8.009E-03 | .250 | .158 |
| V1850.12 | .313 | .364 | .153 | -9.97E-02 | .543 | 4.924E-02 | -.349 | -.294 | .200 | -.247 | 7.694E-02 | .150 |
| V2700.04 | .601 | -.296 | -.152 | -.512 | .279 | 9.252E-02 | -4.57E-02 | .134 | -5.32E-02 | -.263 | -.183 | 5.190E-02 |
| V2200.03 | .361 | -.445 | .383 | .134 | -.445 | .252 | .109 | -7.59E-03 | -.237 | -.234 | -4.82E-02 | .209 |

Extraction Method: Principal Component Analysis.

a. 12 components extracted.

b. Only cases for which VAR00001 = 1 are used in the analysis phase.

Fig 16.  A dead enset plant due to enset bacterial wilt

Fig 17. Healthy enset plants