The McKnight Foundation Collaborative Crop Research Program Project

"Integrated Management of Bacterial Wilt of Enset (Ensete ventricosum (Welw.) Cheesman) caused by Xanthomonas campestris pv. musacearum in Ethiopia"

Year Two Report

Southern Agricultural Research Institute (SARI) in Ethiopia

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ANNUAL PROGRESS REPORT — NARRATIVE AND APPENDICES

"Integrated Management of Bacterial Wilt of Enset (Ensete ventricosum (Welw.) Cheesman) caused by Xanthomonas campestris pv. musacearum in Ethiopia"

I. Overview

Enset (*Ensete ventricosum* (Welw.) Chessman is a perennial, herbaceous and monocarpic crop belonging to the family Musaceae. Enset is an orphan and little researched food crop cultivated only in Ethiopia. The project was initiated with the overall objective of improving food security, income and livelihood of enset farmers through integrated enset bacterial wilt management and improving the capacity of partner institutions, farmers, development agents and researchers through providing research facilities, training, field visits, experience sharing, and long term training. Detailed baseline survey was carried out in eight districts namely Guragie, Siltie, Dawuro, Sidama, Wolayita, Gedeo, Hadyia and Kembata-Tembaro. Data was collected on the number of enset clones and names of the clones, purpose each clone grown at farmers field, the reaction of each clone to the pathogen, farmer perceptions of causes and modes of enset xanthomonas wilt (EXW) transmission, means of disease management, and farmer's knowledge on symptom identification.Furthermore, EXW distribution map is produced. The survey revealed that farmers' average land holding is 0.86 hectare out of which 22.7 % is apportioned for enset production. Farmers use different clones for different uses like for kocho, bulla and fibre productivity, medicinal values, and rate clones based on their reaction to EXW. Farmers conserve a number of enset clones ranging from 3 to 28, on average 9 clones per family farm in southern Ethiopia. The highest number of enset clones (66) was recorded in Dauro zone and the lowest number (26) in Gedeo zone. Enset contribute 23.36 % of the total gross farm income in the study location. On average 40 % of the sample respondents' reported bacterial wilt infection in their enset field.

To charactorize the pathogen and assess virulence spectrum, evaluation of the efficacy of antibiotics against X. campestris p.v. musacearum in vitro was conducted. All the in vitro tested antibiotics reduced the growth of bacterial culture significantly as compared to the control for both Gurage and Hagereselam isolates but in varying extent. Accessions with different reaction level were established for epidemiological study. Suckers of eleven enset clones with differential reactions to the wilt pathogen were established at three different sites and data collection is underway. To investigate how long the EXW would survive in an acidic medium in fermented kocho when infected plants are harvested to be processed into starch food; sampling of kocho at 15 days interval indicated presence of the pathogen from the first day of processing to 90 days after processing. However, the number of colonies declined with the delay in sampling date. In ordered to identify markers for resistance/tolerance to the EBW disease 456 clones were established at Areka Agricultural Research Center for morpho-agronomic characterization. Moreover, in order to choose a subset that represents as much of the variability in the full set of 456 accessions a multi-locational trial including clones from the whole collection site were planted in three different locations. A total of 100 enset clones were used for this experiment. With the purpose of assessing on-farm genetic resources management of enset semi-structured interview methodology with 120 farmers' was employed in Kembata, Gurage and Wolaita

provinces. Quantification of clonal diversity per farm was done by a participatory zigzag sampling in the diagonal direction of the plot and the number of varieties planted in the field were counted. To assess molecular diversity, sucker multiplication of 456 enset accessions is underway. Botanical seeds were collected from 'Mazia' and wild enset clones from Dawro and sowed in pot at SARI to assess morpho-genetic variability and response to environmental stresses. The seedlings were inoculated with the pathogen and one month after inoculation, disease symptom was developed including seedlings from Mazia botanical seeds.

Three sets of enset clones screening for resistance to bacterial wilt are on-going with promising results. In the first set 25 enset clones collected from Gurage zone were evaluated for their reaction in pot experiment. Data on disease incidence, incubation time and area under disease progress curve was collected and clones showed wilt symptom but at varying lintensity and 8 out of the 25 clones were found to be tolerant. In the second and third sets eighty and one hundred fifty enset clones respectively, were planted under field conditions. Their reaction to enset bacterial wilt using artificial inoculation was evaluated. From 80 enset clones 21.50% of the clones exhibited relatively resistance/tolerant reactions, 22.50% showed moderately tolerant/ susceptible to the disease and 56.25% of the clones. This result was in agreement with the farmers ranking to host-pathogen reaction. Crossing block was established at Areka ARC for hybridization of Maziya (tolerant) and Mesena moderately tolerant with good culinary quality.

By using the existing formal and informal administrative structures, demonstration and dissemination of integrated bacterial wilt control measures through collective action was started at benchmark sites. Baseline information on the status of the disease in the area was ccollected. Training on the management of enset XW was provided to 95 extension agents (10 female and 85 male) drawn from six zones and 25 districts of the SNNP and Oromia Regional States. Moreover, for the purpose of clean planting material procurement, about six thousand half-corms of the EXW tolerant clone 'mazia' were planted in three hectares at Areka Agricultural Research Centre and 2000 suckers distributed to Gedeb district bench mark site. Over all the project, in its existence of over a year now, has shown highly promising results which need to be consolidated and disseminated through further support from the McKnight Foundation in the coming years.

II. Narrative

Baseline survey: Detailed baseline survey was carried out in eight districts namely Guragie, Siltie, Dawuro, Sidama, Wolayita, Gedeo, Hadyia and Kembata-Tembaro. Survey data collected on the host-pathogen included number of enset clones and names of the clones, purpose each clone is grown, the reaction of each clone to the pathogen, farmer perceptions of causes and modes of disease transmission, means of disease management, and farmer's knowledge on symptom identification. Purposeful sampling techniques were employed to selected zones, districts, Peasant Associations and a total of 320 sampling units/households, 40 from each Zone were selected randomly. Farmers average land holding is found to be 0.86 hectare out of which 22.7 is allotted for enset production. Farmers were able to rate clones differently for kocho, bulla and fibre productivity; medicinal values; and for clonal reaction to bacterial wilt disease. Farmers conserve a number of enset clones ranging from 3 to 28, on average 9 clones per family farm in southern Ethiopia. About 66, 65, 59, 44, and 23 clones in Dawuro, Kembata, Hadiya, Gurage, Wolayta and Gedeo respectively were identified and found to be conserved in farmer's fields. According to the survey result, enset contribute 23.36 % of the total gross farm income in the study location. On average 40 % of the total sample respondents' reported bacterial wilt infection in their enset field.

Pathogen characterization and virulence spectrum study: Some preliminary investigations on bacterial wilt of enset was conducted to evaluate the efficacy of antibiotics against X. campestris pv. musacearum in vitro experiment. Five antibiotics were tested in vitro at three concentrations (0.1, 0.5 and 1%). All the in vitro tested antibiotics reduced the growth of bacterial culture significantly as compared to the control for both Gurage and Hagere selam isolates but in varying extent. Pathogen virulence spectrum studies that have been started shall be strengthened. Molecular characterization of the pathogen to determine diversity shall be carried out. Early detection tools such as LFD that were developed elsewhere shall be adopted, tested and extended to farmers for disease tracking and control. Bio-control strategies for EXW shall be tested and effective ones identified.

Study on enset bacterial wilt disease epidemiology: A set of 456 clones representing 12 enset growing provinces was established at Areka Agricultural Research Center for morpho-agronomic and molecular characterization using augmented design. Eight suckers which have similar size were taken from each clone and planted using a spacing of 3m and 1.5m between rows and plants, respectively (a plot size of 5.25 m^2). Quantitative and qualitative data collection is underway. Molecular charactorization will commence soon. A subset with 100 entries (91 new entries, 6 standard and 3 local checks) that represents as much of the variability in the full set of 456 accessions was used for a multi-location trials. The trial was planted at three locations, Areka, Angacha and Hawassa using simple lattice design. With the aim of assessing on-farm genetic resources management of enset; semi-structured interview methodology with 120 farmers' was employed in Kembata, Gurage and Wolaita provinces. Onfarm clonal diversity was quantified using participatory zigzag sampling along the diagonal direction of the plot in Angacha and Geta districts and 65 and 44 enset clones with their indigenous knowledge were collected from Angacha and Geta respectively. Collection from Wolayta will be started.

Characterization of enset clones: Collection and characterization of enset clones: A set of 456 clones representing 12 enset growing provinces was established at Areka Agricultural Research Center for morpho-agronomic and molecular characterization using augmented design. Eight suckers which have similar size were taken from each clone and planted using a spacing of 3m and 1.5m between rows and plants, respectively (a plot size of 5.25 m^2). Quantitative and qualitative data collection is underway. Molecular charactorization will commence soon. A subset with 100 entries (91 new entries, 6 standard and 3 local checks) that represents as much of the variability in the full set of 456 accessions was used for a multi-location trials. The trial was planted at three locations, Areka, Angacha and Hawassa using simple lattice design. With the aim of assessing on-farm genetic resources management of enset; semi-structured interview methodology with 120 farmers' was employed in Kembata, Gurage and Wolaita provinces. Onfarm clonal diversity was quantified using participatory zigzag sampling along the diagonal direction of the plot in Angacha and Geta districts and 65 and 44 enset clones with their indigenous knowledge were collected from Angacha and Geta respectively. Collection from Wolayta province and participatory charactorization and evaluation will be started in the coming year.

Enset breeding and selection for wilt resistance/tolerance and other desirable traits

Evaluation of enset clones for wilt resistance: Large scale screening of enset clones for resistance to bacterial wilt three sets of trials are on-going with promising results. In the first set twenty five enset clones collected from Gurage zone were evaluated for their reaction against Exw in pot experiment. All the 25 enset clones showed wilt symptom but at varying levels. Out of them 8 clones were found to be tolerant and the rest 17 were susceptible or highly susceptible to the disease based on disease incidence, incubation time and area under disease progress curve (AUDPC). In the second set 80 clones were transplanted at the experimental field of Hawassa Agricultural Research Centre for disease inoculation and subsequent evaluation. Out of the 80 clones tested, about 26% showed high resistance/tolerance reaction with an average disease index of 10-20%. 29 % of the clones showed moderate tolerance/resistance reaction (average disease index of 30-40%) and 45% of the clones were susceptible with an average disease index of >50%. Moreover, the result of farmers' opinion assessment on these enset clones reactions is also supportive of results of this study. In the third set, one hundred fifty clones were inoculated with bacterial suspension. Disease assessment was started at 15 days after inoculation and continued within 15 days interval. The number of infected plants per clone at each disease assessment period has been recorded. All inoculated enset clones did not show disease symptoms during the first 30 days after inoculation. However, accessions developed disease symptoms at various intensity levels 30 days after inoculation. Further replicated trials with 484 enset clones will be conducted at screen house and experimental fields of Awassa Agricultural Research Centre, Southern Ethiopia.

Evaluation of seedlings from botanical seed against *Xanthomonas* wilt: Four hundred botanical seeds were collected from 'Mazia' and wild enset clones. The seeds were sown in pots at Hawassa ARC and seedlings have been raised. Enset seedlings raised from botanical seeds were artificially inoculated with virulent strain of enset wilt bacteria by September 2013. All the seedlings show the disease symptom after one month from inoculation. Data collection is underway.

Floral biology, phenology and breeding system, and pollination ecology of enset accessions from Ethiopia: For effective enset breeding, there is a need to understand the crop's reproductive biology as well as the breeding procedures. One hundred twenty plants in the Areka maintenance field have been tagged. The morphology of the separate floral parts assessment started and data collection continued. This activity is an on-going as such the remaining plants shall be used for studying the remaining flower morphology, phenology and breeding system studies.

Variety development for wilt resistance/tolerance and other desirable traits: Crossing block with two parents Mazia and Messena was established at Areka ARC. Mazia is resistant /tolerant to Xcm with less culinary quality and Messena is moderately tolerant to Xcm with higher quality of kocho. Mazia used as male and female parent alternatively. The crossing technique from banana will be adapted for emasculation and collecting viable pollen. F1 will be evaluated against EXW, for culinary quality and other desirable traits.

Demonstration and dissemination of integrated bacterial wilt control measures through collective action

Multiplication of planting materials: Recently released six varieties and recommended disease tolerant clone Mazia, were multiplied at Areka Agricultural Research Centre. Last year about 2000 suckers were distributed to Gedeo zone bench mark site. This year near to three hectare of land has been covered by the six varieties and Mazia. In 2014 the multiplied suckers will be ready for distribution.

Setting up benchmark sites for piloting collective action: In Gedeo Zone, Hallo Hartume kebele of the Gedeb Woreda was selected as a benchmark site for demonstration and dissemination of enset bacterial wilt control measures. Data on incidence of enset bacterial wilt in the entire kebele were recorded before the implementation of collective action for eradication. Focused group discussion with key informant farmers were made about modes of enset bacterial wilt transmission, causative agent of the disease, and their indigenous knowledge to control the disease. Important actors for integrated EXW management at the levels of Hallo Hartume Kebele and Gedeb woreda were identified. The selected actors who were about 60 received training on sustainable integrated enset bacterial wilt control measures. Task forces were established using the formal amd informal administrative structures.The task forces took the responsibility to lead and mobilize farmer communities for the control of bacterial wilt at the Hallo Hartume Kebele. This activity is an on-going as such demonstration and evaluation of collective action for the remaining bench mark sites will be commenced this year.

Lessons learned

In the two year period of the project, we have had some emerging insights that the enset-XW pathosystem is not an all losing game. There appears to be co-habitation of the host and pathogen in enset farming systems and wild habitats, the delicate equilibrium of which is affected by the direction and level of anthropogenic interventions. We perceived that interactions among biophysical (climatic, species diversity, etc) and socio-economic entities (values, practices, etc) in enset agro-ecosystems may exacerbate or ameliorate the XW disease incidence, spread and socio-economic impacts. A large repertoire of traditional knowledge and information on host clonal diversity, the various economic and cultural uses of this diversity and their interaction with the management of the pathogen were noted. From farmers report and our small screening works, there are already important indications that some clones are resistant/tolerant to the pathogen.

There are diverse widely used and location specific traditional EBW management practices including taboos. Some of these are likely to help while others worsen disease epidemics. Understanding and documenting these practices may help in their validation and subsequent education of farmers in scaling up of best practices. Farmer participatory evaluation of clones, control measures, crop and product management practices as they affect EBW incidence and spread shall be dealt with in the coming months and years. We created demand for our services on integrated management of enset bacterial wilt in Ethiopia and, as a result, we were able to project our image as a repertoire of information and as an entity worth reckoning for enset bacterial wilt menace in Ethiopia. The overall goal of the project was to improve food security, income and livelihood of enset farmers while also ensuring enset-based sustainable agricultural systems. The project, in its existence of over a year now, has shown highly promising results which need to be consolidated and disseminated through further support from the McKnight Foundation in the coming years. We created demand for our services on integrated management of enset bacterial wilt in Ethiopia and, as a result, we were able to project our image as a repertoire of information and as an entity worth reckoning for enset bacterial wilt management in Ethiopia.

I. Annual Work plan

Table 1. Year one Work plan for the project integrated management of enset (Ensete ventricosum (Welw.) Cheesman) bacterial wilt caused by Xanthomonas campestris pv. Musacearum in Ethiopia

	Activities	Objective	Milestones/Timelines	Outputs	Budget ($)
	Inception work shop				
		To bring partners and different stakeholders together for creating better working environment.	**Sensitization workshop conducted at the beginning of the budget year**	**Workshop report submitted,**	**10,800**
1	**Baseline survey and disease mapping**				**21199.99**
		To conduct baseline survey to determine the prevalence, intensity and socio-economic impact of enset bacterial wilt and to characterize bench mark sites	Baseline survey completed, data compiled and report made ready at the end of the first year.	Geographic distribution, prevalence and intensity of the disease determined, and disease hot spots identified. Farmers' perception and awareness on the disease, its impact and management analyzed, and improved. Over all enset production constraints documented and publicized to the public.	**21199.99** = SARI
2	**Germplasm Collection, Characterization and Variety development and selection for wilt resistance/tolerance and other desirable traits**				**25188.6**
2.1	Collection and Conservation of enset clones for future use	To collect, and assess the genetic diversity of cultivated and wild enset clones and their relatives and to identify duplicated clone.	Germplasm from seven location collected and their passport data documented	No. of clones included to the genebank reported and avail the material for further evaluation multiplied.	5369.74= SARI
2.2	Morpho-Agronomic, culinary quality and other use-value based and molecular characterization, and evaluation of enset clones for EXW resistance	To evaluate enset genotypes based on their morphological, use value, culinary quality and for their resistance/tolerance to bacterial wilt	Morphological data collection continued.	Elite enset genotypes with different merits identified, grouped and ready for further evaluation	3000= SARI

No.	**Activities**	**Objective**	**Milestones/Timelines**	**Outputs**	**Budget ($)**
2.3	Assessment of genetic diversity of previously collected enset accessions using SSR markers and evaluating the accessions for marker disease associations.	To study the genetic diversity among enset accessions using SSR markers	Molecular characterization for 456 clones completed and documented.	Diversity of the clones with different merits identified	3000= SAR|I
2.4	Evaluation of enset accessions against EXW and understanding Mechanism of Resistance	To evaluate enset clones against disease resistance/tolerance.	First phase evaluation of previously collected enset clones for EXW completed, Second phase started.	Clones from phase one having different reaction level identified.	2000= SARI
2.5	Molecular characterization of newly collected enset clones	To study the genetic diversity among enset accessions using molecular markers	Second phase Molecular characterization of newly collected enset clones started	Data collection continued and progress report submitted.	3500 = Holleta Biotech
2.6	Variety development for wilt resistance/tolerance and other desirable traits	continue the ongoing variety development process through crossing and selection of enset clones that combine high yield, disease resistance/tolerance and culinary traits	Morpho agronomic data collection continued.	Data collection continued and progress report submitted.	2000=SARI
2.7	Disease reaction, biochemical and metabolic profiling	To reveal the biochemical and metabolic changes in response to Xcm infection, in screening resistant/tolerant enset genotypes	Disease reaction of genotypes, biochemical and metabolites playing role in Xcm tolerant/resistance will be documented at the end of 2014/15	MSc Thesis report and one reviewed journal article	3000
2.8	Participatory Varietal Evaluation**,** Popularization and Promotion of Enset clones	To develop new varieties that farmers adopt to improve their livelihoods, Enhance in-situ conservation, expanding genetic diversity,	Planting material for participatory variety selection multiplied and planted in the respective locations.	Data collection continued and progress report submitted.	3000= SARI
	Activities	**Objective**	**Milestones/Timelines**	**Outputs**	**Budget ($)**
3	**Host development and host-pathogen interaction as affected by crop husbandry and harvest and post-harvest management practices**				**9322.4**

	(Epidemiology):				
3.1	Genotype X environment interaction	To understand the impact of environmental factors and host genotype on bacterial wilt development	Data collection completed and first year report ready for submission.	Annual report on GXE interaction submitted	5290= SARI
3.2	Insect vector and nematode transmission	To identify diversity and role of insects and nematodes in enset bacterial wilt transmission	Nematodes collected, reared and Isolated. Role of insects in EXW identified Evaluation started	Number of preserved isolates reported Insects having a role for EXW transmission known and reported	4032.40= SARI
4	**Pathogen characterization, early detection, and bio-control strategies**				**18471.61**
4.1	Pathogen characterization	· To characterize enset bacterial wilt pathogen	Isolates from different enset growing sites collected and preserved. DNA extracted and ready for molecular analysis	No. of collected strains reported Progress report submitted.	5424.449= Holleta BIO tech
4.2	Virulence spectrum studies	To evaluate virulence level of different strains on the host	Data collection continued	preliminary report on the virulence level of the strains submitted	3000= SARI
4.3	Evaluation of Microbial agents for enset bacterial wilt disease control	To test the efficacy of microorganisms as bio-control agents against the wilt pathogen	Microbial agent evaluation for EXW started	Data on their reaction compiled and report submitted	3000= SARI
4.4	Development of early detection tools	· To adopt and/or develop early detection tools	· Early detection tools adopted and their effects tested. New tools tested	Data for each detection tools collected and preliminary report submitted	3000= SARI
4.5	In vitro regeneration of disease free enset planting materials from enset bacterial wilt infected plants using meristem culture	To produce enset bacterial wilt free planting materials in vitro from infected enset plant and finally tested for pathogen availability using different diagnostic techniques.	In vitro regeneration of disease free planting material at the mid of the second year.	In vitro regeneration of disease free planting material developed. Report and one MSc theses and publication of at least one peer-reviewed journal article.	4047.161= SARI
	Activities	**Objective**	**Milestones/Timelines**	**Outputs**	**Budget ($)**
5	**Scaling out (seedling multiplication, training, mass mobilization and collective action)**				**23017.4**

5.1	Demonstration and dissemination of integrated bacterial wilt control measures through collective action in benchmark site of the country	To get a common understanding among the respective partners and RK leaders about the overall objectives of the activity	Field visits, farmer's field days, preparation and distribution of posters, leaflets, manuals etc, In the remaining bench mark site task force established Training given in all bench mark sites Data collection, monitoring and evaluation continued	IDM strategy will be developed and manual prepared Task force composition and their responsibility identified and reported. Status of farmers' involvement on the campaign and their awareness reported. Progress report submitted.	11615.66= SARI
5,2	Information and communication technology (ICT) based early warning and information exchange for bacterial wilt disease management	To develop an ICT method for early warning and information exchange about enset bacterial wilt	Training for contact person given. Early warning tools to the bench mark site introduced and action research started.	· Report on the type of warning tools introduced to the bench mark site and the progress reported.	11401.74= SARI

IV. Budget

(Format attached: McKnight-CCRP 11-283 Year III budget, 2013.doc**)**

V. Appendices

Appendix A - RESEARCH REPORT

1. Statement of the problem and systemic context.

Enset (Ensete ventricosum (Welw.) Cheesman) is an indigenous, little researched staple food crop known for its tolerance to transient drought, high productivity, gender equity and environmental sustainability. Enset is a multi-purpose plant with a range of utilities including food, feed, construction and medicinal uses. Kocho, a fermented starch resulting from pulverized pseudostem and corm, is the primary product which is often baked into bread. Enset fiber is the main byproduct resulting from decortications of the pulp from leaf sheathes of the pseudo stem. Enset also plays an important role as a feed for animals during dry spell. Fresh enset leaves are selectively cut from the standing crop and fed to livestock during feed shortages. Leaves for livestock feed can also be obtained as byproducts during the entire enset plant harvest (corm and pseudo stem processing and fermentation into starch food). Among all other agricultural enterprises, enset farming systems support the largest human population density in Ethiopia, which in some areas such as Gedeo exceed 1000 persons per square kilometer.

Despite such importance of the crop, enset production in Ethiopia has received little attention from research and development. As a result, enset production, processing and utilization have been constrained by a host of problems along the value chain. Of all constraints, bacterial wilt disease is the most economically important, putting the sustainability of enset farming systems in jeopardy (Shank and Chernet, 1996). It is reported that up to 80% of enset farms are currently infected with EXW. This problem directly affects the livelihood of more than 20 million enset growing farmers in the country.

Though EXW is the most important problem in enset farming system, there are also good opportunities like rich indigenous knowledge on the clonal diversity and utilization, rich enset biodiversity, established partnership on bacterial wilt management, existing biotechnology facilities in the country, government structure and 1 to 5 group organization at a grass root level, for mass mobilization to control EXW. Effective management of enset bacterial wilt problem necessitates a better understanding of the ecological complex in which the host and pathogen co-evolved, especially focusing on how the interaction between and among bio-physical entities in this complex affect host-pathogen relationship and how this relationship is modified because of anthropogenic interventions in enset farming systems and wild habitats.

To this end, a research project **'Integrated Management of Bacterial Wilt of Enset (Ensete ventricosum (Welw.) Cheesman) caused by Xanthomonas campestris pv. musacearum in Ethiopia'** project, in just over a year of its operation, has displayed encouraging results, which need to be capitalized on, consolidated, amplified and extended. Because of the perennial nature of the crop and the seriousness of the problems all the proposed objectives have not been met. Therefore, there is a need for renewal of EXW project to answer the research questions with its own specific objectives.

2. Literature Review

Enset (*Ensete ventricosum* (Welw.) Cheesman) is an orphan or little researched food crop cultivated only in Ethiopia. Enset serves as staple or co-staple food for about 17 million people in Ethiopia, which accounts for 20% of the more than 82 million people (Tsegaye 2002). The edible parts of the plant are the underground stem (corm) and pseudo stem, which are pulverized and fermented into a starch-rich product called kocho. Kocho is mainly consumed after making pancake-like bread. The corm can be harvested at almost any stage of the crop, and cooked and consumed in the same way with other root and tuber crops, relieving hunger during periods of critical food shortages. Kocho can be stored for a long time (for 10 years and even more) without being spoiled (Brandt et al, 1997)

Enset is well known for its tolerance to transient drought and for its high productivity; hence it is considered as one of the priority crops for food security in the country. Owing to its morphology, growth habit and cultivation practices, enset can be grown with minimum tillage helping reduce soil erosion, land degradation and loss of productivity. Unlike cereals and other root and tuber crops, soils under enset production are known to display a positive nutrient balance, making the crop suitable for sustainable agriculture (Woldetensaye, 2001).

Enset is perhaps one of a few crops that is cultivated by men but harvested, processed and marketed solely by women. Hence, interventions on this crop are likely to impact the lives of women and children more than interventions on any other crop, thereby ameliorating gender disparity in access to resources (Negash, 2001).

However, enset production and productivity are constrained by various factors of which bacterial wilt caused by Xanthomonas campestris pv. musacearum, is the number one bottleneck (Quimio, 1991). There have been attempts to devise control options against the disease and develop clones with desirable agronomic traits and optimize agronomic practices. Currently, some tolerant clones have been identified and some cultural practices have been evaluated to control enset wilt disease. However, these clones, more often than not, fail to combine disease tolerance with culinary quality and other desirable agronomic traits, calling for further cross-breeding and selection works. Moreover, the enset-XW pathosystem, under different agro-ecological conditions in Ethiopia, remains poorly understood, hindering adequate prevention and control of the disease. Although some fragmented surveys were undertaken in different parts of the country, the socio-economic impact of bacterial wilt remains largely undetermined. Unlike other diseases, control options are limited. In addition, there is lack of awareness on available management practices and early diagnostic tools that are essential for the production of disease free planting materials. There have also not been concerted efforts for country wide coordination of disease diagnosis and management. Therefore, disease management practices that were successful in some areas have not been scaled up, leading to fragmented, inefficient and unsustainable disease management efforts.

Furthermore, the genetic diversity of enset and the bacteria causing wilt in Ethiopia has not been sufficiently understood, constraining the development of robust, efficient and sustainable disease management interventions. Although some enset clones were tested and found to possess some level of resistance/tolerance to the pathogen, their use by farmers remains limited due, presumably, to lack of broader resistance/tolerance to different strains of the pathogen that occur in different enset producing regions of the country. Epidemiology of the disease and application of biological control method for disease management are areas that merit further investigation. In addition, national efforts on enset bacterial wilt management have not been linked with regional and international undertakings on the management of bacterial wilt disease on banana, undermining synergy and economy of scale in the generation of new knowledge and use of the already available information.

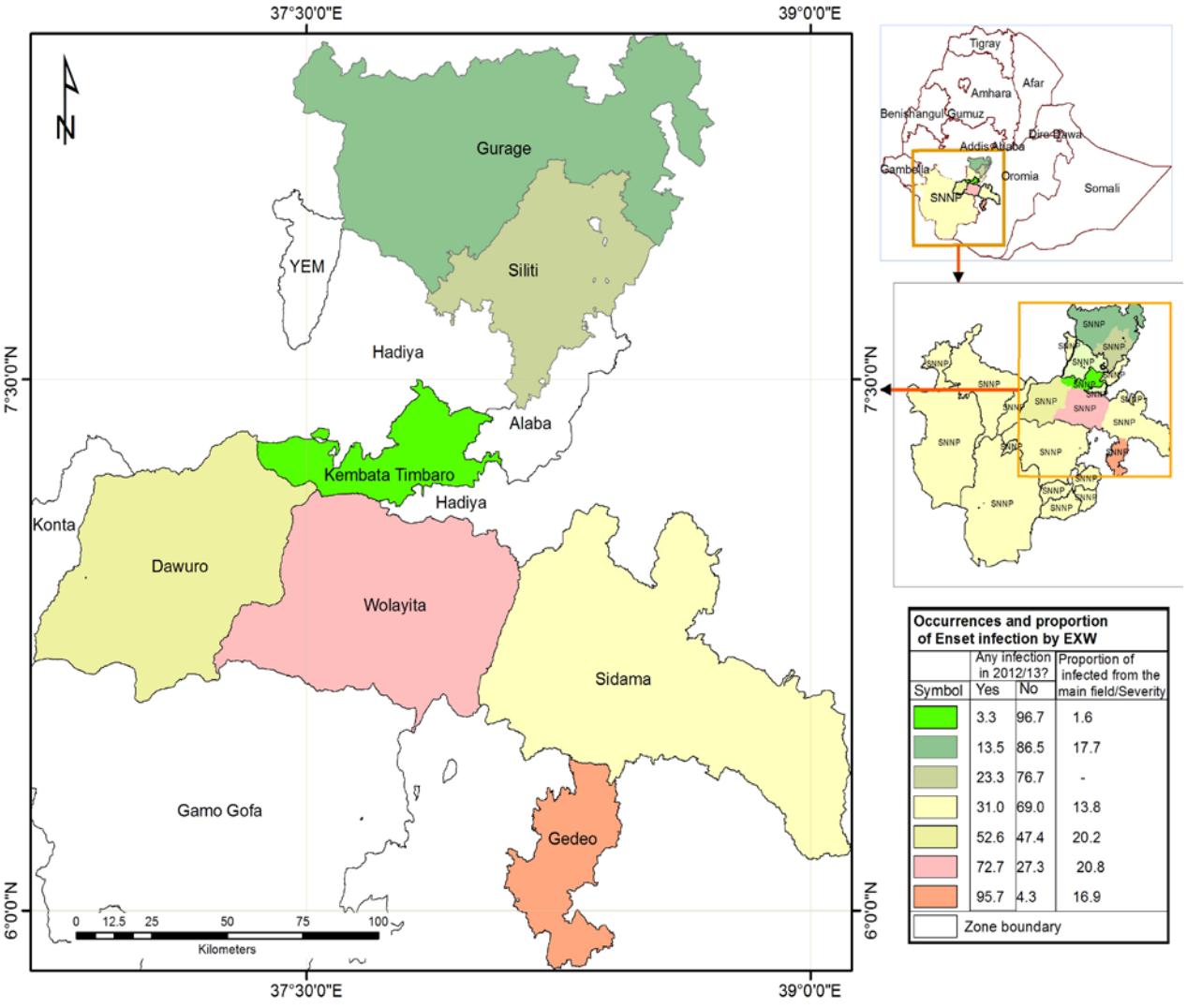
In this project, baseline surveys for the remaining provinces will be conducted to determine the prevalence and intensity of bacterial wilt, and its socio-economic impact in different enset producing regions of Ethiopia. Farmers' indigenous knowledge and research outputs on the pathosystem, and available enset wilt control measures will be assessed, evaluated, documented and made available for future use. Molecular and phenotypic characterization of enset clones and bacterial strains will be carried out. Attempts will be made to identify molecular markers for resistance/tolerance to the disease in resistant enset clones, using microsatellite techniques. Cross breeding between resistant enset clones and clones having other desirable traits shall be made. F1 plants shall be raised from botanical seeds and characterized for their morpho-genetic variability and response to environmental stresses. This will be followed by large scale screening of enset clones for resistance to bacterial wilt taking the pathogen diversity into consideration. Early diagnostic tools that would be developed under the MFCCRP banana bacterial wilt project shall be used to multiply clean planting materials of elite and tolerant clones in vitro.

Biological and cultural control methods will be integrated and demonstrated in major enset growing areas through participation of farmers and key stakeholders. The project shall study and make use of existing traditional community organization and formal administrative structures for mass mobilization in order to control the disease nationwide. Above all, the project shall document what works and what will not in communal actions for the control of the disease. The project will train various stakeholders, notably women, school children, male farmers and development agents on the pathosystem and management practices. This, in addition to improving food security of farmers in enset producing regions, will have an indirect benefit in improving the research and development capacity of various stakeholders on enset, especially in reference to enset XW management.

3. Research design and method, findings and implication of the research findings

Objective 1. Baseline survey: Baseline survey was carried out in major enset growing areas of sothern Ethiopia; eight Zones namely Guragie, Siltie, Dawuro, Sidama, Wolayita, Gedeo, Hadyia and Kembata-Tembaro have been covered. Purposeful sampling techniques were employed to select zones, Woredas, Pas and a total of 320 sampling units/households, 40 from each Zone were selected randomly. Disease map showing EXW distribution and severity has been produced. EXW disease distribution map has been produced (figure 1) and the survey needs to be extended to other potential enset producing areas such as South West and Oromiya zones, Ethiopia.

Figure 1. EXW disease map



Household characteristics: From the total population about 80 % of the respondents were male headed and 20 % female headed households with mean age of 48 years (Table 2). About 56 percent of the selected respondents in the study area never have access to formal education. Consequently, substantial proportions (37.5 %) of respondents were found to be illiterate and 18.5 % of them are able to read and write without having formal education (Table 2).

Table 2. Demographic characteristics of sample respondents

Variable	Category	Zone							
		Gedeo	Wolayta	Silti	Gurage	Kembata	Sidama	Dawro	Total
Sex of HHD	Male	44	43	67.6	89.7	73.8	87.9	86.8	80.3
	Female	4	8	32.4	10.3	26.2	12.1	13.2	19.7
Age of HHD	Mean	42.1	44.6	49.7	44.6	50.1	46.6	48.7	48.0
Education status	Illiterate	9	22	45.4	32.4	41.7	27.3	40.5	37.5
	Read and write	10	1	21.2	21.6	21.7	6.1	18.9	18.5
	Grade 1-4	10	5	12.1	10.8	5.0	18.2	10.8	10.5
	Grade 5-8	8	10	15.2	29.7	13.3	42.4	16.2	22.0
	Grade 9-10	7	6	6.1	5.4	13.3	3.0	8.1	8.0
	Above 10 grade	3	4	0.0	0.0	5.0	3.0	5.4	3.0

Source: computed from survey data, 2012/2013

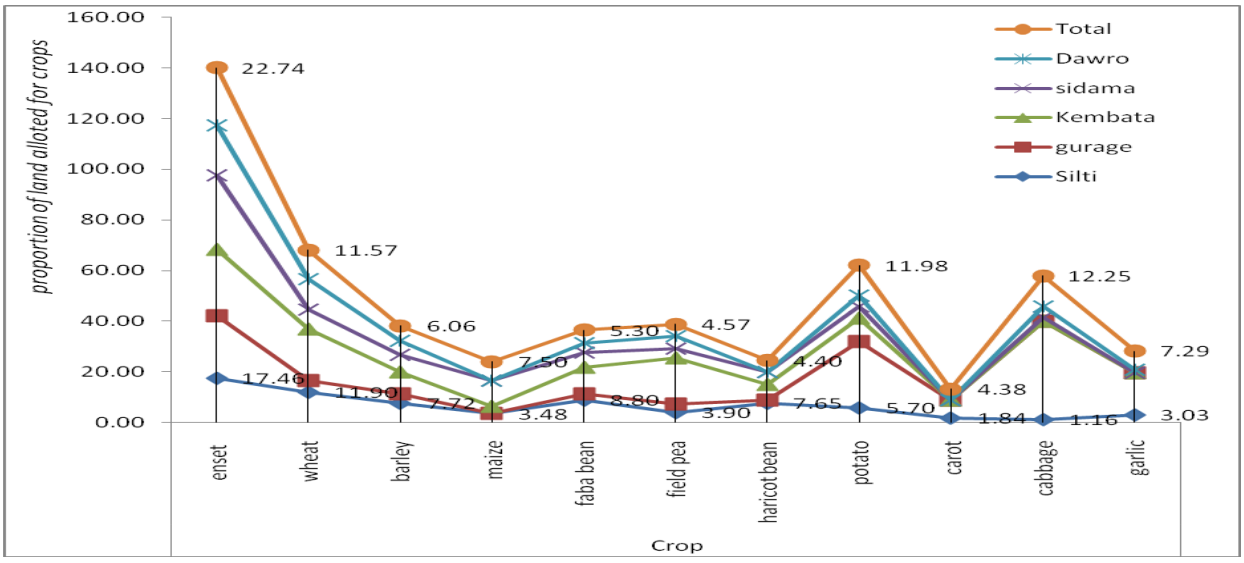
Farming system and the role Enset: Enset based farming system is the backbone of Ethiopian economy particularly in the Southern and South-western part of the country. The crop is highly drought tolerant and resilient to various environmental shocks being an ideal crop for food security, in Ethiopian highlands, where population density is highest (up to 300 per square kilometer). According to the information from this study, farmers' average land holding is found to be 0.86 hectare. Enset, Wheat (durum), food barley, Irish potato, faba bean and Field pea (Table 3) are among the major crops cultivated by smallholder farmers with different degree of combination. From the result portrayed in Figure 2, enset represented about 22.7 hectare of the total land holding that is by far greater than other competing enterprises.

Table 3. Type of crop cultivated and area allotted for each crop (timad=0.25ha)

Crop area cultivated (timad=0.25ha)	Zone					
	Silti	Gurage	Kembata	Sidama	Dawro	Total
Total land holding	3.268	3.493	2.075	3.259	5.761	3.434
Enset	0.570	0.866	0.545	0.949	1.136	0.781
Wheat	0.389	0.160	0.421	0.257	0.683	0.397
Barley	0.252	0.121	0.179	0.229	0.307	0.208
Maize	0.114	.	0.063	0.328	.	0.258
Faba bean	0.287	0.084	0.219	0.193	0.204	0.182
Field pea	0.127	0.119	0.375	0.125	0.288	0.157
Common bean	0.250	0.045	0.125	0.167	.	0.151
Potato	0.186	0.917	0.195	0.146	0.247	0.412
Carot	0.060	0.245	.	.	.	0.150
Cabbage	0.038	1.357	.	0.051	0.237	0.421
Garlic	0.099	0.582	.	.	0.070	0.250

Source: computed from survey data, 2012/2013

Figure 2**.** Summarizes area share of major crops from the total land holding



Source: computed from survey dat, 2012/2013

Enset is a versatile crop and cultivated by Ethiopian farmers for various reasons. The crop among others used as food, feed, medicinal, ornamental and row materials for industrial. Respondents articulated the source and share of their farm income from different crops as presented in Table 4. Enset aside from other (unable to be computed) cultural, social and medicinal values contributed more than 23 % of the gross farm income for as a cash, staple and co staple food for enset producing farmers.

Table 4. Share of Enset for Gross farm Income

Revenue from Crop	Zone	Total

	Silti	Gurage	Kembata	Sidama	Dawro	
Enset	1804.1	3808.4	363.01	4256.2	465.08	1658.4
Wheat	1602.2	771.11	691.69	3835	724.15	1178.7
Barley	889.23	501	500	875	457.33	648.44
Faba bean	580.36	574.8	784.36	2566.7	552	791.22
Field pea	618.75	751.33	375.5	700	706.67	633.82
Potato	547.38	1977.8	876.5	382.14	1277.8	1050.7
Cabbage	137.14	287.5	240	485	250	283.64
Garlic	271.65	283.33	2620	.	693.75	854.48
Total Annual farm Revenue	6450.81	8955.27	6451.06	13100.04	5126.78	7099.4
Share of Enset for Gross farm Income	27.97	42.53	5.63	32.49	9.07	23.36

Source: computed from survey data, 2012/2013

Clone diversity and distribution: Enset farming communities has maintained a diversity of enset germplasms and sustainably utilizing the crop over several decades. Survey results revealed that about 67, 63, 59, 58, 57, 29 and 26 clones in Dawro, Kembata, Hadiya, Gurage, Siltie, Wolayta and Gedeo Zones/districts respectively were recorded and found to be conserved in farmer's fields with various degrees of diversity and abundance. Some clones in different Zones/districts share similar names and this needs further characterization study to verify their similarity. Farmers conserve and cultivate different enset clones for various purposes like kocho, bulla, and fiber yield and quality, medicinal, drought and EXW tolerance/resistance and ritual purposes in various level of abundance. Table 4 portrayed the average number of enset clones cultivated by individual farmers and this ranges from seven to ten across different zones. The number of clones cultivated by individual farmers within Zones/districts ranges from 3 to 28 clones. Average number of clones per farm was 9 and this shows high farm level richness (Table 5).

Table 5 Average number of clones maintained/farmers

Number Of Clones Per Farmer	Minimum	Maximum	Mean
Silti (57)	5	24	9.6
Gurage (58)	3	20	9.3
Kembata (63)	3	14	7.5
Sidama (57)	3	28	9.9
Dawro (67)	3	28	10.1
Total	3	28	9.0

Source: computed from survey data, 2012/2013

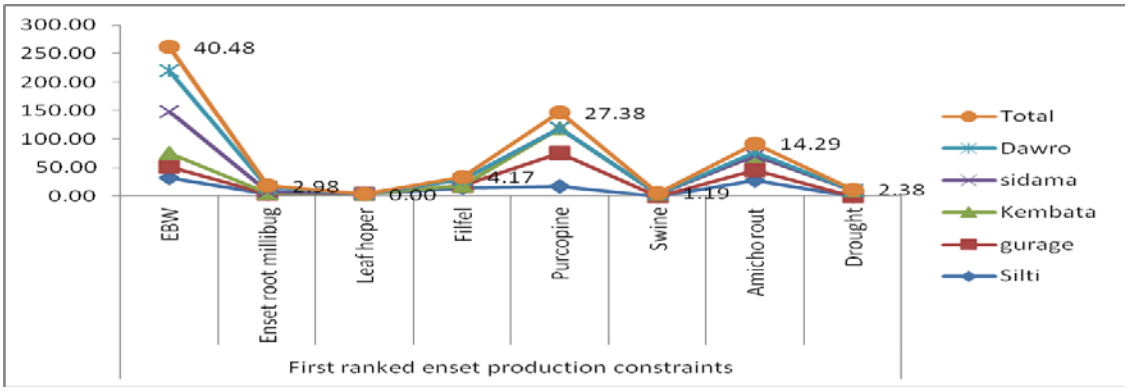
Enset Xanthomonas wilt (EXW) and Enset Production Constraints: Previous research works by various scholars revealed that enset production and productivity is constrained by several abiotic and biotic factors. These comprises of many diseases that attack different parts of the plant caused by bacteria, fungi, nematodes, and viruses, pests and wild vertebrates. Based on the survey result sample respondent identified various enset production traits in their locality and prioritized the most important one. Table 6, summarizes most frequently reported enset production constraints in the study area. Corm rot, porcupine, EXW and Leaf hoper constitute the most important constraints. Farmers also ranked the first most important enset production constraints in their locality from the abovementioned constraints. Large proportion of sample respondents ranked EXW (40.5%), first followed by porcupine (27.4%) and corm rot (14.3%) (Figure 3).

Table 6. Frequently reported enset production constraints in the study area.

Major constraints in enset production	Zone							
	Silti	Gurage	Kembata	Sidama	Dawro	Gedeo	Wolayta	Total
EBW	32.1	19.4	14.3	66.7	84.4	93.1	90.9	42.3
Enset root millibug	7.1	5.6	49.3	60.0	40.6	56.8	22.7	21.4
Leaf hoper	3.6	2.8	0.0	13.3	37.9	36.4	22.7	11.3
Mole rat	21.4	25.0	7.1	60.0	50.0	4.6	4.5	30.4
Porcupine	25.0	86.1	42.9	63.3	43.8	0.0	0.0	51.2
Swine	0.0	0.0	4.8	20.0	0.0	0.0	0.0	6.0
Corm rot	42.9	83.3	28.6	36.7	78.1	54.4	45.5	54.2
Drought	0.0	8.3	9.5	0.0	0.0	0.0	0.0	4.2

Source: computed from survey data, 2012/2013

Figure 3. Proportion of sample respondents who ranked enset production constraints first



Source: computed from survey data, 2012/2013

Among the Enset production constraints in Ethiopia (Table 6), EXW Disease rated the first in its devastation and distribution in this study area. EXW prevalence and severity has been summarized in Table 7. From the total sample respondents 27.8 % reported existence of EXW in their enset field. The highest (78.7%) and lowest (3.3%) prevalence rate is recorded in Gedeo

and Kembata zones respectively. Consequently EXW disease distribution is highest in Gedeo, Dawro, Sidama, Silti, Wolayta and Gurage with varying degree.

Proportion of Enset infected with EXW per individual holding (severity) has also been computed and in each zone and results revealed on average 28.7 % of enset stands was lost due to EXW Disease. Highest enset damage was recorded in Wolayta (20.8%), Dawro (20.2%), Gurage, (17.7%), Gedeo (16.9%), Sidama (13.8%) in 2012/13.

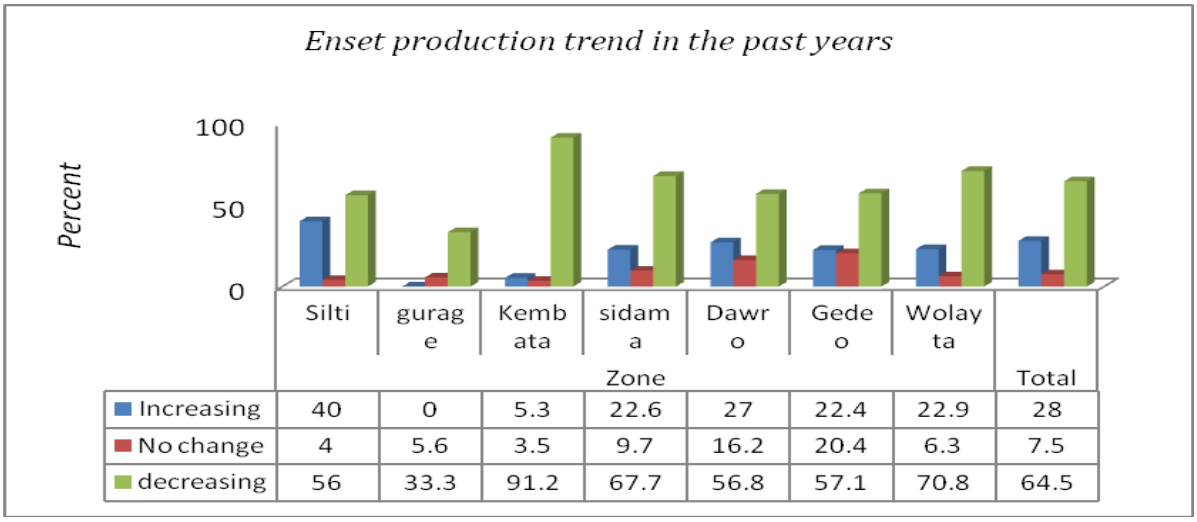
Table 7. Proportion of EXW Disease prevalence and severity

		Zone							Total
		Silti	Gurage	Kembata	Sidama	Dawro	Gedeo	Wolayta	
Infected plant (EXW) in 2012/2013	Yes	23.3	13.5	3.3	31.0	52.6	78.7	21.3	27.8
	No	76.7	86.5	96.7	69.0	47.4	4.3	27.3	72.2
Number if infected Enset (mean)		10.2	38.0	8.7	135.5	141.2	44.8	15.1	128.3
Number of matured Enset in the field (mean)		345.8	214.3	558.3	982.7	699.8	265.5	72.6	448.4
Proportion of Enset Infected with EXW /Field (Severity)		3.0	17.7	1.6	13.8	20.2	16.9	20.8	28.6

Source: computed from survey data, 2012/2013

Enset production in Southern Ethiopia, according to the information from sample respondent has revealed a trend portrayed in Figure 4. More than 64 % of the respondents reply enset production declines and attributed different reasons presented in table 8 for the particular trend noted. EXW disease, poor management, drought and climate change, consumption pressure and fragmented land are among reasons for the declining enset production trend.

Figure 4. Enset production trend in the past few years



Source: computed from survey data, 2012/2013

Table 8. Reason for enset production trend

Reason for the trend	Zone							Total
	Silti	Gurage	Kembata	Sidama	Dawro	Gedeo	Wolayta	
Drought and climate change	21.1	0.0	74.5	14.8	0.0	5.7	52.4	26.7
Occurrence of disease and pests	5.3	6.5	4.3	0.0	18.9	5.7	9.5	7.5
Poor management	31.6	45.2	14.9	11.1	24.3	17.1	4.8	27.3
Reducing of soil fertility	10.5	3.2	0.0	18.5	2.7	22.1	2.4	5.6
EXW disease	0.0	9.7	2.1	7.4	13.5	17.1	0.0	28
Fragmented land	0.0	0.0	0.0	29.6	5.4	17.3	4.8	10.2
Consumption pressure	15.8	12.9	0.0	14.8	18.9	2.9	11.9	11.2
Replaced with other crops	10.5	6.5	0.0	0.0	0.0	11.4	4.8	9.1
Shortage of clean suckers	0	3.2	2.1	0.0	0.0	0.0	0.0	1.2

Source: computed from survey data, 2012/2013

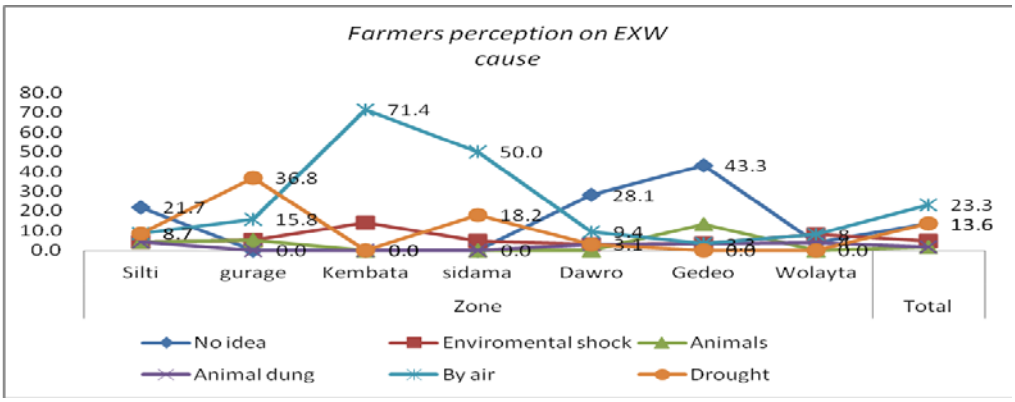
Farmers' perceptions about EXW Disease: More than 41 % of farmers replied that they never recognize the disease early; about 58 % confirmed that they able to identify the disease by observing the symptoms listed in Table 9.Farmers' knowledge on EXW Disease cause showed that their understanding about the cause of EXW varied across zones and seems to be very decisive in managing threat against Enset production. Most respondents reported the cause for EXW as air in Kembat and Sidama, No idea in Dawuro and Gedeo, droudgt in Guragie and Sidama and environmental shock and animals in Kembata and Gedeo respectively are causes for EXW disease (Figure 5). Majority of the respondents depicted that contaminated material, Animal dung; animals, farm tools, insect and air are considered as a major means of EXW transmission (Table 10).

Table 9. Farmers' perceptions

Varible	Category	Zone							
		Silti	Gurage	Kembata	Sidama	Dawro	Gedeo	Wolayta	Total
Identify EXW infected enset early?	Yes	75	48.6	36.7	71.0	73.7	95.7	72.7	58.2
	No	25	51.4	63.3	29.0	26.3	4.3	27.3	41.8
How do you identify if it is EXW or not?	Yellowish leaf	59.1	41.2	44.4	5.3	32.1	38.6	26.9	35.8
	Wilted leaf	18.2	52.9	22.2	89.5	39.3	0.0	0.0	45.3
	Wilting upper part of the leaf	18.2	5.9	22.2	5.3	17.9	2.3	3.8	13.7
	Yellowish leaf and strange smell	4.5	0	11.1	0	0.0	2.3	11.5	2.1
	Shoot bud and entire leaf wilted	0	0	0	0	7.1	4.5	7.7	2.1
	Excretes outh on the psuedostem	0	0	0	0	3.6	0.0	0.0	1.1

Source: computed from survey data, 2012/2013

Figure 5. Proportion of farmers' perception on the cause of EXW.



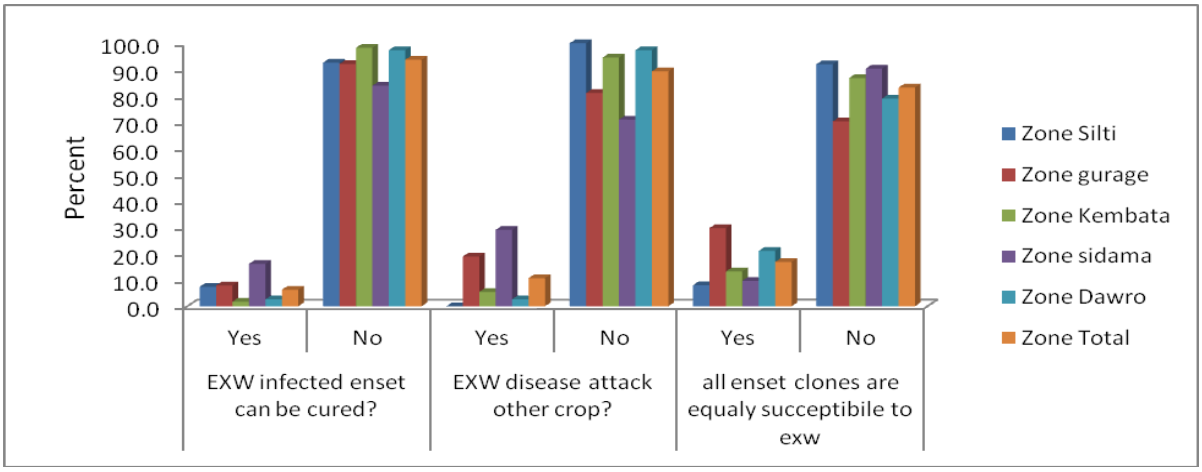
Source: computed from survey data, 2012/2013

Table 10. Summarizes Farmers perceptions on means disease transmission

	Means disease transmission	Zone					Total
		Silti	Gurage	Kembata	Sidama	Dawro	
From one field to another	Contaminated material	33.3	39.1	14.3	40.0	38.7	36.7
	Drought	0.0	0.0	28.6	0.0	0.0	2.0
	Farm tools	66.7	60.8	28.6	28.0	19.4	6.0
	Air	0.0	0.0	14.3	32.0	12.9	13.3
	Insect	16.7	21.7	14.3	0.0	25.8	16.3
	Animal dung	0.0	0.0	14.3	0.0	0.0	1.0
	No idea	0.0	0.0	0.0	0.0	3.2	1.0
	Animals	25.0	21.7	28.6	0.0	25.8	18.3
From one plant to another	Contact between infected and healthy plant	35.7	4.2	44.4	6.5	0.0	11.0
	By contaminated material	42.9	37.5	11.1	35.5	58.1	41.3
	Farm tools	21.4	45.8	33.3	45.1	29.0	36.7
	Air	0.0	0.0	0.0	6.5	6.5	3.7
	Animals	7.1	12.5	11.1	9.6	9.7	10.1
	Human	0.0	0.0	11.1	3.2	0.0	1.8

Moreover in the surveyed areas farmers also identify that all enset clones are not equally susceptible to EXW and listed out the most important clones in each zone with respect to disease resistance (Figure 6 and Table 11).

Figure 6. Proportion of farmers' perception.



Source: computed from survey data, 2012/2013

Table 11. Presents EXW resistance clones when farmers perceptions revealed all enset clones are not equally susceptible to EXW

Silti		Gurage		Kembata		Sidama		Dawuro	
Clone	%	Clone	%	Clone	%	Clone	%	Clone	%
Garado	9.1	Tegeded	4.8	Unjame	85	Gatecho	76.95	Tuzmia	15.15
Bededet	18.1	Agede	61.9	Sesekela	85	Nifo	12	Maziya	93.94
Agede	9.1	Bededet	52.4	Banko	85	Astara	9.1	Shadedine	33.33
Benezhe	18.1	Enkufaye	9.5	Etne	85	Kotecha	42.9	Amiya	6.06
Enkufaye	9.1	Shirteye	33.3	Astera	85	Ado	42.8	Kuruwa	12.12
Shirteye	9.1	Dere	14.3	Sheleke	85	Bera	14.1	Argama	9.09
Ahiro	9.1	Benezhe	9.5	Degumerz	85	Modasho	20.4	Tela	9.09
Enba	9.1	Ginbura	9.5	Gishera	85	Gena	10.7	Yesha maziya	12.12
Zegez	9.1	Astera	4.8			Gosala	14.3	Badedit	9.09
Ager amer	9.1	Nechute	4.8			Dewarama	7.14	Shasha	6.06
Ashekit	16.5	Wanadia	4.8			Berbo	7.14	Baze	6.06
Kombat	16.5	Beker	4.8			Gadami	3.6	Agunta	6.06
						Altecho	3.6	Kuruma	3.03

Source: computed from survey data, 2012/2013

As a concluding remark, the large number of Enset clones recorded and the nature of diversity indicate that the region is rich in terms of Enset clones diversity in Ethiopia. The diversity of Enset is not spread evenly across the region. Some areas in the region pass high varietal diversity while others are characterized by relative varietal paucity. The household characteristics, geographical distance and ethnic differences are responsible for this variation. The distribution of clone is characterized by high level of endemism which has implications for the conservation of Enset diversity. It is suggested that high land areas owing to the high concentration of diverse and unique landraces there should be given a high priority for collection and in situ germplasm conservation. We also concluded that the names given by the Enset growing farmers to the

different clones are generally consistent, distinguishing different Enset clones linguistically, phenotypically and in terms of their utilization values. It will support further characterization of the clones using other techniques by avoiding redundancies and optimizing the efficient conservation and sustainable use. Because of the biophysical and socioeconomic diversity in enset farming system in the country, there is a need to collect and document baseline data from the remaining major enset growing areas.

Pathogen characterization and Virulence spectrum study

Preliminary investigations on bacterial wilt of enset: This study was conducted to evaluate the efficacy of antibiotics against X. campestris pv. musacearum in vitro to X. campestris pv. Musacearum. Sensitivity of X. campestris pv. musacearum to five antibiotics was conducted by using paper disk diffusion assay techniques. Filter paper discs 0.6 cm diameter were cut with the help of cork borer and autoclaved at 180^0c for 1hr. Solutions of five antibiotics namely Chloramphinicol (CAPH), streptomycin sulfate, Tetracycline, Amoxicillin and Gentamycin were prepared in three concentrations (0.1, 0.5 and 1%)., at a concentration of. Then 20µl of each solution was dropped to each disk and left for 10 minutes to evaporate. Sterilized distilled water was used for the control disks. All the tested antibiotics are known to be effective against gram negative bacteria. Bacterial suspension of X. campestris pv. musacearum (10^8 cfu/ml by using spectrophotometer) was prepared as inoculums for this test. One milliliter of this suspension was poured in sterilized Petri dishes on to which about 20 ml of autoclaved YDC agar cooled to about 50ºC in a water bath was poured. The Petri dishes were gently shaken to mix the bacterial cell suspension uniformly and allowed to solidify.

The paper discs were then placed on the solidified nutrient agar containing the bacterium in Petri dishes. In each Petri dish the five antibiotics and the control were placed having similar concentration per Petri dish. These Petri dishes were labelled and then incubated at 28^oC for 48 hours. This experiment was conducted for two isolates of Xcm which were obtained from Gurage zone and Hagere selam (Sidama zone). The experiment was laid out as CRD in a factorial arrangement in lab experiment for both isolates with antibiotics as a main factor and their concentration as a sub factor. The experiment was conducted with three replications. The different antibiotics and their concentrations were evaluated by the diameter of inhibition zones around the discs.

In vitro Efficacy of Antibiotics against Xcm indicated that all the five antibiotics tested were inhibited the growth of Xcm pathogen culture tested in vitro all concentrations as compared to the control. However, they showed variable reactions to the pathogen.

In addition in vitro evaluation of antibiotics for Gurage isolate reveled that all the toxicants at all concentrations reduced the multiplication of X. campestris pv. musacearum significantly (<0.001) as compared to control for the Gurage isolate, but they varied greatly in their effect (Table 13). The diameter of inhibition zone ranges from 0.53cm (Gentamycin at 0.1%) to 3.87cm (Amoxicillin at 1%). The interaction effect between antibiotics and concentrations indicated that, Amoxicillin at a concentrations of 1%, Tetracycline at 1%, Amoxicillin at 0.5%, Tetracycline at 0.5%, Amoxicillin at 0.1%, and CAPH at 1% concentrations being the most effective antibiotics in inhibiting the growth of the Gurage isolates of Xcm culture, in which the inhibition zones were 3.87, 3.20, 3.07, 2.97, 2.70 and 2.63 cm, respectively. Similarly, Streptomycin sulphate at 1%, CAPH at 0.5%, Tetracycline at 0.1 %, Streptomycin sulphate at 0.5% and Streptomycin sulphate at 0.1% concentration were found to be moderately effective having an inhibition zone of 2.47, 2.30, 2.23, 2.17 and 1.77 cm respectively. Thus, Amoxicillin and Tetracycline were found to be the most effective antibiotics at all concentrations.

On the other hand, Gentamycin at 0.1%, Gentamycin at 0.5%, CAPH at 0.1 %, Gentamycin at 1% and Streptomycin sulphate at 0.1%, were comparatively less effective in inhibiting the growth of the bacterial culture, even if they were significantly inhibited the bacterial growth as compared to the control, with an inhibition zone of 0.53, 1.300, 1.43 and 1.47 cm respectively. Gentamycin was found to be the least effective antibiotics in inhibiting the growth of Gurage isolates of Xcm with inhibition zone of 1.38 cm, followed by, Streptomycin sulphate and CAPH were comparatively moderately effective with the diameter of inhibition zone of 2.52 and 2.58 cm, respectively. Overall, the effect of all antibiotics in reducing bacterial growth significantly (<0.001) increased with increase in concentration. There were bacterial growth around all control disks (no inhibition zones were observed) (Fig. 7).

Moreover in vitro evaluation of antibiotics for Hagere Selam isolate showed that the diameter of inhibition zone for Hagereselam isolate was presented in Table 12. Analysis of variance showed significant effect of antibiotics on the diameter of inhibition zone. Similar to the Gurage isolate, all the antibiotics at all concentrations were reduced the multiplication of X. campestris pv. musacearum significantly (<0.001) compared to the control for Hagere selam isolate, but they varied greatly in their efficacy. All the antibiotics showed lower to higher activities against Xcm. The maximum diameter of inhibition zone was observed in Tetracycline at a concentration of 1% (2.73 cm) and the minimum was for Streptomycin sulphate at 0.1% concentrations (0.40 cm). Tetracycline at rate 1%, CAPH at rate 0.5%, Tetracycline at rate 0.5% and CAPH at rate 1% are the most effective antibiotics by inhibiting the growth of Xcm for HS isolate with an inhibition zone of 2.73, 2.63, 2.47 and 2.47 cm, respectively.

Similarly Tetracycline at a concentration of 0.1%, CAPH at 0.1%, Amoxicillin at 1%, Gentamycin at 1% and Amoxicillin at 0.5 % are moderately effective for the inhibition of the growth of this isolate having an inhibition zone diameter of 1.77, 1.73, 1.63, 1.53 and 1.43 cm, respectively. This result shows that Tetracycline and Chloramphinicol were found to be the most effective antibiotics for inhibiting the growth of Xcm for HS isolate significantly.

In contrast, Streptomycin sulphate at 0.1%, Gentamycin at 0.1%, Streptomycin sulphate at 0.5%, Streptomycin sulphate at 1%, Amoxicillin at 0.1% and Gentamycin at 0.1% were found to be comparatively least effective for the inhibition of the growth of Xcm for Hagereselam isolate with an inhibition zone diameter of 0.40, 0.57, 0.67, 0.77, 0.80 and 1.00 cm respectively. On the other hand, Streptomycin sulphate was found to be the least effective antibiotics for the inhibition of the growth of this pathogen at all concentrations with an average inhibition zone diameter of 0.61cm followed by Gentamycin with 1.03 cm inhibition zone. This result revealed that the diameters of inhibition zones increased with the concentration of antibiotics. But, the diameter of inhibition zones for all antibiotics at 0.1% concentration were significantly (<0.001%) lower than 0.5 and 1% concentrations. In all the control treatments no inhibition zones were recorded around the disk (Fig.7).

Generally, this result revealed that there was variation between the antibiotics for the inhibition of bacterial culture growth of Xcm for Gurage and Hagereselam isolates. All the antibiotics reduced the multiplication of Xcm significantly as compared to control for both isolates but they varied greatly in their effect. Similar findings were reported by Maher et al. (2005), for Xanthomonas campestris patovars. Amoxicillin was found to be the most effective antibiotics in inhibiting the growth of Gurage isolate, but it was moderately effective for HS isolate. Tetracycline was effective for both isolates. In contrast Gentamycin and Streptomycin sulphate were found to be the least effective for both isolates. For both isolates as the concentration of antibiotics increase from 0.1% to 1% the inhibition zone also increases. Maher et al. (2005) indicated that, Streptomycin sulphate at 0.1 and 1% concentrations was found to be the most effective for X. campestris pv. citric through in-vitro test. However, this research revealed that Streptomycin sulphate was found to be least effective for Hagereselam isolate at all concentrations and moderately effective at 1% and 0.5% concentrations and least effective at 0.1% concentration for Gurage isolate of Xcm.

Table 12**.** Inhibition zone of antibiotics against the growth of Gurage and Hagere Selam isolates of X.campestris pv. musacearum, antibiotics*rate interaction

	Inhibition Zones (cm)							
	Gurage				Hagere Selam			
Antibiotics	Rate (%)				Rate (%)			
	1	0.5	0.1	Mean	1	0.5	0.1	Mean

Amoxicillin	3.87^{a}	3.07^{cd}	2.70^{cde}	3.21^{a}	1.63^{dc}	1.43^{e}	0.80^{g}	1.29^{b}
Tetracycline	3.20^{b}	2.97^{cbd}	2.23^{g}	2.83^{b}	2.73^{a}	2.47^{b}	1.73^{c}	2.31^{a}
Chloramphinicol	2.63^{fed}	2.30^{fg}	1.43^{ih}	2.12^{c}	2.47^{b}	2.63^{ba}	1.73^{c}	2.28^{a}
Strept. sulphate	2.47^{feg}	2.17^{g}	1.76^{g}	2.13^{c}	0.77^{g}	0.67^{hg}	0.40^{i}	0.61^{d}
Gentamycin	1.47^{ih}	1.30^{i}	0.53^{j}	1.10^{d}	1.47^{de}	1.00^{f}	0.57^{hi}	1.01^{c}
Control	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{e}	0.00^{j}	0.00^{j}	0.00^{j}	0.00^{e}
Mean	2.27^{a}	1.97^{b}	1.44^{c}		1.51^{a}	1.37^{b}	0.87^{c}	
LSD	0.39	0.16	0.26		0.086	0.21		
0.12								
CV (%)				9.28				7.51
SEM±				0.176				0.09

LSD, Least Significance Difference; CV, Coefficient of variation; SEM, Standard Error of Means; Means with different superscripts within the same column and class are statistically different at 1% level of significance.

Several experiments showed that in vitro and in vivo evaluation of antibiotics was found to be effective for the control of different Xanthamonas species (Talwar et al., 1996). This finding also revealed that in vitro evaluation of the tested antibiotics show significant inhibitory effect on both isolates of Xcm. A research by Singh et al. (2007) reported that Streptomycin at 30 mg concentration were found to be the most effective followed by Chloramphenicol at 30 mg concentration, and Gentamycin and Tetracycline were intermediately effective against Xanthomonas axonopodis pv. malvacearum.

Chloramphenicol was least effective for in vitro inhibition of X. oryzae pv. oryzae (Khan et al., 2012). This research also revealed that Chloramphenicol was less effective against inhibition of Gurage isolate but it was effective against Hagere selam isolate. Similarly Gentamicin was reported to be effective against some isolates of X. maltophilia (Khardori et al., 1990). But in this research Gentamicin was less effective for both isolates of Xcm. Streptomycin and chloroamphenicol were also tested for the control of black rot of cauliflower caused by Xanthomonas campestris pv. campestris and they were effective control of the pathogen (Lenka and Ram, 1997).

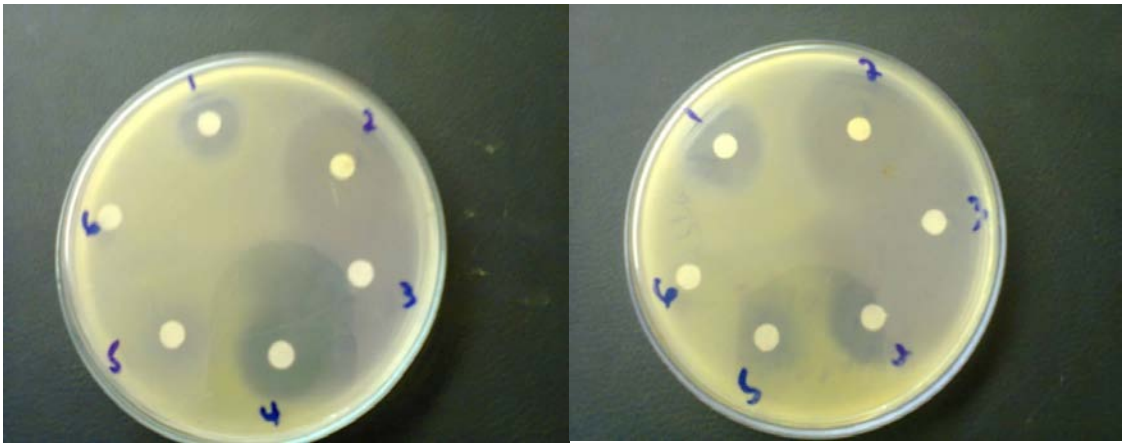


Figure 7. Inhibition zone of test antibiotics against X.campestris pv. musacearum for Gurage and Hagereselam isolates

Pathogen virulence spectrum studies that have been started shall be strengthened. Molecular characterization of the pathogen to determine diversity shall be carried out. Early detection tools such as LFD that were developed elsewhere shall be adopted, tested and extended to farmers for disease tracking and control. Bio-control strategies for EXW shall be tested and effective ones identified.

Studies on enset bacterial wilt disease epidemiology

Genotype × Environment interaction: Eleven enset clones differing with their reaction to enset XW, moderately tolerant (Gewada, Endale, Zerieta, Kellisa, Messena), susceptible (Gulumo and Arkiea), resistant /tolerant (Yanbule, Mazia and Halla) and one local variety from each location were included and planted using completely randomized block design with four replications at three locations (Hawassa, Hosanna (Bobicho), and Hegereselam (Bongodo FTC)). Artificial inoculation with Hagereselam virulent isolate (HSI) of Xcm suspension with a cell concentration of 10^8cfd/ml will be done after six months of transplanting. The trial will be repeated at all locations. Data will be collected on disease incidence, incubation period, soil type and actual weather conditions during the experimental periods. Data will be subjected to statistical analysis using the SAS software. Genotype, environment and pathogen interaction will be identified.

Role of diseased enset plant harvesting, processing and utilization on disease transmission: This activity was designed to see how long the EBW would survive in an acidic medium in fermented kocho when infected plants are harvested to be processed into starch food. The ultimate objective is to attest wether kocho movement such as the case in marketing would surve as the means for EBW dissemination. Matured bacterial wilt infected enset plants were collected from the farmers' fields of H/Selam (2485m a.s.l) and Bulle (2526m a.s.l) woredas of Sidama and Gedeo zones respectively. Both districts are adjacent and in these

locations the strain of Xcm is the most virulent. Bacterial wilt diseased plants were uprooted and all leaves (wilted), dead outer leaf sheath and roots from corms were removed before transporting to the Hawassa Experimental Station. Women having traditionally experienced and who were knowledgeable about the preparation of kocho were selected and provisionally employed to carry out enset processing (scrape, pulverize the stem and corm) following the traditional farmers' practices. The scraped and pulverized masses were thoroughly mixed with small amount of previously fermented kocho (as starter/initiator of fermentation) and placed in pits lined with enset leaves/plastic and left for fermentation at ambient temperature. Kocho was sampled from the pits periodically (15 days interval from the date of processing until final fermentation stage) and the samples were tested for the presence of the bacterial wilt pathogen. The pH of kocho was also determined in the soil laboratory using pH meter.

For the bacterial wilt pathogen examination, kocho sample was taken from the pit at 15 days intervals starting from the 1st date of fermentation (0, 15, 30, 45, 60, 75, 90 and 105) up to final fermentation day. Kocho sample was tested by the dilution plate method. About 0.5kg fresh weight of kocho sample was collected from the pit and mixed thoroughly, and then from mixed fresh mass 2 gm of sample was taken in 100ml of sterile water and diluted serially to obtain different concentrations of the pathogen distributed in the suspension. From each appropriate dilution one ml quantity of the suspension was added/poured (spread-plated) and/ or streaked to petridishes, to which melted and cooled (45^{o}C) YPSA nutrient media (20 ml) is added and mixed thoroughly with the suspension and then allowed to set. The inoculated petri dishes were incubated at 25 - 30^{0}C for 36 hours after which the appearance of pathogen is observed and colonies were counted using colony counter. For the determination of pH, some amount of fresh kocho was squeezed and liquid/susspenssion was tested using pH meter.

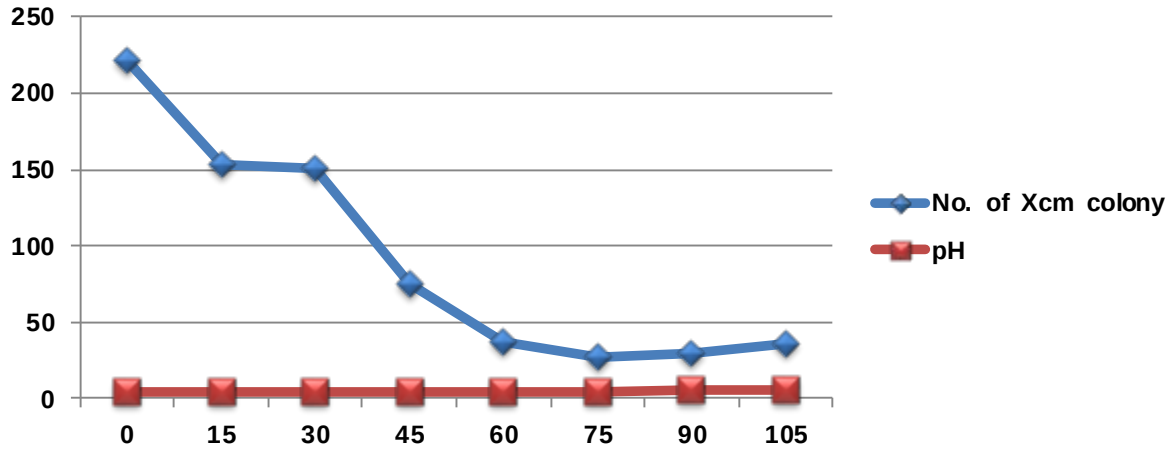
Results of this study indicated that bacterial wilt pathogen was existed in processed kocho obtained from bacterial wilt infected ensets. In the due course of studies the number of colonies of pathogen were observed on all the examined samples of kocho. In this study the pooled maximum number of bacteria colonies (183 to 221.5) were recorded on fermentation day 0 to 30 and the colonies numbers were reduced as per fermentation days increased. The mean pooled pH of kocho was found to be between 4.07 to 5.21 (Table 13).

In the due course of the studies Xcm colonies and kocho pH levels were corelated to some extent. However, they were not corelated negatively (Figure 7). Relatively at low kocho pH level, the colonies number of Xcm were minimal, so the effect pH on the colonies development was not significant.

Table 13**.** Xcm colonies and pH levels in fermentated kocho processed from

BW infected enset

Fermentation		pH			Colony	
Day	**H/Selam**	Bulle	Pooled	**H/Selam**	Bulle	Pooled
0	4.35	4.49	4.42	225	218	221.5
15	4.01	4.12	4.07	183	124	153.5
30	4.12	4.09	4.11	195	107	151
45	4.64	4.25	4.45	50	99	74.5
60	4.35	5.16	4.76	43	30	36.5
75	4.76	4.77	4.77	25	26	26.5
90	5.02	4.97	5	30	30	30
105	5.46	4.95	5.21	40	31	35.5
Average	4.59	4.6	4.59	98.88	83.125	91.29



Figuer 7**.** Xcm colonies and pH level in kocho obtained from infeted enset.

This study indicate that bacterial wilt pathogen was existed in kocho processed from bacterial wilt infected ensets. In all samples of kocho taken from the first fermentation day 0 up to 105 days, the Xanthomonas colonies were recorded at varying levels, seeming colonies were independent of pH. These results are accordance with earlier reports of the survival and transmission mechanisms of BW pathogen. The survival and transmission mechanisms of BW pathogen in different substances has bee studied and reported by various authors. According to Quimio and Mesfin (1996) Xcm can survive in the soil for about 3 months, in arid conditions where decomposition of the debris slow. Mwebaze et al. (2006) cited that the survival of Xcm was declined rapidly in non-sterile soil as compared to sterile soil, indicating that Xcm has limited ability to survive saprophytically in soil in the presence of other competing

microorganisms. The pathogen was also found to survive on the surface of contaminated knife for up to 3 and 4 days under humid and dry conditions, respectively (Ashagari 1985).

From this study it was found out that farmers uproot and discard when BW disease occurs in thier enset fileds. However, if the plants are matured or old enough for harvesting, such plants are not discarded but immediately harvested, processed and fermented into kocho. In kocho processed from matured BW diseased enset the pathogen survives for quite over three months. As one to three months fermented kocho is ready for use.

Farmers process kocho from matured BW diseased enset for incom genaration by selling and/ or for his own familiy consumption. Some women also pay kocho as a wage for daily labor when workers perform kocho processing or for other tasks (planting, weeding etc.) in the enset fields. In these cases the pathogen can be transmited within the fields and across locations through contaminated kocho. Women process the BW infected enset in the midst of healthy plants. During the cutting and transporting the infected enset within the healthy plants, the chance of Xcm contamination and transmission is very high. From the present study, the overall results suggest that kocho obtained from BW infected ensets would play role in the disease transmission. As a results, input of this study can be used in the integrated EBW management stratategies, through sensitization of farmers/women not to harvest and utilize the product (kocho) of BW infected ensets.

Characterization of enset clones

Morphological characterization: Till now there is no morphological descriptor for different Ensete ventricosum clones in the country. In order to determine diversity within and among the clones, 456 clones from Wolaita, Kembata and Hadiya, Sidama, Gamo Gofa, Gurage, Keffa, Sheka, Jimma, Yem Special woreda, West& South West Shewa, Western Arsi and Dawro zones were established at Areka Agricultural Research Center for morpho-agronomic characterization (Figure 1). Eight suckers which have similar size were taken from each clone and planted using a spacing of 3m and 1.5m between rows and plants, respectively (a plot size of 5.25 m^2). Augmented design was employed. Both quantitative and qualitative data will be collected one year after transplanting. The four plants from each plot will be used for data collection. Moreover, in order to choose a subset that represents as much of the variability in the full set of 456 accessions a multi-locational trial including clones from the whole collection site were planted in three (Areka, Angacha and Hawassa on station) different locations. A total of 100 enset clones (91 new entries, 6 standard checks and 3 local checks) were used for this experiment. The clones have been planted in plot size of 5.25m x 3 m. Simple lattice design with two replication were employed. The results of phenotypic characterization shall be related to and interpreted against molecular characterization.

Assessment of genetic diversity and on-farm evaluation of enset clones: Ethiopia is cited as one of the centers of enset diversity. In order to assess the on-farm genetic resources management of enset semi-structured interview methodology with 120 farmers' was employed in Kembata, Gurage and Wolaita provinces. From this provinces based on enset diversity and distribution Angacha, Geta and Bolososore districts were selected. At kebele level a representative farmers group comprised of 20 members were interviewed. For quantifying on-farm diversity, direct on-farm monitoring and participation with 120 farmers were made. Quantification of varietal diversity per farm was counted by a participatory zigzag sampling in the diagonal direction of the plot with the farmer and all encountered varieties were counted. Altitude and other related climatic data were collected. Soil samples were taken from 40 farms from Kembata province and will be subjected to analyses of soil pH, P, available nitrogen, organic matter and exchangeable potassium.

The variation among zones with respect to the total number of clones recorded in each zone (Richness of the zones) and in number of Enset clones per farm visited (Farm richness) is summarized in Table 14. Kembata with a total of 65 named clones recorded is the richest zone followed by Gurage with 44 and Wolaita with 30 clones (Table 14). Average number of clones per farm ranged between 9.3 for Kembata to 8.5 for Gurage and 7.0 for Wolaita had high farm level richness (Table 14).

There was also a considerable difference among the clones with regard to number of farms where the clone was grown in individual zones (clone abundance) (Table 15). Siskela and Gimbo, from Kembata, Amerate and Nechewe from Gurage and Halla and Tuzuma from Wolaita (in Table 15) was the most abundant clone as it were recorded on more than 75% of the farms surveyed. As indicated in Table 16, clone abundance also varied across the zones surveyed. Few clones were well represented in some zones, but virtually missed from the others. For example, Amerate was encountered on 33 of the farms visited in Gurage. Clones which are used by many famers in any zone tend to be introduced to other zones and have wider distribution.

The extent of clone diversity described in this study is also comparable with earlier reports. Admasu (2002) described 146 named Enset clones from three zones of SNNPR (52 clones from Sidama, 55 clones from Wolaita and 59 clones from Hadiay). Almaz (2001) described 146 different named Enset clones from three zones of SNNPRS (66 clones from Kefa-Sheka, 30 clones from Sidama, 45 clones from Hdaiya and six clones from Wolaita). More over Genet (2004) described 111 Enset clones from nine Enset growing regions of Ethiopia. Bizuayehu (2002) studied 79 clones from Sidama. Although our sample size was different from many of the samples used by previous studies, 23 of the Sidama clones included in our study are similar to the ones studied by Bezuayehu (2002).

In addition 65 and 42 clones were collected from Angacha, and Geta districts (Table 16). This year collection from Sodo province and transplanting to permanent field for evaluation will be carried out. The remaining activities like participatory characterization and evaluation will be commenced with in their work plan. Collected data will be subjected to descriptive statistics, analysis of variance, log-linear regression, cluster, and discriminant analysis. In 10 volunteer farmers field from each kebele the best fifteen clones for different use value and which are ready for harvest will be identified and evaluated. Farmers will be fully involved throughout the project time.

Table 14. Enset clone diversity in the three zones, Southern Ethiopia, Expressed as richness

Number of clones per Farm	Number of farms (N=40)		
	Kembata	Gurage	Wolaita

≤ 5 clones	1	6	2
6-10 clones	39	23	22
11-15 clones	0	9	14
≥15 clones	0	2	2
Total (Richness)	65	44	30
Mean Richness/Farm	9.3	8. 5	7
Minimum Richness	4	2	4
Maximum Richness	10	12	9

Table 15. Name of the most abundant and well distributed enset clones in SNNPRS

		No. of surveyed farm/district (N=40)		
No.	**Name of clone**	**Kembata**	**Gurage**	**Wolaita**
1	Halla			34
2	Amerate		33	
3	Sisqela	31		
4	Gimbo	25		
5	Astara	22		
6	Nechewe		22	
7	Tuzuma			22
8	Guarye		21	
9	Nekaka			21
10	Unjame	20		
11	Sabara		16	

Table 16. List of enset clones collected from Kembata Tembaro and Gurage Provinces

No	**Accession local name (Gurage collection)**	**No**	**Accession local name (Kembata collection)**	**No**	**Accession local name (Kembata collection)**
1	Shertia	1	Gambala Siskela	45	Aganche

2	Zegewerete	2	Wellanche	46	Mandaluka
3	Bazerie	3	Gunze	47	Wolagala
4	QuashQuashie	4	Ferchase	48	Bekaka
5	Amerate	5	Dirbo	49	Nejawro
6	Zara Badadate	6	Torore	50	Zobera
7	Anzana	7	Abatmerza	51	Felegede
8	Yergie	8	Sheleqe	52	Morala
9	Nechewe	9	Qerqere	53	Dereqeta
10	Tederader	10	Mariye	54	Gomorsa
11	Gumbura	11	Sebera	55	Luqande
12	Ahira	12	Gishira	56	Bulla Siskella
13	Guarye	13	Gagabo	57	Sorpie
14	Qumida	14	Mesmesa	58	Gimbewa
15	Gimbewe	15	Etine	59	Oniya
16	Badediet	16	Dego	60	Ginjona
17	Orad	17	Fechache	61	Shelequmie
18	Kembata	18	Tabare	62	Wojo Woea
19	Beneze	19	Eskuris	63	Lenbona
20	Tegaded	20	Wachiso	64	Qeshela Dirbo
21	Agade	21	Astara	65	WojoDirbo
22	Ferezie	22	Henwa		
23	Aywegne	23	Unjame		
24	Kandwa	24	Weshemeda		
25	Kessete	25	Badade		
26	Terie	26	Hargema		
27	Emerye	27	Hella		
28	Chehewot	28	Tessa		
29	Eymerete	29	Korbo		
30	Lemate	30	Derga		
31	Sebara	31	Lekaka		
32	Ayite quaquafe	32	Disho		
33	Awiea	33	Woea		
34	Dere	34	Qye Qiqle		
35	Badedet	35	Neche Qiqle		
36	Astara	36	Goderete		
37	Mariye	37	Cherkwa		
38	Yesherafire	38	Qoyina		
39	Hiniba	39	Sinera		
40	Qibenar	40	Gambala Merza		
41	Wanade	41	Dego Merza		
42	Yegetie Fereze	42	Ginawa		
43	Kanchewe	43	Moche		
44	Ankafe	44	Wongorate		

Assessment of genetic diversity among enset accessions using SSR markers: The application of molecular markers is crucial and more efficient for selection in breeding programs as well as to assess genetic diversity. Therefore, the present study will be designed to assess the diversity of enset accessions from different enset growing parts of the country using SSR analysis. The

experiment is expected to start this year. But preliminary activities like sucker multiplication of 456 enset accessions and proposal review at University level was carried out. Leaf materials of 456 accessions will be taken from healthy suckers. SSR markers that will be identified and characterized for enset in this study will be used to analyze the genetic diversity among 456 enset accessions. The molecular marker analyses will be carried out at Holleta Biotechnology, Ethiopia and BecA-ILRI, in Nairobi, Kenya.

Objective 5. Enset breeding and selection for wilt resistance/tolerance and other desirable traits

Evaluation of Enset Clones for Reaction to EXW under greenhouse condition: For large scale screening of enset clones for resistance to bacterial wilt three sets of trials are on-going with promising results. In the first set a total of 25 enset clones (Table 17) were evaluated for their reaction to Xcm pathogen at Hawasa University plant protection site. Twelve one year old suckers of each of the 25 clones were collected from enset growing areas of Gurage zone and grown in pots at Hawasa University. They were collected from similar environmental condition from farmer fields. The suckers were developed from a single corm for each clone. The clones were evaluated for their reaction to the pathogen under artificial inoculation. Lemat and Nechewe clone were included as tolerant/resistant checks (Gizachew, 2008) while Astra was used as a susceptible check (Gizachew et al., 2008). The suckers were watered at two days interval for two weeks after transplanting and two times per week after two weeks post inoculation.

Inoculum preparation and inoculation: Bacterial ooze was collected from the inoculated plants used in pathogenicity test. The exudates were collected aseptically at the cut end of petioles and leaf sheaths with the help of tooth pick and suspended in sterilized distilled water. A loopful of the suspension was streaked on YDC plate for multiplication of inoculum. The plates were incubated at 28°C for 24 hours. Pure bacterial colonies showing light yellow mucoid growth typical of X. campestris pv. musacearum from the plate was re-cultured on YDC agar and incubated at 28°C for 2 days, to produce enough bacterial culture for inoculation.

After two months of transplantation (at 4-7 leaf stages) the enset clones were inoculated with 3 ml of 2 days old bacterial suspension at the base of young leaf petiole by using hypodermic syringe. The concentration of bacteria was adjusted to 10^8 cfu/ml using spectrophotometer. The control plants were similarly inoculated with the same amount of SDW. The inoculated leaves and plants were labeled with marker. Ten suckers were inoculated with the pathogen and two suckers were inoculated with SDW as a control for each clone. A total of 25 enset clones were grown and 250 suckers were inoculated.

Table 17. Characteristics of the 25 enset clones that were evaluated for reaction to Xcm pathogen

No.	Clone	Purpose	Midrib color	Leaf sheath color	Height (m)	Diam	Age
1	Lemat	Kocho	Black	Green with black spot	2.4	1.6	12
2	Oret	Kocho, Amicho	Red	Red	2.5	1.3	9
3	Astara	Medicine, Amicho	Red	Red	2.4	1.3	9
4	Nechiwe	Kocho	Red at top	Green	2.4	1.3	7
5	Yeregye	Kocho	Red	Light red	2.5	1.4	8
6	Gimbewe	Kocho	Deep red	Deep red	2.4	1.5	9
7	Agade	Kocho	Grey	Red	2.7	1.4	12
8	Beresye	Kocho	Red	Red with black spot	2.4	1.3	8
9	Kanchwe	Kocho	Grey	Red	2.4	1.3	7
10	Yegendye	Kocho	Green	Green with black spot	2.5	1.3	8
11	Terye	Kocho	Red	Grey	2.4	1.3	8
12	Wenadye	Kocho	Red	Red	2.5	1.3	9
13	Sheberat	Kocho	Grey	Red	---	---	7
14	Kechere	Kocho	Light red	Light green	2.5	1.2	8
15	Badedat	Kocho	Red	Red	2.4	1.4	9
16	Yekeswe	Kocho	Red at top	Green with black spot	2.3	1.2	8
17	Zober	Kocho, Amicho	Red	Red	2.5	1.4	9
18	Yeshrakike	Kocho	Red	Red	2.6	1.3	10
19	Kibenar	Medicine, Amicho	Green	Green	2.3	1.2	9
20	Ferezeye	Kocho, Amicho	Red	Red	2.5	1.4	9
21	Teguaner	Kocho, Amicho	Red	Red	2.4	1.3	11
22	Ewene	Kocho	Red	Red	2.4	1.3	10
23	Demolejat	Medicine	Deep red	Deep red	---	---	9
24	Gezwet	Kocho	Green to grey	Red	2.3	1.3	8
25	Bushrat	Kocho	Grey	Red at top	2.4	1.4	7

These data were taken from direct measurement and interview with enset farmers.
Vernacular names of enset clones were according to given by enset farmer in Gurage zone.
Diam; Diameter of the girth (m), Age; Average age of the clone to reach maturity

Disease assessment: Disease data was taken as of 10 days after inoculation, then at 7 days interval for one month. The number of suckers showing wilt symptom, the time of initial symptom (incubation period) and complete wilting date were recorded. The percentage of wilted plants (wilt incidence) at each assessment period was calculated according to the formula:

Incidence = (NW/NT) ×100, where, NT is the number of total tested plants and NW is the number of wilted plants.

The reaction of each clone was categorized into four resistance levels based on average wilt incidences at 35 DAI (days after inoculation) as follows (Tripathi et al., 2007): Highly Susceptible (HS): 70 - 100% plants wilted, Susceptible (S): 40 - 69% plants wilted, Moderately Resistant (MR): less than 40% plants wilted and Resistant (R): none of the plants wilted. Furthermore, date of complete wilting, incubation time and average wilt incidence were used for evaluation. Similarly, percentage of wilted plants at each assessment period was used to calculate area under disease progress curve (AUDPC) using the following formula (Jeger and Viljanen-Rollinson, 2001).

Area under Disease Progress Curve (AUDPC) = $\sum_{i=1}^{n}[(Di_{i+1}+Di_i)/2]\times[t_{i+1}-t_i]$, in which Di_i = Percentage of wilted plants at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations.

All the inoculated enset clones showed symptom of EXW at different assessment periods, while all the control plants inoculated with water did not show any wilt symptom in all clones and at all assessment period. None of the evaluated clone was immune to the pathogen. Many reports indicate that there was no completely resistant enset clone to Xcm pathogen (Dereje, 1985, Gizachew et al., 2008), except for Mezya which has better resistance to the pathogen (Fikere and Gizachew, 2007). Similarly, no banana cultivar was found to be completely resistant to Xcm (Ssekiwoko et al., 2006, Biruma et al., 2007, Smith, et al., 2008, Tripathi, et al., 2007).

All inoculated clones symptom (yellowing and chlorosis) development started in the inoculated leaves. Symptom development after artificial inoculation was similar to those observed in young plants following natural infection in the field. Significant differences ($P \leq 0.0001$) were observed in incubation period, wilt incidence at 35^{th} day, complete wilting, average incidence and area under the disease progressive curves among the 25 enset clones evaluated for their resistance to Xcm (Table 18). Symptom development starts at 10^{th} day after inoculation and the mean incubation period of the clones varied from 16.2 to 42.2 days.

Table 18. Mean Incubation time, complete wilting, wilt incidence at 35 DAI and disease rating for the enset clones

No.	Clone	No. Inc[a]	Incid35[b]	Mean Incub[c]	Compt[d]	Rating
1.	Gezwet	10	0.0^{h}	42.2^{a}	71.0^{a}	R
2.	Gimbwe	10	10.0^{hg}	40.9^{ba}	66.0^{bac}	MR
3.	Terye	10	20.0^{fhg}	38.5^{bac}	67.0^{bac}	MR
4.	Agade	10	30.0^{fheg}	39.5^{bac}	63.0^{bdac}	MR
5.	Yeshrakinke	10	30.0^{fheg}	39.8^{bac}	70.0^{ba}	MR

6.	Kechere	10	30.0^{egdf}	37.9^{ebdac}	64.0^{bdac}	MR
7.	Badedat	10	30.0^{egdf}	38.6^{bac}	68.0^{bac}	MR
8.	Ferezye	9	33.3^{feg}	38.0^{bdac}	67.8^{bac}	MR
9.	Kibinar	10	40.0^{fdeg}	32.6^{ebdgcf}	63.0^{bdac}	S
10	Yegendeye	10	50.0^{fdec}	38.5^{bac}	70.0^{ba}	S
11.	Zober	10	50.0^{fdec}	34.8^{ebdagcf}	62.2^{bdec}	S
12.	Ewane	10	60.0^{bdec}	34.3^{ebdagcf}	56.0^{fdeg}	S
13.	Wenadeye	10	60.0^{bdec}	31.6^{edhgcf}	60.0^{fdec}	S
14.	Astara	10	60.0^{bdec}	31.2^{eidhgcf}	66.0^{bac}	S
15.	Beresye	10	70.0^{bdac}	29.5^{eidhgf}	53.6^{fhg}	HS
16.	Shebrat	10	70.0^{bdac}	29.5^{eidhgf}	52.4^{fhg}	HS
17.	Teguaner	10	70.0^{bdac}	35.7^{ebdacf}	53.2^{fhg}	HS
18.	Demolejat	10	70.0^{bdac}	29.2^{eihgf}	62.0^{bdec}	HS
19.	Nechwe	10	80.0^{bac}	22.8^{ij}	51.4^{hg}	HS
20.	Kanchwe	10	80.0^{bac}	28.1^{ihgf}	55.0^{fheg}	HS
21.	Yekeswe	10	90.0^{ba}	27. 8^{ihgf}	54.0^{fhg}	HS
22.	Bushrat	10	100.0^{a}	26.7^{ihg}	63.0^{bdac}	HS
23.	Oret	10	100.0^{a}	23.8^{ihj}	64.0^{bdac}	HS
24.	Lemat	10	100.0^{a}	18.4^{j}	57.4^{fdeg}	HS
25.	Yeregye	9	100.0^{a}	16.2^{j}	47.8^{h}	HS
CV%			12.38	15.7	12.5	
R^2			0.91	0.63	0.64	

[a] Number of inoculated enset plant; [c] Incubation period; [b] Wilt incidence at 35 days after inoculation; [d] Mean complete wilting date after inoculation; R, Resistant; MR, Moderately resistant; S, Susceptible; HS, Highly susceptible; Means with different superscripts within the same column and class are statistically different at 5% level of significance according to DMRT.

In this experiment the tested enset clones were categorized in to four disease ratings (resistant, moderately resistant, susceptible and highly susceptible) based on their wilt incidence at 35 days after inoculation. Accordingly, lower incidence, longer incubation period, complete wilting and slow disease progression were associated with resistant clones and the reverse hold true for susceptible clones.

The various enset clones showed significant differences in susceptibility to X. campestris pv. musacearum. The wilt incidence at 35th DAI ranged from 0 to 100% for the evaluated enset clones. Gezwet was the only resistant clone to Xcm with no wilt incidence at 35 days after inoculation, mean incubation period of 42.2 DAI and complete wilting 71 DAI. Seven enset

clones namely Gimbwe, Terye, Agade, Yeshrakinke, Kechere, Badedat and Ferezye were moderately resistant to Xcm. These clones showed wilt incidence of less than 40% at 35 DAI and an incubation period of 37.9-40.9 days. On the other hand, complete wilting for these clones ranged from 63-70 DAI and there were no significant difference between them at 5% level of significance for incubation period and complete wilting.

Six enset clones, namely Kibinar, Yegendeye, Astara, Ewane, Wenadeye and Zober were susceptible to the pathogen with incubation period of 31.2-38.5 DAI and complete wilting from 56-70 DAI. These clones did not vary significantly from each other in disease parameters. The rest eleven enset clones were found to be highly susceptible to Xcm pathogen with wilt incidence of 70-100% at 35 DAI, incubation period of 16.2-35.7 DAI and complete wilting of 47.8-64.0 DAI (Table 18). The average wilt incidence over the assessment periods ranges from 20.11 to 70.30%. The maximum average wilt incidence was recorded on Yeregye (HS, 70.3%) and Lemat (HS, 64.29%). On the other hand, BWE incidence was the lowest on Gezwet (R, 20.11%) and Gimbwe (MR, 22.09%).

Disease progress was rapid on highly susceptible and susceptible clones, whereas relatively slow progress was noted on resistant and moderately resistant enset clones (Fig. 10). Similarly the disease progress curve was steeper initially for resistant and moderately resistant clones, while it increases faster for susceptible and highly susceptible enset clones. AUDPC vary significantly ($P<0.0001$) between the clones. The highest AUDPC (86.19) was recorded on clone Yeregye (HS) but the resistant clones Gezwet had the lowest (21.25) AUDPC (Table 19). There was no significant difference ($P \leq 0.0001$) between the clones that are grouped in the same disease rating.

Generally, this result partially agrees with previous findings. However, Gizachew et al. (2008) reported Gezwet was susceptible, while in this experiment it was resistant but Astara was susceptible clone in both case. Lemat and Nechwe showed a relative tolerance to Xcm (Gizachew et al., 2008) conversely both were susceptible to the pathogen. Dereje (1985) reported that Agade was more susceptible to Xcm as compared the other, but here it was moderately resistant clone. Yeshrakinke was found to be moderately resistant clone, which is in agreement with Anita et al (1996), which reported it was tolerant clone to Xcm. In contrast, Gizachew et al. (2008) reported that Yeshrakinke was susceptible clone. This variation might be due to the variations of isolates of Xcm pathogen though, this experiment was conducted in only one pathogenic isolate or it might be due to the genetic variation within clones (a single clone may contain several sub clones). The susceptible (Nechewe) and the tolerant (Lemat) checks used in this experiment was found to be susceptible to the pathogen.

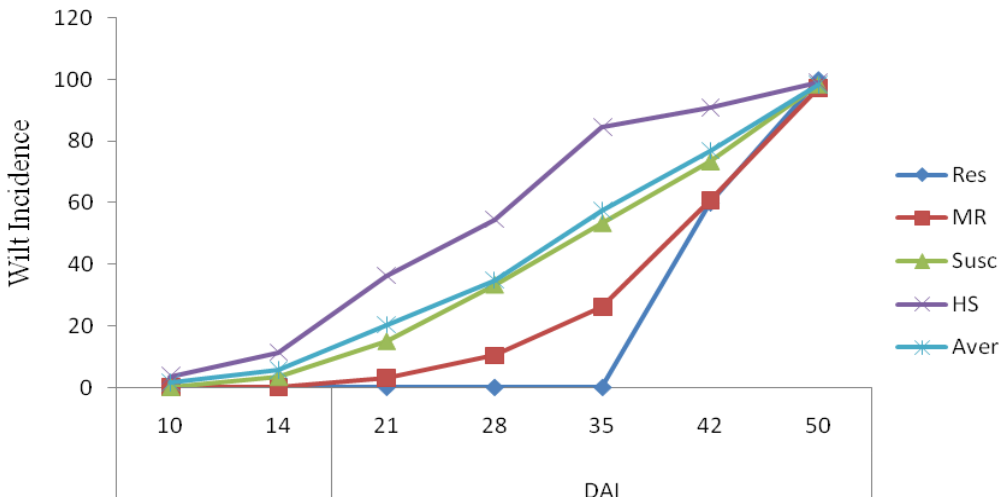


Figure 10**.** Mean disease progress curve for resistant, moderately resistant, susceptible and highly susceptible clones as compared to the average progress curve.

Evaluation of enset clones against bacterial wilt under field condition: Two sets of trials are on-going with promising results. In the first trial, a total of 80 corms of enset clones locally known to tolerate bacterial wilt disease were assessed and collected from some major enset grown areas of the southern region were used for this study (Table 20). All collected enset corms were planted at Areka Research Centre conditions for suckers' production using recommended agronomic practices. A year old enset suckers were transplanted at the experimental field of Hawassa Research Centre in the 2011 cropping year under rain fed conditions. Six suckers from each healthy and uniform sized enset plantlets were transplanted at spacing of 1.5m x 1.5m between plants and rows respectively and each row was considered as a plot. All agronomic recommendations were practiced accordingly. To confirm and correlate the study (conducted under research conditions) results, farmers perceptions were assessed and recorded.

Table 19. Arcsine transformed wilt incidence of the 25 enset clones at different disease assessment periods and their AUDPC value

Wilt Incidence (%)										
		DAI[a]								
No.	**Clone**	**10**	**14**	**21**	**28**	**35**	**42**	**50**	**Mean**	**AUDPC**
1.	Gezwet	0	0	0	0	0	50.77	90	20.11^{i}	77.25^{a}
2.	Gimbewe	0	0	0	0	13.29	51.33	90	22.09^{i}	63.38^{cb}
3.	Terye	0	0	0	13.29	26.57	57.1	76.72	24.81^{hi}	46.13^{gefh}
4.	Agade	0	0	13.29	13.29	32.9	50.77	90	28.61^{ghefi}	66.06^{b}
5.	Yeshrakinke	0	0	0	26.57	32.9	57.1	76.72	27.62^{ghfi}	86.19^{a}
6.	Kechere	0	0	0	13.29	32.9	51.33	90	26.79^{ghi}	23.0^{kl}
7.	Badedat	0	0	0	0	32.9	50.77	90	24.81^{hi}	30.0^{kijl}
8.	Ferezye	0	0	15	30	34.62	42.12	90	30.25^{ghefi}	49.50^{gefd}
9.	Kibinar	0	26.57	26.57	32.9	39.23	57.1	90	38.91^{gcefd}	54.88^{cebd}
10.	Yegendye	0	0	13.29	26.57	45	50.77	76.72	30.34^{ghefi}	34.25^{kij}
11.	Zober	0	0	26.57	26.57	45	63.43	90	35.94^{ghefd}	27.38^{kjl}
12.	Ewane	0	0	13.29	32.9	50.77	57.1	90	34.87^{ghefd}	47.63^{gefh}
13.	Wenadye	0	0	26.57	51.33	51.33	57.1	90	39.48^{cefd}	52.63^{cefd}
14.	Astara	0	0	13.29	38.67	50.77	76.72	90	38.49^{gcefd}	28.25^{kjl}
15.	Beresye	0	0	32.9	39.23	57.1	63.43	90	40.38^{cebd}	26.50^{kjl}
16.	Shebrat	0	13.29	26.57	25.69	57.1	63.43	90	39.44^{cefd}	56.75^{cebd}
17.	Teguaner	0	0	0	13.29	57.1	57.1	90	31.07^{ghefi}	40.75^{gih}
18.	Demolejat	0	0	32.9	39.23	57.1	76.72	76.72	40.38^{cebd}	30.88^{kijl}
19.	Nechwe	13.3	26.57	45	57.1	70.39	45.45	90	49.69^{cb}	41.63^{gifh}
20.	Kanchwe	0	0	32.9	45	63.43	76.72	90	44.01^{cbd}	30.25^{kijl}
21.	Yekeswe	0	0	19.62	45	76.72	90	90	45.91^{cbd}	37.13^{ijh}
22.	Bushrat	0	0	39.23	39.23	90	90	90	49.78^{cb}	40.63^{gih}
23.	Oret	0	13.29	32.9	50.77	90	90	90	52.42^{b}	50.38^{gefd}
24.	Lemat	26.6	39.23	50.77	63.43	90	90	90	64.29^{a}	21.25^{l}
25.	Yeregye	13.3	42.12	76.72	90	90	90	90	70.30^{a}	60.25^{cbd}
CV(%)									13.64	11.09
R^2									0.92	0.96

[a] days after inoculation; Means with different superscripts within the same column and class are statistically different at 5% level of significance according to DMRT.

Bacterial wilt pathogen (Xcm) isolate was collected from naturally BW infected enset fields of Hegere Selam, of Sidama zone. From collected fresh isolates, the Xcm suspensions were prepared in plant pathology laboratory, Hawassa ARC. The cells concentration of Xcm in suspension was adjusted to 10^8 cfu/ml. After transplanted a year old enset (after well established) plants were artificially inoculated with 5ml Xcm suspension at the bases of youngest leaf petioles using hypodermic syringe with needle. Maziya and Arkia enset clones previously known as resistance/tolerant and susceptible to wilt disease respectively were included as check. After artificial inoculation agronomic practices were also applied as per needed. The data on disease symptoms (yellow, wilt, bacterial ooze etc) appearance, plants reaction/ leaves, plant dead etc were observed and recorded periodically two weeks since artificial inoculation.

Table 20. Location and amount of enset clones collected from different locations

No.	**Location**	**Quantity**	**No.**	**Location**	**Quantity**	**Total**
1	Sidama	10	7	Silte	1	
2	Gedeo	2	8	Gurage	13	
3	Wolayita	14	9	Yem	7	
4	Hadiya	9	10	Sheka	9	**88**
5	Dawro	8	11	Jimma	4	
6	KT	10	12	M/Arsi	1	
S/total		**54**	**S/total**		**34**	

Among the collected enset clones and planted corms some (8) were not produced required amount of suckers at Areka ARC. Therefore, 80 enset clones were investigated at Hawassa ARC conditions. All the tested enset plants were showed bacterial wilt infection after 30 to 45 days of artificial inoculation. Later on a wide range of bacterial wilt disease infection was recorded on all enset clones with varying type of reactions. Hence, they were clustered in to three reaction types. Group one comprised 21.50% of enset clones which were exhibited relatively resistance/tolerant reactions, and group two has 56.25% of the clones with susceptible reactions to the pathogen. Group three consisted of 22.50% of enset clones which was showed moderately tolerant/ susceptible to the disease (Table 21).

Bacterial wilt disease symptoms did not successfully progress in enset clones which were exhibited relatively resistance/tolerant reactions to the pathogen. On the relatively resistance/tolerant enset clones first wilting/yellowing symptoms were recorded on the inoculated leaves.

Table 21 Enset clone reaction to bacterial wilt under artificial inoculation condition (Hawassa), 2012/13.

No.	Reaction Type	Average Disease Index (%)	enset clone %
1.	Resistance/Tolerant	10-20	21.25
2.	MTMS	21-60	22.50
3.	Susceptible	>60	56.25

MR = moderately tolerant, MS = moderately susceptible

The enset clones which were showed BW symptoms at initial stages, observed to recover/revive from infection and became healthy at the end of disease assessments (after some period of time) (Table 22) while on the most of the enset clones that progressed symptoms ultimately died. In this regard, Quimio and Tessera (1996) also observed that 'Genticha' enset clone was recovered from BW infection at 12 to 16 weeks after artificially inoculated

Generally no one completely free from the BW disease symptoms yet among the all tested enset clones (80) in Hawassa field conditions using Hagere Selam Xcm isolate. This is in accordance with Ashagari (1985) report any suggested that complete resistance enset clone to BW. Enset clones with low disease index (0-20%) were considered as a resistance/tolerant to the pathogen (Table 22). The enset clones with high disease index (60-100%) were found highly susceptible to BW pathogen. There were also some enset clones (Table 22 & 23) observed as partially tolerant/susceptible to the pathogen recorded moderate disease index (25.-58%).

Table 22. Enset clone with moderately susceptible (MS) reaction to bacterial wilt

No.	Clone name	Average BW infection (%)				
		1*	2	3	4	5
1	Yamarat	5.00	38.75	50.00	66.25	53.33
2	Siskela	0	12.50	15.00	25.00	58.33
3	Toracho	0	13.75	17.50	20.00	55.00
4	Ashikit	0	20.00	32.50	41.25	55.83
5	A/merza	0	12.50	12.50	15.00	50.00
6	Bozeria	2.50	22.50	23.75	26.25	52.50
7	Lobo	2.50	22.50	26.25	28.75	50.83
8	Addo	5.00	17.50	26.25	28.75	56.66
9	Ganticho	2.50	51.25	57.50	56.25	57.33

1* Month after artificial inoculation

Table 23. Enset clones with moderately tolerant (MT) reactions to bacterial wilt

No.	Clone name	Average BW infection (%)				
		1*	2	3	4	5

1	Bededet	0	46.25	51.25	66.25	35.00
2	Ayasse	0	22.50	26.25	27.50	33.33
3	Gemo	0	21.25	22.50	26.25	25.00
4	Aniya	0	11.25	13.75	13.75	33.33
5	Sorphe	5.00	37.50	47.50	67.50	27.00
6	Wolanchoa	2.50	18.75	26.25	31.25	38.33
7	Beradi	2.50	23.75	26.25	26.25	37,00
8	Wogu	2.50	11.25	11.25	10.00	39.16
9	Kuuro	5.00	23.75	29.00	30.00	30.00

1* Month after artificial inoculation

The source of these enset clones are local farmers. Contributions of local farmers towards the maintenance of enset genetic resources were also appreciable. The recent statistical data of Areka Agricultural Research Centre indicate that there are over 600 different enset vernaculars which were collected from different areas and maintained at an experimental station, Areka (Anonymous, 2009). Farmers in enset farming communities grow and maintain diversified of enset clones in the same field/homestead. Growing the different enset clones in the same field is a direct reflection of the number of different usage. Clonal diversity may secure the stable food in times of unfavorable environmental conditions include provide wide range of resistance/tolerant to the BW disease. Accordingly an ordinary household keeps 200 to 300 enset plants in his homestead which comprised of fife to ten types of clones.

To notice the enset clones reactions to the pathogen correlation among the farmers' field conditions and experimental field situations, the assessment of farmer's comments/perceptions was made in different of these enset clones collected areas. In general the results of farmers opinion assessment on these enset clones reactions is also supportive of results of this study (Table 24)

Several studies have also described that in the enset in the enset farming communities there are several enset clones resistance/tolerant to the BW disease. Fikre and Gizachew (2007) were suggested that Mazia enset clone is resistant/tolerant to Xcm pathogen while Arkia enset clone was highly (100%) susceptible. Similarly a number of enset clones include Buacho, Wonigoro, Bazeriet, Dere, Anikefye, Eminiye, Lemat Nechwe (1) Abate, Arkya, Mezya Ado, Disho, Genticha, Hala, Kembate and Mezya were resistant/tolerant to the pathogen (Gizachew et al 2008).

Table 24**.** Enset clone that showed tolerant reaction during on station evaluation and farmers perception on their response against EXW.

No.	**Clone name**	**Average BW infection (%)**					**Farmers' perception (N=40)**	
		1*	**2**	**3**	**4**	**5**	**Tolerant (%)**	**Intermediate (%)**

1	Lemat	0	10	11	10.5	20	0	33.33
2	Nechuwe	0	10	10	10	0	25	50
3	Unjeme	0	17.5	17.5	18.75	0	85	0
4	Tikur enset	2.5	31.3	32.5	33.75	0	50	30
5	He'lla	0	10	12.5	13.75	5	36.5	30.7
6	Dirbo	0	20	32.5	35	4	59.4	15.62
7	Wachiso	0	12.5	13.8	15	0	20	80
8	Hae'la	2.5	12.5	17.5	22.5	3.33	53.5	40
9	Falakia	2.5	10	13.8	15	12.5	22.2	77.8
10	Gefetano	0	10	10	15	17.5	34.2	40.8
11	Godere	0	10	12.5	13.75	0	50	22.5
12	Amiya	0	10	10	12.5	17.5	10	90
13	Yesha	0	10	10	10	3.33	88.9	11.1
14	Alagena	0	10	12.5	11.25	0	29.6	33.3
15	Gisiro	0	10	12.5	15	0	60	25

The results of study reveal that there are several enset clones with resistant/tolerant reactions to EBW in the country, although no one free of the BW symptoms among the evaluated in the course of studies. Enset clones that were showed disease symptoms at initial period of incubations after artificial inoculations were recovered from infection and became healthy at the final disease assessments, although the exact mechanisms is not immediately known. The enset clones with low disease index were considered as resistant/tolerant to bacterial wilt disease and can be used as a suitable component in the integrated enset bacterial wilt disease management.

In the second set, suckers of a total of 150 enset clones were planted under field condition at Hawassa ARC to evaluate their reaction to enset bacterial wilt using artificial inoculation. Disease assessment was started at 15 days after inoculation and continued within 15 days interval. The number of infected plants per clone at each disease assessment period has been recorded. All inoculated enset clones was not showed disease symptoms during the first 30 days after inoculation. However, accessions developed disease symptoms at various intensity levels 30 days after inoculation. Further replicated trials with 484 enset clones collected from south and south western Ethiopia will be conducted. The screening for wilt resistance will be conducted at screen house and experimental fields of Awassa Agricultural Research Center, southern Ethiopia.

Evaluation of botanical seed for resistant/tolerant to Xanthomonas wilt: Four hundred botanical seeds were collected from 'Mazia' and wild enset clones. The seeds were sown in pots at Hawassa ARC and seedlings have been raised. Enset seedlings raised from botanical seeds were artificially inoculated with virulent strain of enset wilt bacteria by September 2013. All the seedlings show the disease symptom after one month from inoculation (Figure 11). The plants are in the field and data collection continued for the coming two months.

Clonal strains found to tolerate/resist the disease shall be reproduced and advanced for further research and development uses. The experiment with more number of seeds with more clones will be repeated this year.

Figure 11. Enset seedlings from botanical seeds for EXW evaluation at Hawassa

Floral biology, phenology and breeding system, and pollination ecology of enset accessions from Ethiopia: For effective enset breeding, there is a need to understand the crop's reproductive biology as well as the breeding procedures. One hundred twenty plants in the Areka maintenance field have been tagged. The morphology of the separate floral parts assessment started and data collection continued (Figure 12). This activity is an on-going as such the remaining plants shall be used for studying the remaining flower morphology, phenology and breeding system studies. Crossing for effective breeding system identification shall be made when the desired plants have come to flowering. Field management and data collection will be continued.

Variety development for wilt resistance/tolerance and other desirable traits: Crossing block made of two parents Mazia and Halla were established at Areka Agricultural Research Center experimental field (Figure 13). Mazia is resistant /tolerant to Xcm with less culinary quality and Halla is moderately tolerant to Xcm with higher quality of kocho. Mazia used as male and female parent alternatively.

Figure 12. Enset maintenance field for floral biology, phenology and breeding system study

Plants will be tagged and hand pollinated. The crossing technique from banana will be adapted for emasculation and collecting viable pollen. F1 will be evaluated for tolerant/resistant to bacterial wilt, culinary quality and other desirable traits. Hybrids that combine resistance/tolerance to EXW with high quality yield will be developed.

Figure 13. Established crossing block (Mazia and Halla) at Areka

Demonstration and dissemination of integrated bacterial wilt control measures through collective action

Training on integrated EXW management and enset production technologies: Training on the management of enset XW was provided to 95 extension agents (10 female and 85 male) drawn from six zones and 25 woredas (districts) of the SNNP and Oromia Regional States. Leaflets (3,000), posters (1,300) and manuals (500) describing improved enset production and on EBW management options were prepared and distributed to trainees.

Multiplication of planting materials: Recently released six varieties and recommended disease tolerant clone Mazia, were multiplied at Areka Agricultural Research Centre. Last year about 2000 suckers were distributed to Gedeo zone bench mark site. This year near to three hectare of land has been covered by the six varieties and Mazia. In 2014 the multiplied suckers will be ready for distribution.

Setting up benchmark sites for piloting collective action: In Gedeo Zone, Hallo Hartume kebele[1] of the Gedeb Woreda was selected as a benchmark site for demonstration and dissemination of enset bacterial wilt control measures. Hallo Hartume has a population of 1150 households. Data on incidence of enset bacterial wilt in the entire kebele were recorded before the implementation of collective action for eradication. Focused group discussion with key informant farmers were made to gain insights on farmers' perception about modes of enset bacterial wilt transmission, causal agent of the disease, and their indigenous knowledge to control the disease.

Important actors for integrated EBW management at the levels of Hallo Hartume Kebele and Gedeb woreda were identified. At kebele level, there were 22 farmer development team leaders, 4 recognized lead farmers, 8 kebele administrators, 4 community based organization leaders/elders, 3 development agents, 3 women affairs leaders, a school director and 5 youth association leaders that can be brought onboard for mass mobilization.

At Gedeb woreda level, there are 5 administrative leaders, 4 woreda agricultural experts and an EBW focal person/expert that may be co-opted for mass mobilization activities. After discussion with partners, woreda and Kebele level EBW eradication task forces were formed. The selected actors who were about 60 received training on sustainable integrated enset bacterial wilt control measures. The task forces took the responsibility to lead and mobilize farmer communities for the control of bacterial wilt at the Hallo Hartume Kebele. The actors set up by-laws that govern members of the communities towards the management of enset bacterial wilt. Monitoring and evaluation of what works and what not in mass mobilization will be carried out to document and scale up best practices.

Appendix B – Publications Summary & Training and Outreach Summary.

Training and Outreach Summary

I. Long term training

Two junior researchers from Areka Agricultural Research Center have joined Hawassa University and Haramaya University for studies leading to MSc degrees. One of them would be focusing on enset breeding/pathology and the other on enset tissue culture. An enset breeder from the same research center has registered for PhD degree in plant breeding at Addis Ababa University through a supplemental grant from the McKnight Foundation. One Msc student has comleted his study from Hawassa University.

II. Short term Training

A. Training and experience sharing on banana floral biology, breeding methodologies and integrated disease management

Enset is an orphan commodity and less investigated although it has great local importance. Consequently, information on its improvement is very scarce. Collaborative effort of local and international scientists who work on related crops such as banana and plantains is of paramount importance to develop enset that is resistant to bacterial wilt having desirable traits. Nowadays promising results have been registered in different types of disease resistance from various banana and plantain-breeding programs through crossbreeding. The Uganda banana and plantain breeding programs can be mentioned. The enset researcher (Breeder) have been visit Kawanda Agricultural Research Center with the aim of to train and share the experience of banana floral biology, breeding system methodologies, integrated disease management activities and to adopt them for enset breeding program (Figure 14).

The entire visit gave us much exposure on how the banana works conducted on both institutions and we gained the necessary information we required for the enset work going on. And from this we will introduce new activities that were not included before and/or revised our activities in which their methodologies were not clearly stated. Moreover, such kinds of visit improve the capacity and fill the gap of researchers they engage their full time for enset research program and help them to be competent in different disciplines.

Figure 14. Experience sharing visit at Uganda (Kawanda Agricultural Research Center (A, B &C) and Bioversity International (D))

B. Tissue culture

Significant progress has been made in successfully running the tissue culture laboratory at Areka Agricultural Research Center. Regarding enset, callus initiation for two enset varieties are in progress. To facilitate protocol optimization for enset, a researcher skilled in tissue culturing enset was brought inform Holetta Agricultural Research Center to train research and laboratory staff at Areka Agricultural Research Center.

Appendix C. Theory of change for the Project "11-283: Enset bacterial wilt"

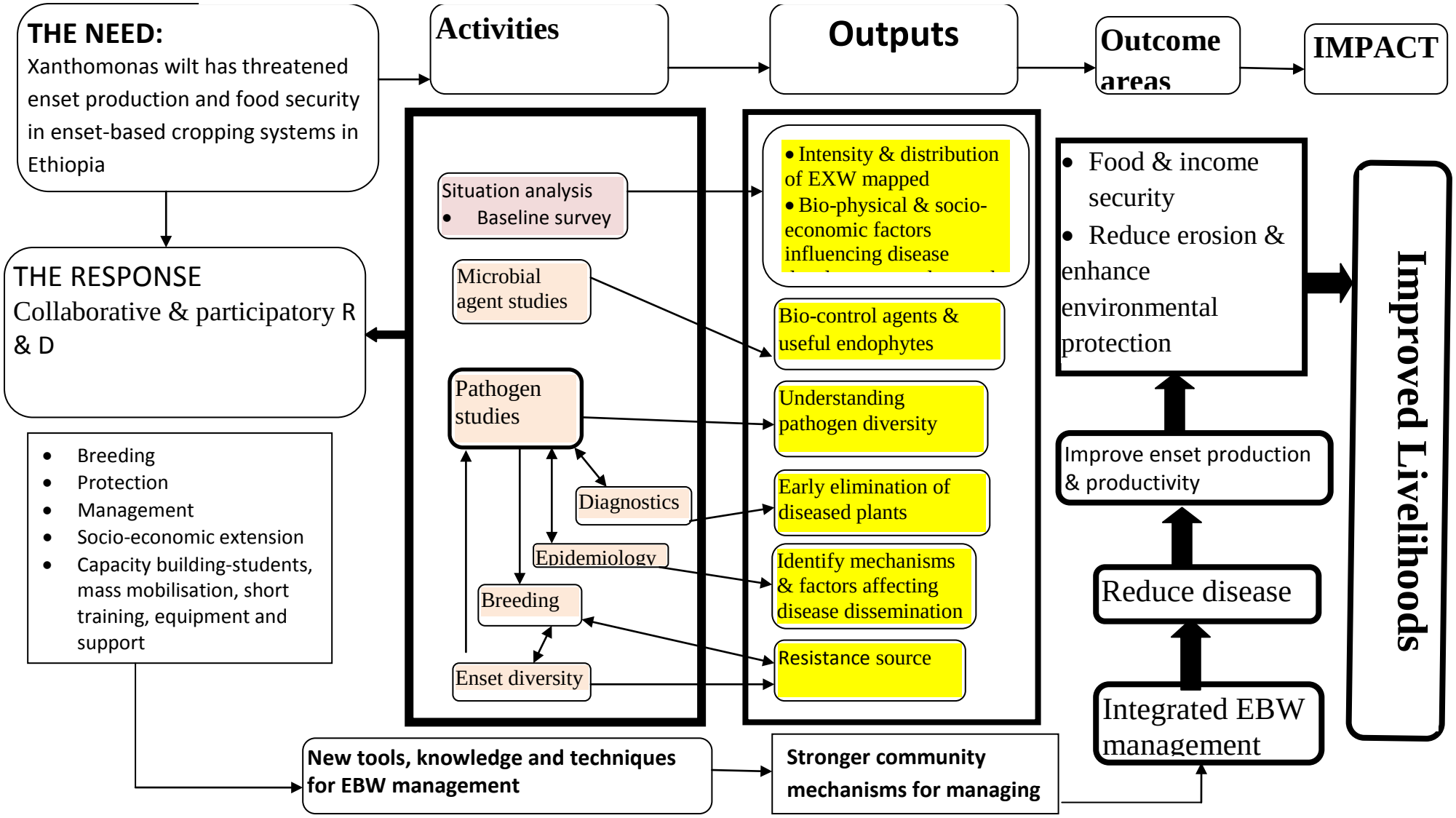


Table 1. Monitoring and evaluation matrix of integrated management of Xanthomonas wilt caused by Xcm in Ethiopia

Narrative summary	Monitoring & evaluation objective	Indicators	Information to be collected	Methods of collecting information	Tools for collecting information	Executing bodies
Output:						
Status (distribution, prevalence and intensity) of enset bacterial wilt in different agro-ecological zones of the country and existing farmers' perception and their indigenous coping mechanisms to manage the disease Biophysical and socio-economic information on enset-based farming system made available Gender role in enset cultivation, processing & utilization understood Literature reviewed and documented	To assess whether the objectives of the project are addressed and research questions answered according to the physical and financial plan	GIS based survey conducted Two peer reviewed articles published	Whether the output is in accordance with the objective and methodology of the research project	Close supervision of the research team at the time of surveying Post survey assessment of collected and compiled data	Communication with the surveying team Reports at different levels	PL, AC, RT, LS
Genetic diversity among enset germplasm collected from different enset growing areas of the country determined Clones and/or hybrids having merits of bacterial wilt resistant/tolerant and other desirable characters identified Parents selected and crosses made	To assess whether the objectives of the project are addressed and research questions answered according to the physical and financial plan	One PhD & M.Sc. Thesis and four peer-reviewed journal articles Three pairs of parental crosses	Whether the output is in accordance with the objective and methodology of the research project	Close supervision with a PhD and MSc student Timely submission of thesis Comments of internal and external examiners Field visit and monitoring and evaluation of the experiment at the start and final stage of the project life time.	Communication with students and other involved institutes Reports at different levels	PL, RT, LS, university advisors and researchers
Influence of the biophysical environment, and crop husbandry practices on disease development and spread elucidated	To assess whether the objectives related to the epidemiology of the pathogen as per the the physical and financial plan	Two peer-reviewed journal articles	Whether the output is in accordance with the objective and methodology of the research project	Field visit and monitoring and evaluation of the experiment at the start and final stage of the project life time.	Reports at different levels	PL, RT, LS, researchers

Genetic diversity among strains of the pathogen collected from different enset growing areas of the country determined Microorganism(s) having a role as a biological control agent against Xanthomonas wilt identified Early detection tools evaluated and adopted Enset bacterial wilt free planting materials from infected enset plant produced in vitro and finally pathogen availability using different diagnostic techniques tested.	To assess whether the objectives of the project are addressed and research questions answered according to the physical and financial plan	One M.Sc. Thesis and three peer-reviewed journal articles	Whether the output is in accordance with the objective and methodology of the research project	Close supervision with MSc students Timely submission of thesis and dissertation Comments of internal and external examiners Field visit and monitoring and evaluation of the experiment at the start and final stage of the project life time.	Communication with students and other involved institutes Reports at different levels	PL, RT, LS, university advisors and researchers
Integrated disease management strategies demonstrated & disseminated Effectiveness of collective action for EBW management documented and workable approaches recommended Effective integrated EBW control approaches scaled up	To assess whether the objectives of the theme are addressed and research questions answered according to the physical and financial plan	Distributed posters, leaflets, manuals, number of trainings given, mass media coverage Established task force & by-laws Started campaigns. One peer-reviewed journal article	Change in the level of Xanthomonas wilt incidence and severity in those targeted areas	Field visit and impact assessment and Field visit and monitoring and evaluation of the experiment at the start and final stage of the project life time.	Communication with TF, community members, RK, and other involved institutes Reports at different levels	PL, RT, LS, and researchers

PL - Project Leader; AC = Advisory Committee; RT = Regional Team; LS = Liaison Scientist

Table 1. Monitoring and evaluation matrix (continued)

Narrative summary	Monitoring & evaluation objective	Indicators	Information to be collected	Methods of collecting information	Tools for collecting information	Executing bodies
Activity:						
Baseline survey and disease mapping Types of resistance for Xanthomonas in other systems EXW and BXW epidemiology and management Banana breeding Reproductive biology? Resistance screening methods Survey of XW Enset-based farming system characterization farmers clonal diversity • crop husbandry practices • gender roles Post- harvest handling? Marketing role in livelihoods?	• To assess the progress of each of activities in the project whether conducted as per the physical and financial plan To take early corrective measures to anomalies	• Activities not conducted within the planned period • Activities conducted on time but not using the planned methodology	The progress/ milestone of the activities in accordance with the physical and financial plan	Field visit, review meeting, and/or quarterly reports from the partner organizations	The objective and work plan of each of the activities in the original submitted project	PL, RT and LS of the McKnight foundation, co-investigators of the partner organizations and other higher executives
Germplasm Collection, Characterization and Variety development and selection for wilt resistance/tolerance and other desirable traits	To assess the progress of each of activities in the project whether conducted as per the physical and financial plan To take early corrective measures to	Activities not conducted within the planned period Activities conducted	The progress/ milestone of the activities in accordance with the	Field visit, review meeting, and/or quarterly reports from the partner	The objective and work plan of each of the activities in the original submitted project	PL, RT and LS of the McKnight foundation, co-investigators of the partner

❖ Collection of enset clones In country ❖ Characterization of enset clones On station morpho-agronomic and quality characterization of enset clones ❖ Molecular characterization of enset clones ❖ Screening for wilt resistance ❖ Enset breeding and selection for wilt resistance/tolerance and other desirable traits ❖ Resistance screening ❖ Farmer participatory on farm testing of interesting clones	anomalies	on time but not using the planned methodology	physical and financial plan	organizations		organizations and other higher executives
Disease epidemiology ❖ G × E interaction ❖Insect vector and nematode transmission	To assess the progress of each of activities in the project whether conducted as per the physical and financial plan To take early corrective measures to anomalies	Activities not conducted within the planned period Activities conducted on time but not using the planned methodology	The progress/ milestone of the activities in accordance with the physical and financial plan	Field visit, review meeting, and/or quarterly reports from the partner organizations	The objective and work plan of each of the activities in the original submitted project	PL, RT and LS of the McKnight foundation, co-investigators of the partner organizations and other higher executive
Pathogen characterization, early detection, and bio-control strategies ❖ Pathogen characterization ❖ virulence spectrum of pathogen strains ❖ Biological control of enset	To assess the progress of each of activities in the project whether conducted as per the physical and financial plan To take early corrective measures to anomalies	Activities not conducted within the planned period Activities conducted on time but not using the planned	The progress/ milestone of the activities in accordance with the physical and financial plan	Field visit, review meeting, and/or quarterly reports from the partner organizations	The objective and work plan of each of the activities in the original submitted project	PL, RT and LS of the McKnight foundation, co-investigators of the partner organizations and other higher

bacterial wilt disease by microbial antagonists • Biocontrol agents • Actinomycets? ❖ Evaluation of early detection tools • LFD ❖ In Vitro regeneration		methodology				executives
Scaling out (seedling multiplication, training, mass mobilization and collective action) ❖ Demonstration and dissemination of integrated bacterial wilt control measures through collective action • Development of integrated disease management tools • Mass mobilization, demonstration and dissemination • Evaluation & documentation of collective action approaches for EBW management • Networking and capacity building Information and communication technology (ICT) based early warning and information exchange for bacterial wilt disease	To assess the progress of each of activities in the project whether conducted as per the physical and financial plan To take early corrective measures to anomalies	Activities not conducted within the planned period Activities conducted on time but not using the planned methodology	The progress/ milestone of the activities in accordance with the physical and financial plan	Field visit, review meeting, and/or quarterly reports from the partner organizations	The objective and work plan of each of the activities in the original submitted project	PL, RT and LS of the McKnight foundation, co-investigators of the partner organizations and other higher executives

management						
Input:						
Budget	To assess if appropriate and timely budget is allocated to each partner organizations	Received budget by each project partners on time	Amount and time of budget released	Communication with the partner organization	Soft and hard communication methods	Funding organization and investigators from each partner organizations
Personnel	To assess if appropriate and qualified personnel is available in each partner organizations	Available qualified personnel at each partner organizations	Number and quality of personnel	communication with partner organizations	Visiting and Soft and hard communication methods	PL, Regional representative and laison scientist from McKnight foundation
Facility	To assess if functional facilities are available in each partner organizations	Available facilities for each of the partner organizations (functional laboratory, vehicle, and other relevant inputs)	Status of available facilities	communication with partner organizations	Visiting and Soft and hard communication methods	PL, Regional representative and laison scientist from McKnight foundation

PL - Project Leader; AC = Advisory Committee; RT = Regional Team; LS = Liaison Scientist

Appendix E – Research Questions and Protocols

Research questions

1. What is the extent of the occurrence and distribution of EXW in unaddressed enset growing areas of Ethiopia in the inception phase? What is the magnitude of the menace of EXW on food security and livelihood of farmers in enset based farming systems?
2. Can we get enset bacterial wilt disease resistant/tolerant clones that combine higher yield and acceptable culinary quality? How can we make variety development through hybridization possible for enset?
3. How is the epidemiology of enset bacterial wilt influenced by host genotype, pathogen characteristics, prevailing environmental conditions, biotic factors (insect, nematodes, mole rat, etc) and human activities including farming practices (e.g traditional enset processing tools)?
4. Can we get effective potential bio-agents against Xcm at field condition? What are the most effective tools for early detection and management of EXW?
5. What existing and novel community organizational and administrative structures could be deployed and enset bacterial wilt alert (early warning and action) system put in place to enhance community action and control the disease nationwide?

Protocols

Activity 1. Baseline survey and disease mapping:

Detailed baseline survey was carried out in seven districts each of Wolayta, Gedeo, Kembata, Gurage, Silte, Dawro and Hadiya. Data collected on the host-pathogen including number of enset clones and names of the clones, purpose each clone is grown, the reaction of each clone to the pathogen, farmer perceptions of causes and modes of disease transmission, disease management options, and farmers indigenous knowledge on symptom identification.

Preliminary result revealed that about 65, 59, 44, and 23 clones in Kembata, Hadiya, Gurage, Wolayta and Gedeo respectively were identified and found to be conserved in farmer's fields. Farmers were able to rate clones differently for kocho, bulla and fiber productivity; medicinal values; and for clonal reaction to bacterial wilt disease. In addition, preliminary reports indicated that farmers in Gedeo, Wolaita, Kembata and Hadiya area identified 13 enset clones (Abatmerza, Agina, Alenticho, Bedadia, Bota-meziya, Buzzare, Dirbo, Hawe, Jegeda, Kekere, Kucharkia, Mariya, Mesmesa) as tolerant to enset bacterial wilt. Farmers in this respective location claimed that the kocho yield of these disease tolerant clones was generally low when compared with other enset clones. Moreover, farmers also listed 14 other enset clones (Adinona Aeluwa Argema Astara Bedadia Chamia Gishera Guarye Hargamo Jegeda Kekere Ored Senkutie Tuzuma) that have been used for medicinal purposes even if they have got lower kocho yield.

Disease prevalence has shown variation with different levels. About 75.5 % of the sample respondents' in Gedeo and 21.3 % in Wolayta reported bacterial wilt infection in their enset field. 71% of the sample respondents in Wolayta and 57% in Gedeo reported a declining trend in enset production over the past fifteen years due to bacterial wilt damage followed by wild animals. According to the survey preliminary result, the EXW disease distribution in each zone revealed that Gedeo was the most EXW infected zones (80.4%) followed by Dawro (61.1%), Hadiya (40%), Wolayta (21.2%), and Silte (15%). Because of the biophysical and socioeconomic diversity in enset farming system in the country, there is a need to collect and document baseline from the remaining major enset growing areas.

The survey shall cover South Omo, Sheka, and Bench-Maji zones in SNNPR and Guji, and Borena Zones in Oromia Region using the survey tools developed in the inception phase. In each zone, two major Enset growing woredas shall be purposively selected. In each woreda, one representative Enset growing highland (altitude 2400 – 2800 m.a.s.l.) and one intermediate altitude (1500 – 2400 m.a.s.l.) kebeles (the lowest administrative unit in Ethiopia) with sever EXW problem and enset diversity shall be identified. Ten enset growing households shall be randomly selected from each kebele, interviewed and additional

biophysical (altitude, longitude and latitude) data will be collected on their enset plantation /farms. Almanac Characterization (ACT) (Corbet et al., 2001) and Arc View (ESRI, 1992). Geographical Information System (GIS) software's will be used for mapping the prevalence and distribution of Enset bacterial wilt and the areas surveyed. Data will be analyzed with descriptive statistics using appropriate software. The survey will provide information on disease distribution, prevalence, and severity of EXW, enset clonal diversity, indigenous knowledge on enset and EXW management.

Activity 2. Germplasm Collection, Characterization, and Variety development for bacterial wilt resistance/tolerance and other desirable traits:

Four hundered fifty six accessions were planted for caharactorization in the inception phase at Areka using augmented design. Moreover, 100 randomly selected enset clones were planted at three locations (Areka, Angacha and Hawassa) using 10 x10 simple lattice design to evaluate their variability for kocho, bulla, starch, fiber and culinary quality. In the inception phase of this project, GIS based enset clones collections were made from Gurage, Kembata and Wolayta zones. 65, 42, and 30 enset clones were collected from Kembata, Gurage and Wolayta zones and were planted at Areka and Fereze field genebank for further evaluation. Clonal distribution map development is underway.

Evaluation of enset accessions against EXW was started in the inception phase. Two sets of trials are on-going with promising results. In one trial, suckers of about 88 enset clones collected from some major enset growing areas of the southern region were raised at Areka Agricultural Research Centre. One year old suckers of 80 clones (8 clones did not produce enough suckers) were transplanted at the experimental field of Hawassa Agricultural Research Centre for disease inoculation and subsequent evaluation. About 27 clones with a better reaction to EBW were retained for further evaluation under Hawassa condition.

Out of the 80 clones tested, about 26% showed high resistance/tolerance reaction with an average disease index of 10-20%. Twenty nine percent of the clones showed moderate tolerance/resistance reaction (average disease index of 30-40%) and 45% of the clones were susceptible with an average disease index of >50%. In the second set, suckers of a total of 150 enset clones were planted under field condition at Hawassa ARC to evaluate their reaction to enset bacterial wilt using artificial inoculation. The clones are in the field and shall be inoculated as soon as the plants have recovered from dry season stress with the advent of the main season rain.

Though efforts have been made to collect, characterise and evaluate enset clones from the some enset growing areas, there are areas which were not covered and clones not collected. Therefore, this activity aims to collect cultivated and wild enset clones from unaddressed areas and clones, characterize and evaluate for different uses. This activity will include collection and characterization of enset clones for future use, morpho-agronomic, culinary

quality and other use value based and molecular characterization, evaluation of newly collected accessions against EXW, variety development for wilt resistance/tolerance and other desirable traits, and participatory variety selection.

2.1 Collection and Conservation of Enset clones for future use : Attempts have been made by Areka Agricultural Research Center to collect and maintain the available enset accessions in the country. Currently, a total of 508 enset accessions previosly collected from 12 major enset growing zones of Ethiopia have been maintained at two field gene banks. However, preliminary information indicated that there are still more uncollected clones in those areas. Moreover, there are enset growing areas which were not considered in the previous collection missions. It is also difficult to map previous collection since they lack GIS references. Therefore, GIS based enset clones collection and mapping will be carried out in areas of the country where collections had been attempted but not fully exhausted and in new areas to assess the diversity and distribution of accessions in the country. Passport data including indigenous knowledge about the clones, altitude, latitude, longitude, soil type and etc will be collected. Characterization, evaluation and clustering of accessions will be carried out in designed layouts.

This sub activity will produce accessions with resistance/ tolerance to EXW and better yield will be identified for further evaluation and genetic diversity will be conserved.

2.2 Morpho-Agronomic, culinary quality and other use-value based and molecular characterization: This activity is a continuation of the inception phase. Existing and newly collected accessions will be characterized and evaluated. Genetic diversity will also be assessed using banana SSR markers. Eleven SSR loci from banana which are found to be transferable to enset and are polymorphic will be used for this study. Diversity analyses indices, analysis of variance, and multivariate techniques will be employed to analyze the data. This activity will yield knowledge on enset genetic diversity and increase the efficiency of improvement program for different traits.

2.3 Evaluation of enset accessions against EXW: This activity will evaluate the wilt resistance/tolerance of collected clones from in country. In addition, one resistant enset clone 'Maziya' and susceptible clone 'Arkya' will be included as a check. The screening for wilt resistance will be conducted at screen house and experimental fields of Awassa Agricultural Research Center. For the screen house experiment, uniformly grown enset suckers will be transplanted in plastic pots (22x22cm) having sand, compost and sterile soil (1:2:1ratio). Completely randomized design with three replications will be used. Each year 200 clones will be evaluated in screen house. Moreover, 484 accessions will be evaluated under field condition Awassa using simple lattice design. For the two experiments, plants will be inoculated three months after planting with 3 ml of a 2- day's old bacterial suspension at a cell concentration of 10^8 cfu/ ml of the most virulent isolates (Hagereselam isolate). Data collection will start fifteen days after inoculation and data recording will continue at seven

days interval for two months. Days to symptom appearance, disease severity and incidence will be recorded. This activity will help us to identify enset accessions that are resistance/tolerance to EXW.

2.4 Variety development for wilt resistance/tolerance and other desirable traits: In this study, investigation on enset inflorescence and development of hybrids that are resistant /tolerant to EXW with higher yield and quality through cross breeding will be continued. Two parents Mazia and Messena were selected. Mazia is resistant /tolerant to Xcm with less culinary quality and Mesena is moderately tolerant to Xcm with higher quality of kocho. The objective of crossing is to improve the culinary quality of Mazia and tolerance level of Mesena to Xcm. Reciprocal crossing will be implemented to achieve this objective. Crossing block was established at Areka Agricultural Research Center experimental field. Plants will be tagged and hand pollinated. The crossing technique from banana will be adapted for emasculation and collecting viable pollen. F1 will be evaluated for tolerant/resistant to bacterial wilt, culinary quality and other desirable traits. Hybrids that combine resistance/tolerance to EXW with high quality yield will be developed.

2.5 Disease reaction, biochemical and metabolic profiling studies on enset genotypes inoculated with bacterial wilt disease (Xanthomonas campestris pv.musacearum)

Selection of relatively tolerant genotypes reported by farmers as one of the other criteria for EXW management decisions (Anita et al., 1996). A number of enset genotypes in major enset growing areas have been reported to have relatively high tolerance (Dereje, 1985; Anita et al., 1996; and Hawassa ARC progress report, 2000) and also variable levels of clonal response (Ashagari, 1985; Archaido and Mesfin, 1993; Anita et al., 1996; Welde Michael, 2000; Welde Michael et al., 2008) against Xcm disease have been reported. Recently, differential response of enset clones against Xcm infection have been observed (Hawassa ARC McKnight EXW project progress report, 2013); showing relatively highly tolerant/resistant, moderately tolerant/resistant and susceptible reaction towards Xcm infection. Screening and development of resistant enset varieties is the most appropriate and realistic approaches for managing EXW. Screening for resistance also requires a reliable screening method to unambiguously discriminate resistant and susceptible genotypes. Recognizing this scenario, this work initiated to scrutinize the differential reaction of enset genotypes to Xcm inoculation with their biochemical, and metabolic changes occurring in response Xcm disease.

In vitro regenerated 48 selected enset genotypes for their differential reaction to EXW disease ranging from relatively high tolerant to susceptible will be inoculated with virulent Xcm pathogen and distilled water for controls arranged on pots using RCBD with three replications. Six in vitro generated suckers of a clone will considered as a plot. Disease scoring and leaf sampling for biochemical study and metabolic profiling will be started 10 days after planting with 7 days interval for one month and 15 days interval for three months (Welde Michael, 2000). Biochemical analysis for total protein, peroxidase activity, free

phenolic compounds, total amino acids, proline, and salicylic acid content will be determined using appropriate procedure for each of the biochemical. Metabolic profiling will conducted using appropriate chromatographic techniques available in the county.

2.6 Participatory Varietal Evaluation of Enset clones: Based on farmer's information from the Geta, Angacha and Boloso sore woredas 6 known for their kocho yield and widely distributed clones (Lemat, Nechewe, Siskela, Unjeme, Halla and Ankogena,) and 2 standard check (Yambule, Zereta) will be planted on-station and at three FTC of each woreda using RCBD design with three replications. Combination of three clones will be planted at 56 randomly selected farmers' field of each woreda. Data will be collected on the morpho agronomic characteristics and farmers' preference will be collected. Farmers' variety selection criteria will be identified and used for future improvement and varieties will be selected for wider distribution.

Activity 3. Disease Epidemiology: On-farm activities and farmer participation shall be encouraged. Farmers' traditional disease management approaches such as the use of botanicals shall be tested alongside research available best practices. Role of vectors such as insects and nematodes in mediating EXW disease epidemics as affected by various crop management practices shall be studied and documented and proven disease/pest mitigation strategies promoted. Work on survival of the pathogen in fresh kocho shall continue one more season.

3.1 Genotype × Environment interaction: in the inception phase, eleven enset clones differing with their reaction to enset XW, moderately tolerant (Gewada, Endale, Zerieta, Kellisa, Messena), susceptible (Gulumo and Arkiea), resistant /tolerant (Yanbule, Mazia and Halla) and one local variety from each location were included and planted using completely randomized block design with four replications at three locations (Hawassa, Hosanna (Bobicho), and Hegereselam (Bongodo FTC)). Artificial inoculation with Hagereselam virulent isolate (HSI) of Xcm suspension with a cell concentration of 10^8cfd/ml will be done after three months of transplanting. The trial will be repeated at all locations. Data will be collected on disease incidence, incubation period, soil type and actual weather conditions during the experimental periods. Data will be subjected to statistical analysis using the SAS software. Genotype, environment and pathogen interaction will be identified.

3.3 Insect vector and nematode transmission: Insect and nematodes transmission of enset bacterial wilt will be studied under controlled environment in the greenhouse at SARI. For this purpose, insects and nematodes will be collected and isolated from healthy and diseased enset plants and pathogen isolation will be made by plating surface sterilized insects on semi selective media. Transmission efficiency of each insect from which the pathogen is isolated will be determined in cage experiments. Possible vectors will be identified and results will be further evaluated under field and greenhouse conditions. This will be done in collaboration with Ambo crop protection research center and Addis Ababa University.

Activity 4. Pathogen characterization, early detection, and bio-control strategies: For the Xcm virulence spectrum studies, four cultivated enset clones reported to differ in their susceptibility to the pathogen and a wild clone have been planted in pots. Suckers of each clone are now two months old (since they are transplanted in pots). Pathogens were collected from enset samples of different parts of the country have been isolated. Artificial inoculation of enset clone with Xcm is on-going. Pathogen virulence spectrum studies that have been started shall be strengthened. Molecular characterization of the pathogen to determine diversity shall be carried out. Early detection tools such as LFD that were developed elsewhere shall be adopted, tested and extended to farmers for disease tracking and control. Bio-control strategies for EXW shall be tested and effective ones identified.

4.1 Sample collection, isolation and preservation of the pathogen: Samples will be collected from enset plants with wilt symptoms from visited enset fields mentioned under activity 1. A maximum of 400 samples will be collected. In instances where disease occurrence tends to be rare in the sample farms, attempts shall be made to collect disease samples from other non-sample farms in the same kebele. Isolation of the wilt pathogen will be made on the semi selective media Cellubiose – Cephalexin Agar (CCA) at 25°C.

4.2 Molecular characterization: A maximum of 400 bacterial strains derived from single colonies will be preserved for molecular characterization, virulence spectrum studies, and future use. AFLP analysis will be conducted to study the genetic relationship/diversity among the 280 bacterial strains collected from different locations. Reference strains will be included in the study for comparison purposes. Phylogenetic relations between the Xcm strains collected from different Enset growing regions will be analyzed based on DNA sequence results from AFLP analysis of the previous section. Phylogenetic trees will be constructed for both methods and bootstrapping will be conducted to ascertain the significance of each clustering.

4.3 Virulence spectrum studies: The virulence of X. campestris pv. musacearum strain isolates will be tested by inoculating different Enset clones following the method described by Woldemichael et al. (2008a). At least 100 strains representing different geographic origins will be used for this purpose. Information on host deferential from previous studies (Handoro and Woldemichael, 2007; Woldemichael, 2008a) shall be made use. The experiment will be conducted in a greenhouse using completely randomized design with three replications. Data will be collected on wilt incidence on each clone and the data will be subjected to statistical analysis using the SAS software.

4.4 Evaluation of Microbial agents for the control of Enset bacterial wilt disease: The antagonistic activity of available strains of the microbial agents in Enset will be evaluated under field conditions at Awassa. Soil samples will be collected from Enset growing regions of the country both from diseased Enset farm and supposed to be disease free farm. From the

soil samples, microbial antagonists with special focus on actinomycetes and rhizobacteria isolates will be evaluated for their antagonistic effect on Xcm at field condition. Evaluation will be done in two ways. One is the preventive method where the bio-agents will be inoculated 10, 20 and 30 days before inoculation of the virulent Xcm of a susceptible clone (Arkiya). The second is curative where the bio-agents will be inoculated after disease symptom has been developed. Disease scoring will be done with 15 days interval to monitor the disease progress. Consequently, effective biocontrol agent will be selected.

4.5 Evaluation of early detection tools: In this project, we will evaluate, lateral flow dipstick for early detection of EXW. As a result, effectiveness lateral flow dipstick as early detection tools for EXW will be known.

4.6 In vitro regeneration of disease free enset planting materials from enset bacterial wilt infected plants using meristem culture: Due to EXW there is a possibility of losing susceptible clones with higher culinary value. To mitigate this problem, different researchers have tried to develop protocols for micro propagation of clean planting materials to distribute for end users, but there is no information weather there is possibility of generating disease free suckers from infected enset plant corms and diagnostic techniques to test pathogen availability from suspected plants for the disease. Therefore, this activity is designed to develop a protocol for micro propagation of disease free planting materials. Three month old suckers of susceptible enset clone (Arkeia) and planted in pots with sterile soil mix of 1:1 top soil and red ash under greenhouse condition. Inoculation with Xcm, Hagereselam isolate will be done two weeks after planting. Explants will be collected from diseased plants and will be cultured on MS media. After subsequent culturing and full regeneration of plantlets, Samples will be taken and testing for infection will be done on selective media for the pathogen prepared from yeast peptone glucose agar (YPGA). Protocol for the micro propagation of disease free enset planting material will be developed.

Two M.Sc and one PhD students are under training with this objective.

Activity 5 Scaling out (seedling multiplication, training, mass mobilization and collective action): Scaling out of best practices such as sanitary control measure, cultural practices and use of resistance/tolerant enset clones shall be integrated and promoted. Workable approaches for mass mobilization for EXW shall be monitored, evaluated and documented. Tracking disease incidence using ICT tools shall be mainstreamed in the extension system in enset based farming systems. Extension leaflets and manuals shall be updated as new information is made available.

5.1 Demonstration and dissemination of integrated bacterial wilt control measures through collective action in benchmark site of the country: Awareness creation workshop was organized at SARI HQ and Training on the management of enset XW was

provided to 95 extension agents (10 female and 85 male) drawn from six zones and 25 woredas (districts) of the SNNP and Oromia Regional States. Leaflets (3,000), posters (1,300) and manuals (500) describing improved enset production and on EBW management options were prepared and distributed to trainees. Furthermore, bench mark sites were established at Gedeb woreda in Gedeo zone, Misha woreda in Hadiya and Mareka woreda in Dawro zone. Intervention was started at Gedeb woreda.

Deploying option in conjunction with resistant/tolerant sanitary and cultural control measures are the only advisable methods at hand believed to reduce its spread. Cultural practices such as deep tillage and turning over the soil to expose during dry season prior to planting, proper spacing, and spot rotation of infested sites would reduce disease spread. Generally, strict and procedural application of sanitary control measures through farmers training about the nature, cause, and dissemination mechanisms of Enset wilt should be given at all levels in order to reduce the threat of Enset wilt in major Enset growing areas of the region. In this project, already available farmers' indigenous coping mechanisms will be identified and integrated with improved Enset production and Enset wilt management technologies. The technologies will be demonstrated through frequent theoretical and practical trainings in the selected bench mark sites of Gedeo, Hadiya, Bench-Maji and Dawro zones of Southern Ethiopia where Enset bacterial wilt disease is reported to be economically important.

5.2 Information and communication technology (ICT) based early warning and information exchange for bacterial wilt disease management: Participatory action research methodology will be used in this study whereby various tools such as meetings, focus group discussions, key informants, and interviews will be conducted between several stakeholder involved in the project especially, local communities, service providers, extension agents, and NGOs. Two information delivery methods will be employed in the study area, these include print media (leaflets, brochure, posters), and electronic media (telephone and radio). Where available, a database of telephone number and/or e-mail addresses of the concerned shall also be maintained. These databases shall be used for alerting and forewarning on the incidence of Enset bacterial wilt. A system /software shall be developed to automate/ facilitate such forewarning and information exchange. The parties who avail their phone numbers and e-mails shall be informed of such a database and their consent obtained before making use of the information. Monitoring and evaluation process will be used to examine the usage of the delivery methods.

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