



Student Research Fellowships 2019 – 20

Research Topics

Topics for Master Theses

1. Diversity of vegetable and grain amaranth cultivated by smallholder farmer in Tanzania and Kenya

The World Vegetable Center is implementing a project to promote the cultivation and use of amaranth in Sub-Saharan Africa. In this context, we are investigating the diversity and origin of amaranth cultivars used by smallholder in Tanzania and Kenya. Our team will collect samples from amaranth fields and the task of the student is to genotype the samples, perform a diversity analysis and to determine the origin of the cultivars based on comparisons of the collected samples with reference material.

2. Drought tolerance of pepper

Drought is one of the most important abiotic constraints for plant production. Vegetable cultivars with low yield penalties under water stress that can survive drought spells are highly suitable for smallholder farmers in regions where access to irrigation is not guaranteed. We plan to screen a set of biodiverse pepper accessions to tolerance to water stress and investigate the underlying physiological mechanisms. The tolerant material and screening protocols will be made available to breeders for developing drought tolerant pepper cultivars.

3. Exploring Bacterial spot resistance gene diversity in pepper germplasm

Bacterial spot (caused by *Xanthomonas* spp.) is one of the most devastating diseases of pepper and tomato grown in tropical and subtropical environments. Once present, the disease is almost impossible to control and prevent major fruit loss when environmental conditions remain favorable. The study of pathogen has significantly enhanced the scientific understanding of host-pathogen interactions and the molecular basis of the gene-for-gene model. There are at least 6 different bacterial spot resistance genes (Bs) in pepper, which confer race specific resistance individually and horizontal resistance when pyramided. In this study, we will identify pepper lines containing different combination of Bs genes using molecular markers for efficient bacterial spot resistance breeding.

4. Development of user-friendly molecular markers in the Cvr gene regions for marker assisted selection

The *Capsicum–Potyvirus* pathosystem has been well studied and used as a model reference for the study of similar pathosystems in other plant species. Resistance to potyviruses is associated with two major gene groups. First are those with recessive inheritance (*pvr1/pvr2*, *pvr5*, *pvr6*, and *pvr8*) and associated with a mutation of the host factor eIF4E or eIF(iso)4E. The second group of single-gene potyvirus resistance mechanisms comprises those with dominant inheritance patterns (*Pvr4/Pvr7* and *Pvr9*) and

are usually associated with NLR cluster regions. A novel dominant resistance gene, *Cvr1*, was recently identified on chromosome 6, and associated molecular markers were developed, although the markers were not closely positioned to the gene, with the CVMV3 marker being 3 cM away. Since then, 3 other *Cvr* genes have been indentified, two with dominant inheritance and one with recessive. In this study, we will conduct targeted sequencing of the *Cvr* regions in WorldVeg sources of ChiVMV resistance and develop new molecular markers tightly associated with resistance.

5. Test CRISPR as a tool for crop improvement – Investigating gene functions defining tomato fruit quality

Quality is a key trait in vegetable breeding. In tomato, in addition to nutrient content, fruit color is an important trait defining processing quality. Lycopene, the pigment responsible for the deep-red color of ripe tomato fruits, is a product of the beta-carotene biosynthesis pathway. We are using CRISPR/Cas9 to investigate signaling genes regulating beta-carotene and lycopene biosynthesis to better understand the channeling of metabolites through this pathway.

6. Understanding domestication traits of legume beans

During crop domestication, humans have modified traits related to seed storage, seed dormancy, and seed scattering, among other traits. Understanding these domestication traits will increase the understanding of the selection history of traits of legume beans during the last millennia. Understanding these domestication traits will also help to improve the procedures for seed multiplication and seed storage of crop wild relatives of legume crops such yard-long bean, lablab, and soybean.

This study will consist of a systematic assessment of different seed traits that could have been modified by domestication. Seed characteristics of cultivated legumes such as yard-long bean, lablab, and soybean will be compared with the seeds of their wild relatives. This assessment could include the comparison of seed morphology, seed maturation, seed longevity and possible traits related to seed dormancy.

7. Screening crop wild relatives for heat tolerance under global climate change (MSc/PhD)

Crop wild relatives become increasingly important for trait discovery, intercrossing with cultigens, and breeding, because they often possess traits to tolerate abiotic stress or to resist insect damage and disease infections. Among these traits, heat tolerance is an important one to adapt horticultural cropping systems to global warming and the increase in heat spikes under climate change. This is especially relevant for open-field systems in tropical-lowland areas in Southeast Asia, which are exceptionally vulnerable to the negative effects of heating because current temperatures are already high.

This interdisciplinary research will allow the student to develop a broad range of research tools to enhance the use of crop wild relatives in crops such as *Capsicum* peppers, eggplant, or mungbean for trait discovery and breeding.

8. Seed health treatments for tropical vegetables

Seed health is key to ensure the distribution of vegetable seed. However, for most vegetable species no seed health protocols yet exist, which puts at risk the distribution of the seeds of these species by genebanks and breeding programs. This research consists of a literature review to make recommendations on 1) seed health treatments to the genebanks of WorldVeg in Tanzania and Taiwan and to the breeding programs of WorldVeg on cucurbits, *Allium*, okra, and pulses; 2) identify gaps for further research in seed health treatments for specific vegetable crops.

9. Optimal seed saving methods for Solanaceae crops

Seed drying methods are essential to maintain seed viability for short and long-term seed storage in genebanks, and by seed producers and farmers. To develop optimal seed saving methods, it would be necessary to test different drying methods for their effectiveness, economic feasibility, and practical implementation. The development of the seed drying methods consists of monitoring seed moisture content in different drying methods, measuring relative humidity and seed germination after different drying methods.

The results can be applied to develop optimal seed storage methods for farmers, seed producers, and germplasm collectors of eggplant, nightshade, tomato, and *Capsicum* peppers.

10. Global diversity assessment of traditional vegetables (MSc/PhD)

Genetic variation of crop species is shaped by the historic interactions between humans and plants and is the basis for contemporary and future food systems. The genebank of the World Vegetable Center maintains a collection of 60,000 varieties of more than 400 vegetable species including many traditional vegetables such as amaranth, spider plant, and sponge gourd. This part of human history maintained at WorldVeg can be used for breeding and research to develop new vegetable varieties and to promote new vegetable crops.

The extent to which WorldVeg genebank collections cover the geographic and climate ranges in which traditional vegetables occur, indicates the relevance of the WorldVeg collection in safeguarding this genetic variation. This information also allows to target geographic and climatic gaps for germplasm collection.

For this study, a set of traditional vegetables will be selected to determine their geographic and climate ranges, and to determine how well these ranges are represented by the WorldVeg collections as well as other genebanks. The geographic and climate ranges will be determined with an Eco geographic analysis and with the use of Geographic Information Systems (GIS).

The results of this study could contribute to a global study of diversity of traditional vegetables.

11. Explora: optimizing core subsets of genebank collections for breeding and screening purposes (MSc/PhD)

Core and mini-core collections of crop species have helped researchers to select diverse subsets of genebank accessions for trait discovery and breeding activities. The selection of core collections depends on the objectives of researchers. Therefore, ideally, researchers should use a core selection tool that maximizes molecular and phenotypic diversity in a subset of genebank accessions while this tool allows them to define agro morphological ranges for their screening and breeding purposes. Such a tool can enhance the use of genebank collections of the World Vegetable Center (WorldVeg).

Explora is a R-based tool with a user-friendly interface that is developed to help researchers to select diverse subsets of genebank accessions that fit to their breeding and screening purposes. *Explora* allows researchers to limit diverse subsets by specific objectives such as a specific fruit color, or fruit size, while maximizing the molecular and phenotypic diversity within these subsets under these constraints. The algorithms of *Explora* can be tested, expanded, and further improved with the use of morphological and molecular data of existing WorldVeg collections such as mungbean (*Vigna radiata*) and okra (*Abelmoschus esculentus*).

At this moment a prototype of *Explora* is developed by Bioversity International. The tool needs to be further developed and tested before it can be made available to researchers in and outside WorldVeg to select diverse subsets of genebank accessions according to their objectives.

12. Heat tolerance screening of tomato and pepper germplasm

Heat stress tolerance is an important trait tomato and pepper production in the tropics. Under high temperatures, fruit set of tomato and pepper is strongly reduced. Pollen development seems to be the most heat sensitive developmental stage in these crops. We use automatized field phenotyping and impedance flow cytometry to screen large core collections of pepper and tomato for heat stress tolerance traits and map the genetic base of the tolerance to facilitate breeding.

13. New sources of resistance to tomato bacterial spot caused by *Xanthomonas* species

Bacterial spot is becoming more important on tomato and pepper in Taiwan and the predominant races of the pathogen have shifted from *Xanthomonas euvesicatoria* race T1 to *X. perforans* races T3 and T4. Several hypersensitive resistance (HR) genes have been identified from tomato wild relatives and these interact with different avirulence genes in the pathogen races. As the pathogen may quickly evolve to overcome these HR genes there is a need to search for new sources of BS resistance that preferably are not strain specific (more horizontal resistance). This project will continue to screen genebank accessions of tomato and near relatives for potential sources of resistance to BS, and will test those potentially resistant accessions with a set of *Xanthomonas* tomato races to identify if the resistances identified are the same as those previously identified, or are new sources.

14. Crinivirus diagnostic tools and identifying new sources of resistance to *Criniviruses*.

New variants of whitefly-transmitted *Criniviruses* are emerging in Solanaceous and Cucurbitaceous crops in different regions. There is a need to be able to diagnose/confirm these infections to genus level and then to species and strain level quickly to be able to decide on management strategies. There is also

need to identify new sources of resistance to these emerging Criniviruses that can be included in resistance breeding programs. This project will evaluate some generic and specific diagnostic tools for emerging Criniviruses in Solanaceous and Cucurbitaceous crops and may screen using viruliferous whiteflies selected germplasm accessions and breeding lines to identify new sources of resistance to *Tomato chlorosis virus* (ToCV) or *Cucurbit chlorotic yellows virus* (CCYV).

15. Biological control of Southern Blight (*Sclerotium rolfsii*) on tomato

Southern blight of tomato (*Sclerotium rolfsii*) is carried over from crop to crop in the soil and in crop debris. It can cause significant losses as there is little durable resistance to the pathogen and fungicide treatments are generally ineffective and unsustainable. An alternative and promising means of managing the disease is through the use of antagonistic or hyper-parasitic microbes as biocontrol agents. This project will continue to assess the efficacy of biological control agents (*Trichoderma* spp., *Streptomyces* spp. and *Bacillus* spp.) for management of **Southern blight** (*Sclerotium rolfsii*) initially under glasshouse conditions with artificial inoculation, with the possibility of moving later to field trials in hotspot locations.

16. Identification of root knot nematode resistance genes in Solanaceous species

Root-knot nematodes (*Meloidogyne* spp.) are one of the three most economically damaging genera of plant-parasitic nematodes on horticultural and field crops. These nematodes cause extensive damage to most of the solanaceous and cucurbitaceous vegetables. An environmentally sound solution for nematode control is the use of plant resistance that mitigates yield losses and reduces the nematode population in the soil. Many commercially available tomato cultivars were incorporated with *Mi* gene against root-knot nematodes. Despite its effectiveness and profitability, the resistance conferred by the *Mi* gene has some critical limitations. Planting a resistant crop for several consecutive years can increase the risk of selection of virulent nematode populations. The World Vegetable Center (WorldVeg) holds a large public germplasm collection of Solanaceous accessions, which can have different genes for nematode resistance. Here, we aim to screen accessions of tomato and its wild relatives at WorldVeg genebank for resistance against to the most prevalent root knot nematode in Taiwan and to identify it.

17. Flooding tolerance rootstocks for tomato grafting

Short-term water-logging/flooding is one of the most important abiotic constraints for vegetable production in some parts of tropical and sub-tropical regions. Several *Solanum* accessions (esp *Solanum torvum*) were found to have a strong recovery index against short term water-logging. We aim to study the tolerance level at different crop growth stages and duration to identify flood tolerant rootstocks for grafting tomatoes.

Topics for PhD Theses

1. Use of wild tomato species as biopesticide factories against the main pests in tomato.

Defensive secondary metabolites (glucose/sucrose esters, allelochemical acyl sugars) from highly resistant tomato species may have a fitness cost to the plants in terms of yield and other horticultural traits. However, those defensive compounds can be extracted and use as foliar protectants to control insect pest on susceptible varieties. For this study, several steps are required:

Determine best sources of secondary plant metabolites (wild tomato relative accessions);

Determine differences in plant developmental stages in terms of secondary metabolites production;

Test different extraction methods;

Evaluate dose response curves with different insect pests.

Bioassays to determine the categories of resistance on different insect pests;

Evaluation of sub lethal effects (i. e. effect on reproductive potential) on surviving populations.