FINAL PROJECT REPORT

Project Title: Consulting for the Pacific Northwest sweet cherry breeding program

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Cooperators: Nnadozie Oraguzie and other members of the cherry team (Matt Whiting, Cameron Peace, Amit Dhingra and Fred Bliss)

Other funding sources

Agency Name: USDA-CSREES NRI Plant Genome
Amount awarded: $400K, Aug 2009 – Aug 2011
Notes: "The development of COS markers for comparative mapping in the Rosaceae and their application for understanding variation in fruit size". PI: Iezzoni. Develop and validate fruit size genetic markers for sweet cherry and new state-of-the-art marker development for cherry. Leveraged with WTFRC/OSCC funding.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae". PI: Iezzoni. Co-PIs include Peace and Oraguzie. Broad umbrella project on genetic marker development and application. Leveraged with WTFRC/OSCC funding.

Total Project Funding: 13,000

Budget History:

Item	Year 1: 2011	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel & expenses	$ 3,000		
Consulting fee	$ 10,000[a]		
Miscellaneous			
Total	**13,000**		

[a]These activities, which began in 2004, have historically been funded as a consulting arrangement. This was done so that Michigan State University would not be a shared "inventor" of the forthcoming sweet cherry cultivars. I then waived my personal "inventor" rights to any cultivars in exchange for a consulting fee that I donate to MSU to help support the MSU tart cherry breeding program.

OBJECTIVES:

1. Assisted in generating breeding populations. This included developing the crossing plan, sourcing germplasm, and making crosses along with the breeding team.
2. Provided horticultural guidance. This is provided by site visits, phone consultations, and sharing results from my cherry research at MSU.
3. Provided genetic expertise. My cherry genetics team is currently developing the genetic infra-structure for the PNW sweet cherry breeding program in collaboration with C. Peace to include the generation of molecular markers and genotyping of many of the parents used in the program. This work is funded by USDA grants.

SIGNIFICANT FINDINGS/ACCOMPLISHMENTS:

- Reviewed and contributed to the crossing plan.
- Made two site visits to Prosser, the first visit was at the beginning of bloom and the second visit was at the start of harvest, to provide organizational and technical assistance and make recommendations.
- Continued to provide specific information on the genetic control of fruit size and self-fertility to C. Peace for validation in the breeding populations.
- Developed a genome-wide set of 250 state-of-the-art high-through-put DNA markers that are also present in apple, peach/cherry, and strawberry to facilitate the transfer and leveraging of information. These markers were tested on a diverse set of sweet cherry germplasm including 40 parents and 65 progeny from the PNW sweet cherry breeding program, confirming the uniqueness of some of the germplasm imported for breeding.
- The RosBREED cherry genome scan was used to obtain genotypic data of thousands of anonymous markers across the genome for 460 sweet cherry selections. This quality checked data will be combined with phenotypic data obtained from Dr. Oraguzie to confirm parentage, deduce ancestral relationships beyond known pedigree records, and identify chromosome regions containing important genes.

RESULTS and DISCUSSION:

Assist in generating breeding populations & provide horticultural guidance.

In March I visited Prosser to help Dr. Oraguzie plan for spring activities. At this time there was currently an open technician position and an effort was underway to hire a new technician. The seedlings in the growth room looked excellent. The seedlings from last year's crosses had not yet been planted so I stressed the need to plant as early as possible in the spring. I also stressed the need to make bloom time observations of the elite individuals to include bloom time, flower bud density, and flower death if there were any freeze events. These observations are critical to begin to develop an assessment of future productivity. I also reviewed the best management practices for collecting quality field data on the breeding material. In particular, I recommended that fruit be evaluated for two harvest dates unless there is prior knowledge of optimum maturity. With the large influx of genetic data from USDA grants, I brought with me an electronic spreadsheet with data on markers that tag key trait regions for over 240 breeding selections. This data had been quality checked by myself and Dr. Peace using FlexQTL™ with the exception of one marker. I left this one marker for Dr. Oraguzie to quality check so that he could use this data to learn the statistical software.

In late June I visited Prosser to coincide with the start of harvest. A new technician, Andrea Young, had just been hired. With Dr. Oraguzie, I discussed my prior recommendations for an April planting date and my recommendation to have virus indexing done prior to crossing. This later recommendation would reduce the possibility that future seedlings are infected with PDV and/or PNRSV. We also discussed with Clint Graf, Roza farm manager, the need to choose a horticulture strategy that keeps the trees at a manageable height. In Michigan, I use a mechanical hedger. I also reviewed best management practices to include tagging each individual tree that will be sampled (Fig. 1) and bringing coolers to the field for transporting samples back to the laboratory. I walked the rows with Dr. Oraguzie to look at the elite selections. Jim McFerson and Tom Auvil joined us for this walk through. Unfortunately the crop load was very low due to spring freeze damage, making it difficult to assess the fruit quality of many of the seedling. Dr. Oraguzie and I had a conference call with the RosBREED database programmer to help further along the sweet cherry web-based database. We discussed the descriptors that would be used for cataloging the phenotypic data and the strategy to be used to proof the data. The goal was to have the 2010 and 2011 phenotypic data complete and quality checked by September 2011 so that it could be used in statistical analysis.

In September, to help Andrea Young learn about activities in a cherry breeding, my research technician, Audrey Sebolt, provided Andrea with sourcing information for breeding supplies and equipment used by the MSU tart cherry breeding program. This included information on tree tags (Fig. 1).

Provide genetic expertise

In a parallel project, my USDA-NIFA funded team (with Dr. Esther van der Knaap and Dr. Dechun Wang) developed more than 250 gene-based anonymous markers that are also present in peach, apple, and strawberry (Cabrera et al. 2009). These gene-based markers were also adopted by the peach, apple and strawberry teams as a way of linking the genetic maps of all three genera (Illa et al. 2011). This has resulted in cherry genetics leading the way in the generation of a common Rosaceae-wide genetic vocabulary, therefore allowing us to leverage information across species boundaries. Thus, due to our development of these gene-specific DNA markers, cherry, which in the past was so far behind in genetics and genomics information, is now fully integrated with peach, apple, and even strawberry. We are currently using this comparative mapping approach to elucidate the full suite of genes that control fruit skin and flesh color, as a first example.

Besides comparative mapping, we used knowledge of the diversity for these ~250 gene-based markers to investigate the genetic relatedness of the sweet cherry selections used as founders in the breeding program (Fig. 2; Cabrera et al. 2011). Discovery of these variants was not only carried out in varieties historically used as parents in the Prosser and British Columbia breeding programs, but also in novel germplasm used in the breeding program that I obtained from the Ukraine and Spain and introduced into the program. This will allow us to follow the unique genetic contributions of this novel germplasm in the breeding populations.

One of the selections that I used initially as a parent is the Spanish selection ‘Cristobalina’. This selection is very early ripening and it contains a unique source of self-fertility. Markers for this self-fertility locus were determined by a group in Spain (Cachi and Wunsch 2011). I provided a summary of the details of the genetic test to Drs. Peace and Oraguzie with the recommendation that screening be done to identify the self-fertile progeny individuals.

A complete set of anonymous genome-wide markers was identified by the RosBREED genetics and genomics team. This genotyping array technology was made available through a commercial partner, Illumina Inc. These arrays were used to genotype 460 sweet cherry selections for more than 5,000

markers. The arrays were run and analyzed by the team at Mich. State Univ. The data analysis revealed that these assays can successfully assess allelic states at ~1,900 positions along the eight cherry chromosomes of any individual scanned (Fig. 3). This is a significant advancement compared to only ~200 genetic markers previously available across the entire genome. In addition, the cost savings for this new high-throughput genetic testing is huge, as any given cherry selection can be screened for these ~1900 markers for ~ $70/sample.

Years ago I focused my research effort on fruit size in cherry as in my experience, large fruited progeny individuals were very rare, suggesting that marker-assisted breeding could significantly increase the efficiency of achieving large fruit size (Zhang et al. 2010). Fruit size data from the PNW Sweet Cherry Breeding Program supports this observation of the rarity of large fruited seedlings. Therefore, using our ever-expanding database, we are concentrating our efforts on the fruit size gene regions on cherry linkage groups 2, 3 and 6 to identify superior genetic markers. This effort is in collaboration with scientists from Bordeaux, France, and is receiving much attention in RosBREED.

Collectively, these efforts provide the building blocks that will allow the cherry team to implement marker-assisted breeding to increase the efficiency and success of the breeding program. These activities also position sweet cherry to be on the forefront of adopting the advances in marker-assisted breeding enabled by the USDA-NIFA-SCRI RosBREED project.

Figure 1 Recommendation for labeling trees. (A) Labels can be printed using a laser printer. (B) Excel sheet that is formatted for the labels to be printed. (C) Image of trees at Amy Iezzoni's tart cherry research trees, each tree that data is collected is labeled. (D) Up-close image of a label.

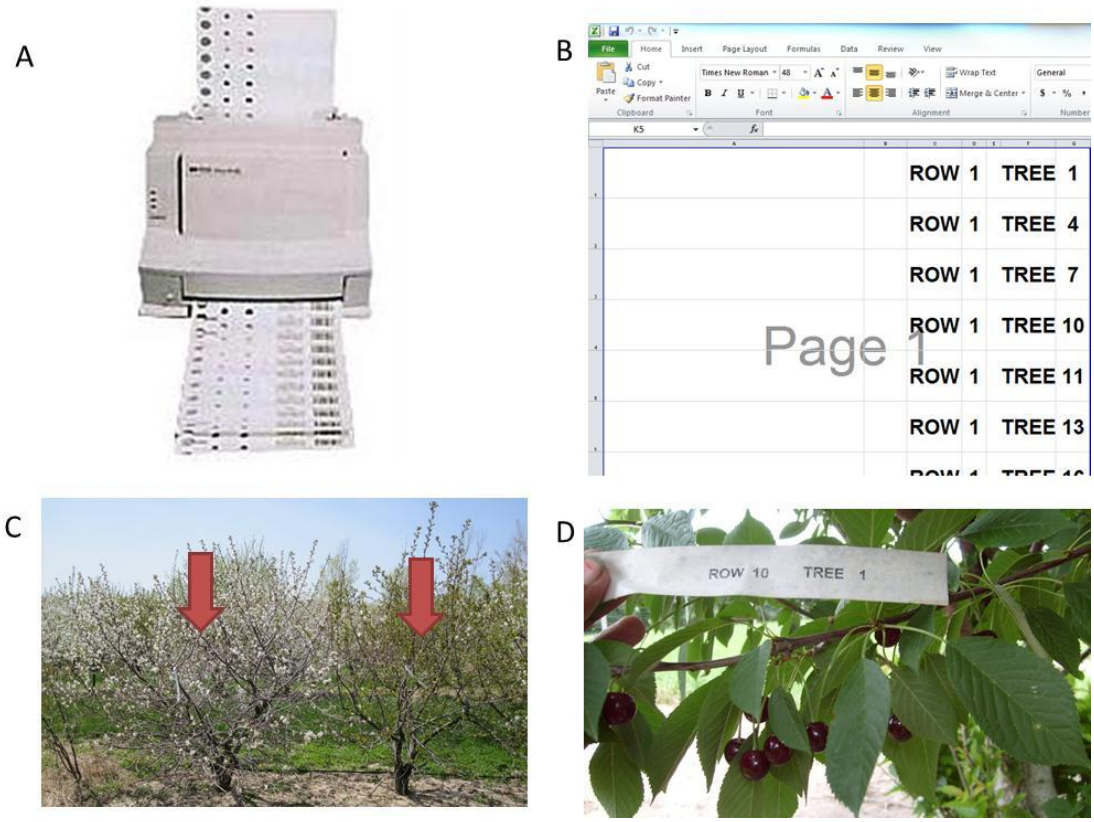


Figure 2 Locations of 17 sweet cherry founder accessions used in the WSU breeding program on the first two principal coordinates determined from the RosCOS haplotypes (Cabrera et al. 2011).

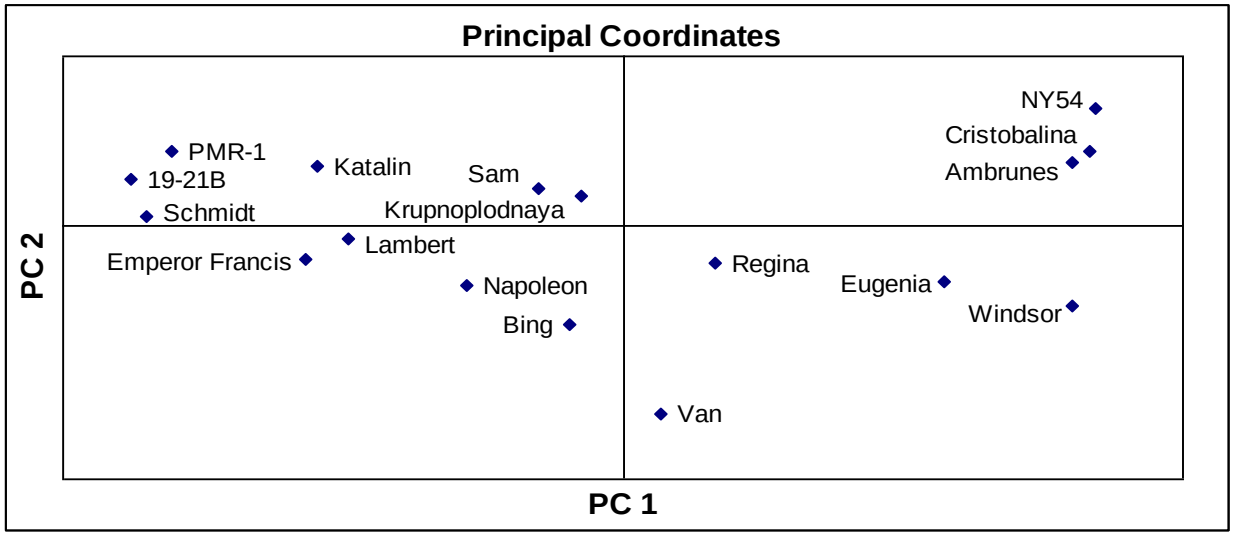
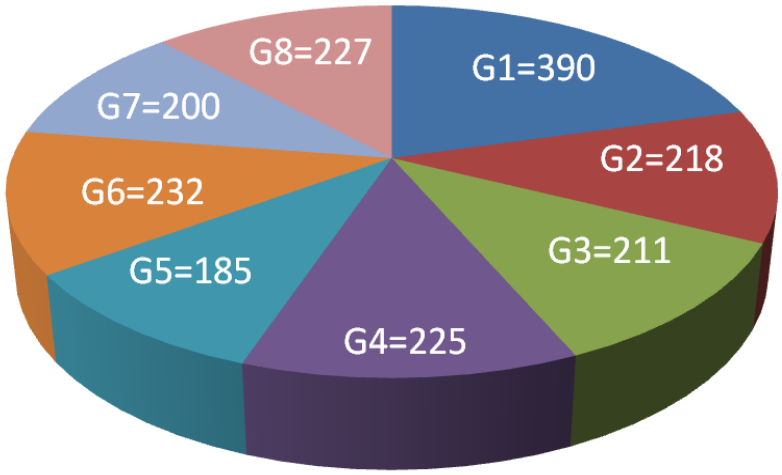


Figure 3 Number of genetic markers for the eight sweet cherry linkage groups (G1-G8) scored using Infinium® SNP array technology. The plant material evaluate was the RosBREED sweet cherry Crop Reference Set that includes 240 progeny from 17 segregating populations (n≥5) and 46 germplasm accessions and cultivars. The number of attempted markers is indicated per linkage group.



LITERATURE CITED:

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Cachi AM and Wunch A. 2010 Characterization and mapping on non-*S* gametophytic self-compatibility in sweet cherry (*Prunus avium* L.). J Exp Bot 62: 1847-1856.

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Zhang G, Sebolt AM, Sooriyapathirana SS, Wang D, Bink MCAM, Olmstead JW, Iezzoni AF. 2010. Fruit size QTL analysis in an F_1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. Tree Genet Genomes 6:25-36.

EXECUTIVE SUMMARY:

The building blocks of a successful breeding program include the use of diverse germplasm, generation of large numbers of progeny populations for evaluation, appropriate horticultural management of the breeding materials, the ability to identify and commercialize superior cultivar candidates, and judicious use of genetics knowledge. The goal of my consultancy with the PNW sweet cherry breeding program was to assist in our ability to excel at all of these objectives so that we can deliver superior sweet cherry cultivars to the Oregon and Washington industries as quickly as possible. This was an important year in the breeding program. The prior problems with seedling growth were corrected; however, the large number of seedlings, although a very good situation, posed new challenges. These challenges include determining strategies for moving the seedlings from the greenhouse to the field, and the need to strategically target financial resources given the growth in the program. Therefore, I provided knowledge and recommendations regarding breeding and horticultural practices that took into account the importance of targeting resources to have the highest impact. Genetic advances made in my NRI project (with Dr. Esther van der Knaap and Dr. Dechun Wang) contributed substantially to the genetics knowledge in cherry through the generation and identification of valuable genetic markers for fruit size and color. Efforts by RosBREED-funded scientists* resulted in the generation of vast amounts of genetic data which, combined with phenotypic data collected by Dr. Oraguzie, will significantly advance our genetics knowledge and contribute to increased breeding efficiency. Continued collaboration, whereby I contribute my time and knowledge of cherry germplasm, breeding and genetics, will help us achieve our collective vision of a cost-effective aggressive and successful sweet cherry breeding program.

*RosBREED team members who have and continue to contribute substantially to this cherry genetics effort are Nahla Bassil, Cameron Peace, Dorrie Main, Umesh Rosyara and Audrey Sebolt.