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	**2010**		
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 includes 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 during harvest, to provide organizational and technical assistance and make recommendations.
- Continued to provide specific information on the genetic control of fruit size to C. Peace for validation in the breeding populations.
- Developed a genome-wide set of state-of-the-art high-through-put DNA markers and 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.

RESULTS and DISCUSSION:

Assist in generating breeding populations & provide horticultural guidance.

In April, I visited Prosser and assisted N. Oraguzie in organizing for spring crossing. This included review of the Best Management Practices, the crossing scheme, pollen viability testing, seeds in stratification and seedlings in the field. Activities over the past year corrected the previous problems with seedling death and at the time of my visit, a large number of healthy seedlings were in the greenhouse. I discussed with N. Oraguzie and C. Graf the possibility of spring and fall plantings. The Prosser team experimented with two planting times (May and October). The spring planted seedlings were slow to break terminal bud and start re-growing; however, the vast majority of the seedlings were actively growing by August (Fig. 1). The plants kept in pots in the lath house until the October planting grew very well over the summer. It is important that these two planting strategies be reviewed next March and in the subsequent years to allow a determination of the best seedling planting strategy.

In July, I visited Willow Drive Nursery (WDV) to see the budded advanced selections and Prosser to tour the seedlings in the field and the greenhouse, and review the fruit evaluation. The bud take at WDN was variable and an early budding date was suggested. Due to various factors (most notably frost damage) the number of fruiting seedlings was less than anticipated. However, I walked those seedling rows that had fruiting trees with N. Oraguzie and we discussed the elite seedlings, phenotyping, and record keeping. My recommendations included increased attention to yield and yield components (including assessments of bloom time and density), the use of field location as a seedling numbering system to minimize human errors, and targeting phenotyping of specific populations to assure that expenditures are spent judiciously.

Provide genetic expertise

My cherry genetics team is currently developing the genetic infra-structure for the PNW sweet cherry breeding program to include a set of state-of-the art high throughput DNA markers. The strategy of our USDA-NIFA funded team (with Dr. Esther van der Knaap and Dr. Dechun Wang) has been to develop gene-based markers that are also present in peach, apple, and strawberry (Cabrera et al. 2009). (See gene list at http://bioinfo.bch.msu.edu/). 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. For example, we have already successfully used knowledge of apple genetics to help us quickly identify the gene likely responsible for fruit skin and flesh color in sweet cherry (Fig. 2) (Sooriyapathirana et al, 2010).

For a genetic marker to be useful in breeding germplasm, it needs to exhibit at least two genetic variants. We have completed a large effort to find these genetic variants in the range of sweet cherry germplasm used in the PNW breeding program and have characterized these variants for over 250 genes in our "common vocabulary" set. 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.

This new set of 96 genetic markers were scored on 40 parents and 65 progeny from the PNW sweet cherry breeding program. I collected branches for this genotyping when at Prosser this spring and brought the branches bay to Michigan for DNA extraction from the young leaf tissue (Fig. 3). Also genotyped were over 400 other parents and progeny contributed from breeding programs in France and Germany, generating a set of over 47,000 data points! Unlike the young germplasm in the PNW program, the plant material provided from the French and German programs has been phenotyped for traits such as fruit size, and rain cracking for multiple years allowing us to answer questions on the genetic control of important traits without having to wait for the Prosser material to flower and fruit. This is also the first genome wide marker data set shared with the RosBREED statistical team, therefore positioning sweet cherry as the "lead crop" in RosBREED integrating high throughput marker data with newly enhanced statistical software.

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. Fruit size data from the PNW Sweet cherry breeding program supports this observation of the rarity of large fruited seedlings (Fig. 4). Therefore, using our ever expanding database, we (my USDA-NIFA Team) are concentrating our efforts on the fruit size gene regions on cherry linkage groups 2, 3 and 6 to identify superior genetic markers (Fig. 5). This activity is in collaboration with C. Peace who has initiated the implementation of genetic screening in the breeding program that targets the fruit size region on linkage group 2 that we identified previously (Zhang et al. 2010).

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.

LITERATURE CITED:

Cabrera A, Kozik A, Howad W, Arus P, Iezzoni AF, van der Knaap E. 2009. Development and bin mapping of a Rosaceae Conserved Ortholog Set (COS) of markers. BMC Genomics 10:562

Sooriyapathirana SS, Khan A, Sebolt AM, Wang D, Bushakra JM, Lin-Wang K, Allan AC, Gardiner SE, Chagne D, Iezzoni AF. 2010. QTL analysis and candidate gene mapping for skin and flesh color in sweet cherry fruit (*Prunus avium* L.). DOI 10.1007/s1 1295-010-0294-x.

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. In addition, this work has positioned cherry as the lead crop in RosBREED for statistical analysis, as the data set we generated is being used by the RosBREED statistical team to test their improved computational software. 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.

Fig. 1 PNW Sweet cherry breeding seedlings planted in May 2010 in the F Block at the WSU-Prosser Roza Experimental Station (photo courtesy of C. Graf).

Fig. 2 The fruit color in sweet cherry is likely controlled by the same gene that controls fruit color in apple and red color around the pit in peach (graphic courtesy of C. Peace).

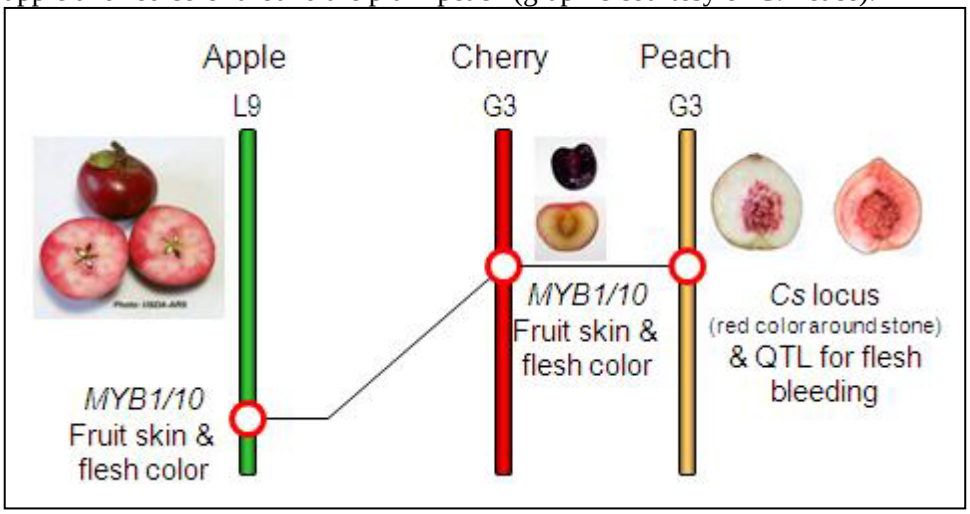

Fig. 3 During my spring visit to WSU-IARDC Rosa Experiment Station, I collected branches of seedlings from the breeding program and brought them back to Michigan for DNA extraction from the young leaves. These seedlings were subsequently genotyped for a set of 96 gene-based markers that are also present in apple, peach, and strawberry.

Fig. 4 Range in fruit size for seedlings from the cross between PMR-1 and Rainier (data courtesy of Jim Olmstead).

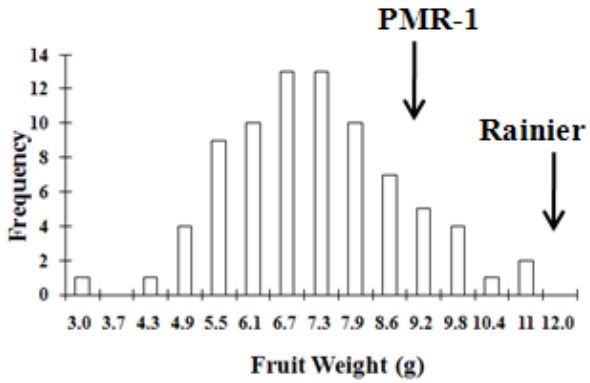

Fig. 5 Genetic regions on sweet cherry linkage groups 2, 3 and 6 that contain genes controlling fruit size. The results are summed over all populations including the Regina x Lapins population from INRA-France.