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 requested/awarded: $400K, Aug 2009 – Aug 2013
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:

Budget History:

Item	Year 1: 2012	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel & expenses	$ 3,000		
Consulting fee	$ 10,000[a]		
Miscellaneous			
Total			

[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:

- Discussed the crossing plan and made recommendations on the implementation of DNA marker-assisted parent selection.
- Visited Prosser at the start of harvest (June) to provide organizational and technical assistance and make recommendations.
- Continued to provide new specific information on the genetic control of fruit size to C. Peace for validation in the breeding populations.
- The RosBREED cherry genome scan was used to obtain genotypic data for thousands of DNA markers across the genome for 480 sweet cherry selections. This quality checked data was combined with pedigree data obtained from Dr. Oraguzie to confirm parentage and deduce ancestral relationships beyond known pedigree records.
- Major genes that may be responsible for the control of fruit size have been identified and are being tested for breeding application.

RESULTS and DISCUSSION:

Assist in generating breeding populations & provide horticultural guidance.

In March 2012, I met with Dr. Oraguzie to plan for spring activities, specifically spring crossing. At this time, the MSU cherry team had transferred to Dr. Oraguzie a quality checked and corrected data file with sweet cherry pedigree information and hundreds of genetic data points for 480 sweet cherry individuals chosen to represent the germplasm in the PNW sweet cherry breeding program. The MSU cherry team also provided the results of genetic analyses using the 2010 and 2011 phenotypic data provided by Dr. Oraguzie. Due to the small number of individuals phenotyped, the genetic analyses were considered preliminary until confirmed using 2012 phenotypic data. However, it was possible to use some of the genetic knowledge gained to plan spring crosses.

I also recommended that self-fertile individuals derived from the cross Rainier × Cristobalina be identified using the available DNA markers. This would identify which of the ~200 progeny should be used for spring crossing to combine early maturity and self-fertility.

In late June, I visited Prosser to coincide with the start of harvest and the Prosser field day. This visit was prior to 'Chelan' harvest, therefore it was possible to observe whether any of the seedlings in the F block were exceptionally early ripening. The following previously identified seedlings were very early ripening (FR1T7, FR9T33, and FR9T89). However, many more newly fruiting seedlings also were very early ripening. These included FR9T46, FR33T94, FR33T019, FR34T30, FR34T50, FR34T56, FR34T74, FR35T102, FR35T121, FR35T122, and FR25T123. The reported pedigree information indicates that the early maturity in these selections is likely due to the use of three early

maturing parents ‘Chelan’, ‘Cristobalina’ and ‘Dzherlo’. The latter two selections that were specifically used to confer early ripening are from Spain and the Ukraine, respectively.

As illustrated by the example of the very early ripening seedlings, the most desirable parents to be used in crosses are now most likely seedlings that are determine to have the best breeding values for the traits of interest. Therefore, a concerted effort is needed to evaluate superior seedlings, not only for elite status but as parental status for crossing in spring 2013. Additionally it would be important to re-evaluate the use of precocious rootstocks for some of the predicted to have the most potential, as those seedlings grafted on Gi5 had significantly more flowers and fruit than those on their own roots. In certain cases, this benefit may be cost effective as it would reduce the number of years per generation.

Provide genetic expertise

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. Therefore, using our ever-expanding database, we are concentrating our efforts on the most important fruit size gene regions that is located on cherry linkage group 2.

In 2012, my USDA-NIFA funded team (with Dr. Esther van der Knaap and) identified a set of candidate genes that may play a major role in the genetic control of fruit size in cherry by controlling the rate of cell division in the fleshy fruit mesocarp. ‘Regina’ and ‘Lapins’ are both heterozygous for one of these genes called PavCNR12 for *Prunus avium* Cell Number Regulator 12. Based on which allele the progeny individuals obtained from both parents, there is a significant mean difference in fruit size (DNA and phenotypic data kindly provided by Elisabeth Dirlewanger and Jose Quero-Garcia from INRA, Bordeaux)(Fig. 1). Those progeny that received two copies of the “2” allele had on average a 2 gram increase in size compared to those progeny individuals that received to copies of the “2” allele. Those progeny individuals that inherited both alleles “1” and “2” had intermediate fruit size values. Diagnostic markers have been developed for this candidate gene, as these markers may increase the precision of predicting which seedlings are most likely to have large versus small fruit.

A complete set of genome-wide DNA markers were identified by the RosBREED genetics and genomics team and this genotyping array technology was made available through a commercial partner, Illumina Inc. These arrays were used to genotype 480 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. 2). 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.

Using these new genotyping tools, the genetic knowledge for sweet cherry was rapidly advanced in 2012. Major accomplishments include the construction of high density linkage maps and the identification of additional chromosome regions containing genes for fruit firmness, fruit size, fruit cracking, bloom time and maturity date (see list of references). Of particular interest are the trait alleles identified in ‘Regina’, as this parent has been used extensively in the PNW sweet cherry breeding program to confer desirable fruit quality traits. Coordination of these sweet cherry research efforts in Europe is provided by Dr. Jose Garcia from INRA, France with funding from the EU Food and Agricultural Cooperation in Science and Technology Action project #FA1104 entitled

'Sustainable production of high-quality cherries for the European market'. As an invited member of this COST Action project, I have enhanced insight into the research activities within the EU community.

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.

Manuscripts and conference posters and presentations from international meetings in 2012 showcasing genetic accomplishments:

Castede S, Campoy J, Le Dantec L, Quero-Garcia J, Rosyara U, Iezzoni A, and Dirlewanger E. 2012.Genetic determinism and candidate genes for flowering date – a phenological trait highly affected by climate change in sweet cherry. Plant & Animal Genome XX, San Diego, CA.

Dirlewanger E, Le Dantec L, Campoy JA, Barreneche T, and Quero-Garcia JK. 2012. The genetic control of fruit quality traits in two *Prunus* species: peach and cherry. 6th Rosaceous Genomics Conference, Mezzocorona, Italy.

Dirlewanger E, Quero-Garcia, Le Dantec L, Lambert P, Ruiz D, Dondini L, Illa E, Quilot-Turion B, Audergon J-M, Tartarini S, Letorumy P, and Arus P. 2012. Comparison of the genetic determinism of two key phonological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. Heredity 00:1-13.

Garcia J, Campoy J, Joly J, Tauzin Y, Rosyara U, Iezzoni A, and Dirlewanger E. 2012.QTL detection for fruit weight, fruit firmness and fruit cracking tolerance in sweet cherry. Plant & Animal Genome XX, San Diego, CA. Poster #P0496.

Klagges C, Campoy JA, Quero-Garcia J, Guzman A, Mansur L, Gratacos E, Silva H, Rosyara UR, Iezzoni A, Meisel LA, and Dirlewanger E. 2012. Construction and comparative analyses of highly dense linkage maps of two sweet cherry intra-specific progenies of commercial cultivars. 6th Rosaceous Genomics Conference, Mezzocorona, Italy.

Peace C, Bassil N, Main D, Ficklin S, Rosyara UR, Stegmeir T, Sebolt A, Gilmore B, Lawley C, Mockler TC,Bryant DW, and Iezzoni A. Development and evaluation of a genome-wide 6K SNP array for diploid sweet cherry and tetraploid sour cherry. PLoS ONE (in press).

Figure 1 Mean fruit size (grams) for 'Regina' × 'Lapins' progeny individuals based on which alleles they inherited for the fruit size candidate gene *PavCNR12*. The *PavCNR12* alleles are identified as "1" or "2" and color coded as blue and green, respectively.

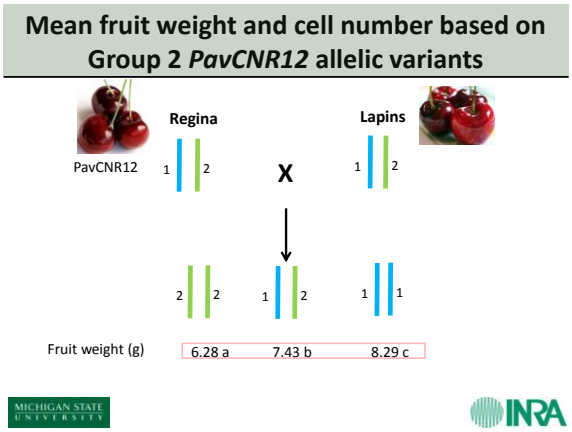

Figure 2 Peach physical map locations of all the polymorphic DNA markers on the eight sweet cherry chromosomes. The DNA markers are from the RosBREED generated SNP array (Peace et al. 2012) evaluated for a diverse set of 240 sweet cherry individuals. The length of the vertical lines illustrates the minor allele frequency (e.g. the longer the line, the more likely the marker will be polymorphic).

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) contributed substantially to the genetics knowledge in cherry through the generation and identification of valuable genetic markers for fruit size and color, and most recently candidate genes that control cell division in fruit mesocarp. 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.