**FINAL PROJECT REPORT**

**Project Title:** Establishing the marker-assisted breeding pipeline for sweet cherry


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**Other funding sources**

**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 for U.S. tree fruit breeding programs. Leveraged with WTFRC/OSCC funding.

**Total Project Funding**: $45,000

**Budget History:**

| Item | Year 1: 2009 | Year 2: | Year 3: |
|---|---|---|---|
| **Salaries** | $ 4,000 | | |
| **Benefits** | $ 1,805 | | |
| **Wages** | $16,675 | | |
| **Benefits** | $ 1,325 | | |
| **Equipment** | | | |
| **Supplies** | $11,195 | | |
| **Travel** | $ 5,000 | | |
| **Miscellaneous**[a] | $ 5,000 | | |
| **Total** | $45,000 | | |

[a] Miscellaneous – development, hosting, and publicizing of a participatory field day to demonstrate MAB methodology and workshops to be held in conjunction with the 2009 WTFRC cherry research review and the 2010 Cherry Institute.

## ORIGINAL OBJECTIVES

1) Establish individual components of the MAB Pipeline not yet in place for the PNW sweet cherry breeding program, particularly the final stages of Cost Efficiency and Trial Use, to ensure that planned MAB efforts confer costs and/or time savings to breeding, and to put theory into practice.
2) Formalize and continue the process of Prioritization, Marker Improvement, Validation, and Utility assessment of new marker-trait associations for cherry as they are discovered and reported.
3) Demonstrate the MAB Pipeline to the PNW sweet cherry producer community through outreach activities, using high impact markers for self-fertility and fruit size.

## SIGNIFICANT FINDINGS

- Modern genetic screening capability integrated with traditional routine breeding operations is now enabled for the PNW sweet cherry breeding program (PNWSCBP), with the establishment of the **Marker-Assisted Breeding (MAB) Pipeline** for this program. From 2010, DNA information can routinely augment crossing decisions to result in a greater proportion of superior seedlings, can routinely support seedling selection decisions as cost-efficient early selection tools to cull inferior seedlings, and can be routinely used in genetic potential descriptions of new cultivars to facilitate industry planting decisions. The infrastructure is now established to efficiently pipeline genetics and genomics advances into routine breeding operations.

- The MAB Pipeline was refined in the last year during preparations for the multi-million dollar federally funded **RosBREED** project, including the previous seven stages being increased to eight. This MAB Pipeline (Figure 1) is to be adopted by numerous U.S. Rosaceae breeding programs, allowing collaborative development of powerful infrastructure and implementation for tremendous benefit to the PNWSCBP and the PNW sweet cherry industry.

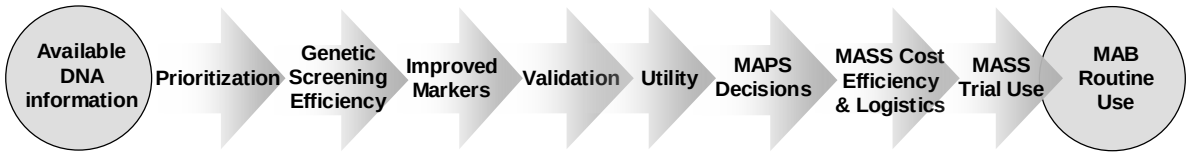


**Figure 1**. The MAB Pipeline

- The first six stages of the MAB Pipeline were formalized for the PNWSCBP in 2009 (addressing **Objective 2**), although even greater formalization will be undertaken in the next four years within RosBREED due to the establishment of powerful infrastructure for each stage. New opportunities for applying DNA information to augment the breeding program were progressed through the Pipeline, and future new genetics and genomics discoveries can be readily channeled in through this Pipeline.

- The final two stages of the MAB Pipeline were successfully implemented for the PNWSCBP (addressing **Objective 1**), using genetic tests for fruit size and *S*-alleles including self-fertility. Cost-efficient and logistically feasible high-throughput genetic screening schemes were identified and successfully trialed, completing the connection between genomics research and routine breeding operations.

**RESULTS & DISCUSSION**

The MAB Pipeline (Figure 1) represents a series of practical stages to convert genomics research into breeding application. This is not the only approach that could be taken, but such a focus on individual stages addresses important considerations that otherwise could impede the efficient use of modern genetics and genomics knowledge and tools to enhance breeding. Indeed, the failure to address considerations such as trait priorities, availability of high-throughput genetic screening services, cost-efficiency, and on-the-ground logistics, has restricted tree fruit marker-assisted breeding to very few examples around the world. The value of the MAB Pipeline approach was recognized by the U.S. Rosaceae genomics, genetics, and breeding community, with stakeholder and international support, in the coordinated development of the first "RosBREED" proposal to the Specialty Crops Research Initiative in August 2008 – led by Dr. Iezzoni. Soon after, the same approach was proposed for this WTFRC-funded cherry project, and in parallel efforts for the Washington apple breeding program. While unsuccessful in the first round, the RosBREED proposal, with the MAB Pipeline approach retained, refined, and reinvigorated, was resubmitted in April 2009 and proved successful in obtaining and directing more than $14 million to targeted application of genomics and socio-economics knowledge for accelerated and streamlined fruit breeding. RosBREED will run for four years from September 2009. In the meantime, with WTFRC funding support in the present project, we have forged ahead with establishing the Pipeline for the PNWSCBP.

The MAB Pipeline consists of eight stages:
(1) Prioritization of reported marker-locus-trait associations is essential to sift through the volumes of available genomics information. Not all genomics discoveries are created equal, and their impact on crop improvement varies by value of a trait to breeding, industry, and consumers, and the strength of association and effect on performance of the tagged controlling genes. Marker-locus-trait associations are specific genetic markers with a known position (locus) in the genome that tag a specific trait of interest.
(2) Genetic Screening Efficiency is identified by locating and testing efficient and logistically feasible methodologies for high-throughput genetic screening (sampling, DNA extraction, genotyping, and timely provision of data to breeder) that suit the idiosyncratic routine operations of breeding programs.
(3) Improved Markers are developed to ensure robustness and amenability to use in the high-throughput pipeline needed for genetic testing of thousands of seedlings.
(4) Validation of robust marker-locus-trait associations is performed in the wider germplasm pool of a crop, beyond the experimental material in which they are usuaully first discovered.
(5) Utility assessment of validated markers is conducted to determine their potential application specifically within a breeding program, detecting the maintenance of marker-locus-trait associations in breeding program germplasm and describing functional marker variants (favorable or not) in each potential breeding parent.
(6) MAPS (marker-assisted parent selection) Decisions are enabled, where the information gained from the previous stage is used to guide crossing decisions by a better understanding of breeding value.
(7) MASS (marker-assisted seedling selection) Cost Efficiency and Logistics calculations and considerations are made to identify optimal seedling selection schemes that integrate available robust, validated, utile genetic tests for some traits into routine breeding operations with phenotypic selection for other traits.
(8) MASS Trial Use is conducted in a high-throughput manner on a subset of breeding program seedlings to transform the pipeline into reality, comparing theory with practice to optimize MASS implementation.

Progress in establishing and implementing each of these stages in 2009 is described below.

1. Prioritization

To facilitate the prioritization of marker-locus-trait associations for application in breeding, and to help direct future marker development research, traits of interest to the PNWSCBP have been placed into the groups of Market-defining, Primary, Secondary, Preferred, and Lineage-specific (Table 1). Traits of highest priority (Primary traits) for MAB in this breeding program are currently fruit size and firmness. Therefore, we need to direct greatest effort toward developing and pipelining marker-locus-trait association for these two traits.

**Table 1**. Assignment of marker-locus-trait associations for application in the PNWSCBP according to trait groups. Within each group, traits are treated equally and simultaneously, and available DNA information should be combined for decision-making.

| Trait groups | Traits in group | DNA information available?[a] | MAB approach |
|---|---|---|---|
| Market-defining | Harvest date | (Yes) | MAPS[b]. Used on parents to predict target market class(es) of resulting seedlings. |
| | Self-fertility | Yes | |
| | Fruit color | (Yes) | |
| | PM resistance | No | |
| Primary | Fruit size | Yes | MAPS & MASS[c]. Seedlings must perform above threshold for each. |
| | Firmness | ([Yes]) | |
| Secondary | Sweetness | (Yes) | MAPS & MASS. Seedlings sought above threshold for each, but weighed together – lower values tolerated. |
| | Acidity | (Yes) | |
| | Taste | (as above) | |
| | Low astringency | (Yes) | |
| | Low bitterness | No | |
| Preferred | Fruit cracking resistant | No | MAPS. Aim for increasing proportion of seedlings to have any of these. Parents and seedlings with rank higher than those without. |
| | Fruit doubling resistant | No | |
| | Bacterial canker resistant | No | |
| | Self-fertile | Yes | |
| | PM resistant | No | |
| | Precocious | (Yes) | |
| | Freestone | ([Yes]) | |
| | Mechanical harvestability | [Yes] | |
| Lineage-specific | e.g. Super-sweet | No | MAI[d]. Use for parent and seedling selection only in certain lineages. |

[a] Marker-locus-trait associations in published reports (unpublished research of PIs) [promising leads from related crops]

[b] MAPS = marker-assisted parent selection (using DNA information of parents to aid crossing decisions)

[c] MASS = marker-assisted seedling selection (using high-throughput genotyping of seedlings to cull those inferior)

[d] MAI = marker-assisted introgression (introducing new traits from unusual sources, usually requiring several generations to combine into elite backgrounds)

RosBREED will apply greater objectivity to the Prioritization process by establishing a method of quantifying the economic value of each trait (with surveys of trait and market segment values and preferences of producers/processors, marketing groups, trade organizations, and consumers), and weighing economic values by the degree to which a breeder can genetically improve the trait.

2. Genetic Screening Efficiency

The four components of high-throughput genetic screening have been developed to a working system, although further refinements will continue to be applied. A successful high-throughput DNA extraction protocol was developed, which is the Silica Bead Method (SBM) as used for the Washington apple breeding program but with minor modifications (i.e., the addition of PVPP to the initial extraction buffer to reduce interfering polysaccharides in cherry leaves, and tripling the amount of template DNA in PCR reactions due to lower extracted yields). SBM involves a simple greenhouse/field tissue sampling method without the laborious step of freeze-drying, and unexpectedly but fortuitously the method is effective for older leaves (unavoidable from mid summer to fall) as well as for DNA-rich young leaves that are usually only available in spring and early summer. To date, this extraction method has been used to extract >1000 samples with >95% success.

The Pacific Northwest Tree Fruit Genotyping Laboratory (PNWTFGL) was established in Pullman in 2009 with the purchase of an ABI 3730xl DNA Analyzer with $100K funding support from the WTFRC and Washington Wheat Commission (WWC), additional equipment provided by the WSU Agricultural Research Center (ARC) support totaling another $100K, and the recent addition of a $90K Laboratory Automated Workstation (a DNA handling "robot") funded by the WWC, ARC, and Dr. Deven See (USDA-ARS Pullman). The PNWSCGL was established to service the PNWSCBP and the Washington apple breeding program as well as supporting research, and is run by Dr. Peace in close collaboration with Dr. Deven See who manages the Western Regional Small Grains Genotyping Laboratory. Despite the availability of such equipment and appropriate technical expertise, successful routine genotyping of sweet cherry on the ABI3730xl eludes us for now (whereas apple works just fine). We continue to troubleshoot, and expect success by the end of 2009. In the meantime, we have used the fallback of large polyacrylamide gels, which are effectively medium-throughput (130-370 data points per day) utilizing the technical expertise currently in the lab. This genotyping system is being used for *S*-genotyping and fruit size genotyping of hundreds to thousands of seedlings in 2009 (described below in Trial Use).

While we continue to develop a streamlined data handling system for the many thousands of datapoints to be collected and provided to the breeder in subsequent seasons, we have already had success in providing data in a suitable and simple format: a color print-out of *S*-genotypes of '04 seedlings allowed the breeder and consultants to cross-reference field performance with parentage while walking the breeding rows during the 2009 fruiting season. By the end of 2009 we expect to have a system of genotypic data provision that can be readily used by breeding personnel to cross-reference marker genotypes with close-packed seedlings in the greenhouse or lath house for ease of culling inferior plants. RosBREED will expand on such efforts for the PNWSCBP.

3. Improved Markers

An efficient genetic test was developed for *S*-genotyping that includes identification of self-fertility in addition to common *S*-alleles. This test is now routinely performed in the lab. The "universal" *S-RNase* gene primers (Tao et al. 1999) are multiplexed with our new $S_4'$-specific marker, "Pav-S4-indel" (forward primer: TGCGAAAAATTGACTTCTGG; reverse primer: TCAAGAACTTGCTTGGATTCG). Standard PCR conditions are used, and alleles are resolved on large polyacrylamide gels. Pav-S4-indel generates a 194 bp fragment for the *S4* allele and 190 bp for the $S_4'$ allele imparting self-fertility.

For fruit size, we are using MSU-developed markers that flank two QTLs for fruit size components (cell number on G2 and pit size on G6) discovered in the 2005-2008 NRI project of Dr. Iezzoni. However, we changed one of the G6 markers for a new one, "Pp-ACS3-SSR". This is a marker for a texture candidate gene that just happens to be located at the pit size QTL, and we are taking advantage of the greater allelic diversity offered by Pp-ACS3-SSR which may help identify new functional pit size alleles.

For other traits, we are creating new markers for reported fruit gene markers, facilitating greater refinement of functional effects and development of predictive markers for use with PNWSCBP germplasm. Examples include the *MYB1* gene associated with cherry fruit color for which we developed a new microsatellite-based marker, the *Pp-ACS3* gene that is the equivalent gene to *Md-ACS1* influencing softening in storage of apple, and the *Pp-PG1* gene (which we call "*PG4*") that is equivalent to the *Md-PG1* gene associated with softening during room temperature ripening of apple and putatively involved in creating air pockets around the stone of peach.

4. Validation

The Parent Set and Diversity Set, which covers most of the currently used and near-future diversity of the PNWSCBP, acts as our Validation material. The Parent Set was slightly refined to include some additional ancestors of important PNW cultivars. In 2009, fruit quality data were collected for many of these parents and ancestors to help establish baseline performance predictions of progeny. A greater level of validation will be achieved in RosBREED, where 480 pedigree-linked representing the U.S. cherry breeding germplasm (the cherry Crop Reference Set) will be comprehensively genotyped and phenotyped.

Markers for *S*-alleles including self-fertility were already validated by the scientific community and in use worldwide. We used our improved multiplex genetic test to screen the Parent Set and thereby confirm *S*-alleles for many cultivars and ancestors, and obtained *S*-genotypes for some cultivars that were previously unknown.

Markers for fruit size were screened on the Parent Set. Fruit size alleles were traced through the generations of the Parent Set, and marker combinations were identified that appear to predict larger and smaller fruit based on the genotypes and fruit size of these parents and ancestors. For example, 'Glacier', 'Tieton', and 'Kiona' have an allele for BPPCT034 observed only in these large-fruited cultivars and their parent/grandparent 'Burlat' (allele 237).

Chloroplast genotyping was used to group cultivars into three lineages. Lineage "B" is the most prevalent in PNWSCBP germplasm carried by 'Van', 'Bing', and daughter cultivars. Lineage "C" is also common, introduced through 'Stella' and 'PMR-1'. Lineage "A" is rare, with no representation in locally grown modern cultivars. A diagram depicting these lineages was provided at the 2009 Cherry Field Day in June so that industry members could see the pedigree relationships of many cultivars and appreciate the power of DNA information such as the chloroplast markers to define genetic groups. However, unlike fruit size markers, chloroplast markers are not known to be trait-associated – instead helping define ancestral genetic relationships among cultivars through the maternal line and ensuring the Parent and Diversity Sets (-> Crop Reference Set) are comprehensive.

5. Utility

Utility assessment requires a pedigree-linked set of germplasm representing the breeding program with enough individuals to achieve sufficient statistical power. While such germplasm does not need to be physically separate from the breeding program, separation allows the trees to survive for longer for extra seasons of performance data to be collected. A separate germplasm planting does not (yet) exist for the PNWSCBP, but the '04 crosses – 245 seedlings from 22 crosses made in 2004, planted in 2006, and with 70% fruiting in 2009 – is a suitable set for current purposes.

The '04 seedlings, and 50 of '05 crosses, were phenotyped in the 2009 season for a range of traits within the routine operations of the breeding program and with funding support from this project. In addition to its value for evaluating performance of seedlings for breeding selection decisions, the comprehensive dataset is very valuable for determining utility of markers that have progressed this far through the MAB Pipeline.

*S*-genotyping was used to determine parentage of ‘04 seedlings and to identify self-fertile seedlings. Many unintended outcrosses, selfs, and incorrect assignments were revealed. Some of the seedlings with correct *S*-alleles for their intended cross may still have arisen from outcrossing, which additional marker genotyping can be used to identify. In fact, additional DNA information (from four fruit size markers) refined seedling parentage verification (Table 2). 104 self-fertile seedlings, carrying the $S_4'$ allele, were observed. Results were discussed at the 2009 Cherry Field Day in June (Prosser, WA), presented at the ISHS Symposium on Molecular Markers in Horticulture (Corvallis, OR), and written in a submitted paper for the journal Acta Horticulturae (Haldar et al. 2009). *S*-genotyping provides an excellent example of many MAB applications:

- Parent and cross choice (e.g. to avoid incompatible crosses)
- Evaluating crossing success (Table 2)
- Marker-assisted seedling selection (MASS; to select for self-fertile seedlings)
- Characterizing advanced selections and new cultivars (*S*-alleles to assign to compatibility groups and thereby speed adoption of new cultivars)

**Table 2**. Crossing success for seedlings of ‘04 crosses of the PNWSCBP, as initially determined by *S*-genotyping and then refined by four additional markers.

| Parentage | According to *S*-genotypes | Proportion by *S*-alleles | According to 4 more markers | Proportion by all markers |
|---|---|---|---|---|
| Intended | 143 | 59% | 166 | 68% |
| Self | 55 | 23% | 24 | 10% |
| Outcross | 28 | 12% | 28 | 11% |
| Does not belong | 17 | 7% | 27 | 11% |
| Total | 243 | 100% | 245 | 100% |

For fruit size genotyping, the four markers for the G2 and G6 QTLs were tested on all ‘04 seedlings (and ‘05 seedling genotyping is underway – currently ¼ complete). Statistical analyses are still underway, and ‘04 data will be added to the MSU dataset to even better define allelic effects across cherry and identify specific utility in the PNWSCBP, in time to inform spring 2010crossing decisions.

In the meantime, interesting and confirmatory results are being achieved, with an example shown in Figure 2. Conclusion: The genetic markers for fruit size developed at MSU in recent years with federal and WTFRC funding support will indeed be valuable for increasing breeding efficiency for large fruit in the PNWSCBP.

The recommended next step is expanding analyses from the 245 ‘04 seedlings to the ‘05 and ‘06 seedlings fruiting in the next couple of years. These 5000-6000 seedlings would represent the “training population” for verifying and characterizing utility of fruit size markers. We can then confidently answer questions of how to most efficiently improve fruit size, namely: how can the breeding program produce and plant a greater proportion of large-fruited seedlings, and what is the effect of genotypic selection for fruit size on other traits of importance (especially firmness and flavor)? Incorporating self-fertility into MASS considerations, we wonder: what is the effect of selecting for self-fertility on achieving enough seedlings with large fruit? According to ‘04 seedling results, we predict that early culling of self-incompatible seedlings would not much reduce the ability to obtain large-fruited seedlings from which to select additional traits of interest (Figure 3).

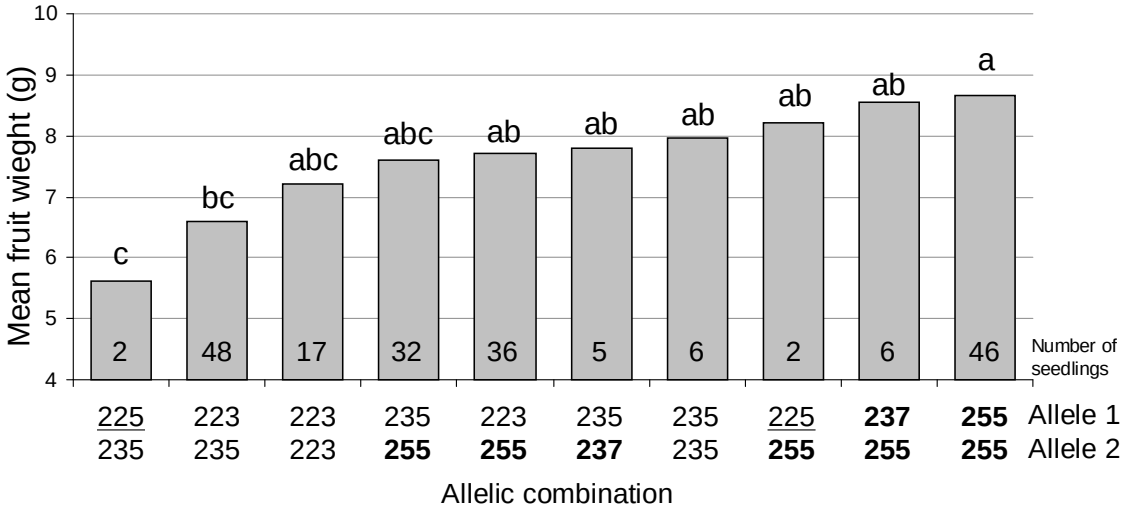


**Figure 2**. Fruit size in the 2009 season of '04 seedlings of the PNWSCBP according to allelic combinations of BPPCT034, a G2 fruit size QTL marker for cell number. Alleles in **bold** (**255** and **237**) were those predicted from their presence in large-fruited Parent Set cultivars to be associated with large fruit in seedlings. The allele underlined was predicted to be associated with small fruit. The **255** allele was also the one associated with large fruit, and 225 with small fruit, in the NY x EF experimental population (Zhang et al. 2009).

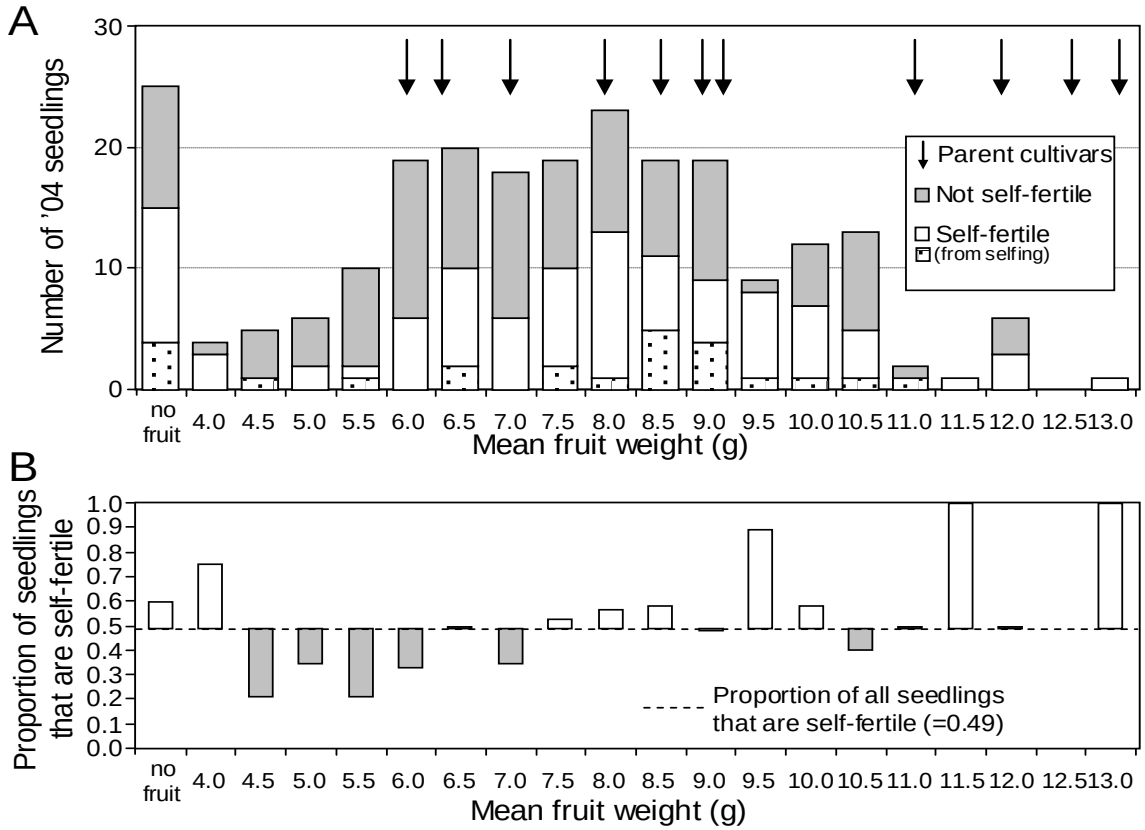


**Figure 3**. Fruit weight distributions observed in the 2009 season for '04 seedlings of the PNWSCBP. (A) Mean fruit weight distributions for self-fertile (including those derived from selfing) and self-incompatible seedlings. (B) Fruit weight distribution of proportions of seedlings that are self-fertile.

6. MAPS Decisions

Using DNA information to aid crossing between pairs of sweet cherry parents that result in a greater proportion of superior seedlings is a very efficient MAB application, even more so than seedling selection. Therefore, particular attention is being paid to obtaining such information and making it available in a format and timely manner to maximize use in crossing decisions. Marker information will be particularly informative for new parents to be used in the breeding program where such information has not previously been available – such as some of the best-performing '04 seedlings.

*S*-genotypes are being used to refine crossing decisions, by avoiding incompatible crosses and by preferring crosses that result in a high proportion of self-fertile seedlings. Selfing as a crossing strategy, which enables efficient doubling up of desirable alleles in seedlings but has been mostly avoided to date, is being reassessed with the observation from 2009 *S*-genotype data that field-planted selfs may perform as well as any other tree (i.e., without exhibiting inbreeding depression in the field). However, in future work, assessment is required of whether selfing results in lower seed set, reduced germination, and/or fewer vigorous seedlings.

Fruit size DNA information will be used from spring 2010 to support the development of families predicted to result in large-fruited combinations (e.g., families with a high proportion of seedlings with two copies of the 255 allele for BPPCT, according to the results shown in Figure 2). If we are able, fruit color markers will be used to predict proportions of fruit color types (mahogany vs. blush, and others) in each cross produced (see second last paragraph of New Marker Identification below).

7. MASS Cost-Efficiency and Logistics

We have used the MASS decision support tool to identify efficient MASS schemes for the PNWSCBP. The tool predicts that the best stage for genotyping will usually be while seedlings are in the lath house in early spring before being field-planted, although MASS instead at the preceding greenhouse stage should usually confer at least 90% of those savings. Culling after trees are already planted also appears worthwhile, – e.g., it typically confers ~75% savings of the best stage if done during the first year in the field, down to 20% or less by the fifth year. As routine field maintenance and fruit evaluation costs are greater than for the apple breeding program, potential savings with MASS are even greater when selecting for traits not expressed until trees reach reproductive maturity.

8. MASS Trial Use

High-throughput genetic screening is underway with '04, '05, '07, and '08 seedlings, to gain practical experience in MASS in the PNWSCBP. We will continue the Trial Use through the winter of 2009, especially for the >1000 seedlings of '05 crosses. We've found so far that:

- Genetic screening at the greenhouse stage will be the most logistically feasible, especially with the use of seedling pots arranged in 8 x 12 arrays to streamline information transfer from genetic screening to culling activities.
- DNA should be discarded following seedling phenotyping, as decisions will be immediately made on information obtained. Any further marker data desired on remained seedlings after planting (e.g., with new useful markers coming pipelined in future years) will be for descriptive purposes only and too late for culling, and thus can be gained by re-extracting and genotyping the small number of target seedlings. This approach eliminates the complication of ensuring individual labels on seedlings are maintained from greenhouse to field, that DNA is kept for an indefinite period, and that DNA samples are sorted to remove culled seedlings.
- Our high-throughput DNA extraction system is effective for older as well as young leaves.
- While the high-throughput genotyping system is still being optimized for cherry, the med-throughput system (large polyacrylamide gels) is a less-efficient, but successful fall-back with the current numbers of seedlings in the breeding program. Greater than a few thousand seedlings to be genotyped in any year will certainly require the high-throughput ABI platform.

- Routine implementation of MAB in the PNWSCBP can be implemented immediately in collaboration with the PNWTFGL in Pullman. While further refinements of logistics will be useful to streamline the process, and new markers are expected to enter the system over time, we can and are using MAB now in this breeding program, and have the opportunity to become one of the first tree fruit breeding programs in the world to routinely conduct high-throughput MASS.

Future routine MAB in the PNWSCBP

Partial support of a dedicated Genetic Screening Technician is recommended to conduct the genetic screening component of future MAB in the PNWSCBP (not to be confused with research assistance). Funds for this position from within operating costs of the PNWSCBP and the WABP, or separately funded to allow breeding programs to continue field operations and field evaluations at current capacity, will ensure the availability of the labor component of the genetic screening service in Pullman. Dr. Peace's research and development program, supported by federal and WTFRC grants and WSU infrastructure, is aimed at establishing the MAB Pipeline and developing new markers for the program. However, this program will not fund a routine genetic screening service through such research grants. Supporting research does not fall under the breeding program's operating budget, but rather under separate research projects such as those led by Dr. Peace (WTFRC-funded) or Dr. Iezzoni (federally funded).

The expectation is that the Cost-Efficiency calculator will identify MAB schemes that provide a *savings* to the breeding program even after the cost of genetic screening is taken into account. Therefore, routine cost-efficient MAB (compared to breeding by traditional phenotypic selection alone) will not only allow genetic screening via the PNWTFGL at no additional cost, but it will also provide a savings to the breeding program – arising from not having to plant, maintain, and evaluate genetically inferior seedlings. We will not implement routine MAB schemes that do not provide a net savings to the breeding program. Understanding this concept of MAB being a net savings and not a cost is critical for all involved.

New Marker Identification

New markers for flavor components of sweetness (overall and individual sugars), acidity, and stringency are under development using MSU data on the NY x EF population and combined with WTFRC-funded research on flavor candidate genes. As expected, the genetic components of sweetness remain difficult to pin down, as this trait is highly affected by non-genetic influences (e.g., maturity and water content). However, using data collected at MSU in 2008 (SSC) 2006-2008 (individual sugars of glucose, fructose, and sorbitol) in collaboration with Dr. Wayne Loescher, we have identified several possible genomic locations of sweetness-related traits, especially for proportions of individual sugars. The sugar profile of cherry fruit, as defined by such sugar proportions and predicted by DNA markers, may be a very important breeding selection criterion. Drs. Jim Olmstead and Dave Rudell reported (at the Plant & Animal Genome Conference in January, San Diego, CA) relationships between individual sugar proportions and SSC/TSS (total soluble solids – total of individual sugars) for ~70 cherry accessions (34 sweets, 19 tarts, and 12 related species) grown at the Davis Repository, CA. Interestingly, while proportions of glucose and fructose remain fairly stable across cultivars from low to high SSC/TSS, sorbitol is extremely associated with SSC/TSS, increasing in proportion almost linearly as SSC/TSS increases – for example, an increase of 1° Brix is associated in sweets and tarts with +1.4% increase in sorbitol on average, at the expense of glucose (-0.5%), fructose (-0.5%), and the minor sugar, sucrose (-0.3%). While sorbitol contributes as much as any sugar to a refractometer reading of SSC, it contributes to perceived (tasted) sweetness only 2/3 as much as glucose, 1/2 as much as sucrose, and 1/3 as much as fructose, according to sugar sensory science. Therefore, to develop cultivars with a pleasant sweet taste, breeding should target a relatively high fructose to sorbitol ratio (F:S) rather than relying only on SSC. Based on these

findings, using SSC as the primary selection criterion for sweetness is predicted to result almost invariably in the development of high sorbitol cultivars if the only parents used are from current elite germplasm. According to the Davis germplasm study, parent material with high F:S and a high SSC is very rare. However, the study of individual sugars of the NY x EF population at MSU indicates that NY54 is one of those rare high SSC + high F:S individuals, and NY x EF seedlings exist that have this sweetness attribute and medium (rather than tiny) fruit size. Such individuals are being used as parents in the PNWSCBP based on this knowledge. Furthermore, the collection of this phenotypic data in the genetically mapped NY x EF population provides the opportunity to dissect the genetic control of F:S and other sweetness attributes and develop predictive markers. Some markers for sugar proportions and ratios, not yet validated as being robust, were identified from QTL analyses of NY x EF and may be useful to track the introgression of high F:S from NY54 into breeding populations and ultimately new cultivars with unique and desirable sugar profiles.

QTLs for acidity and SSC were identified in 2008, but require further analyses to see if the marker-locus-trait associations were maintained in 2009. Phenotypic data were collected again in 2009, and QTL analyses will be conducted in late 2009.

The genetics of fruit skin, blush, and flesh color is being dissected by recent work conducted at MSU using the NY x EF population. The gene that is largely responsible for detecting mahogany vs. blush fruit types appears to be identified, and we are currently developing DNA markers for fruit color prediction. Such markers will likely be used in MAPS rather than MASS in the PNWSCBP. Both of the major fruit color types are desirable and so there is no purpose in culling one or the other at the seedling stage. However, because fruit color type defines the target market class of a potential new cultivar, and each target market class will have specific thresholds for other traits such as size and firmness, prediction of the proportions of seedlings that will fall into each color type for any given cross would be a valuable tool.

Using the genetic map based on the NY x EF population, markers for further traits can be pursued if the traits are genetically variable in the population and if they are measured. Therefore, additional traits measured in 2009 were astringency (0-2 scale, also measured in 2008), freestone (1-5 scale), and scar (tear or dry) Traits measured by the MSU team since around 2006 include harvest date (as well as fruit size and color previously mentioned). QTL analyses will be conducted for such traits in winter 2009. New useful marker-locus-trait associations for entering the MAB Pipeline are expected.

Outreach Activities

With the departure of Dr. Jim Olmstead, the outreach component of the project has not been conducted as planned, and allocated funds, thus far, remain unspent. Participation at the Cherry Field Day, using S-genotyping as examples for various MAB applications, somewhat addressed the outreach objective. We will participate in the 2009 WSHA Annual Meeting (Wenatchee, WA) to provide a poster and props to demonstrate MAB, involving technicians, graduate students, PIs, and the breeder, Dr. Oraguzie.

**References**:

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## EXECUTIVE SUMMARY

### Goals and Outcomes

The main goal of this 2009 project was to establish the marker-assisted breeding (MAB) Pipeline approach for the PNW sweet cherry breeding program (PNWSCBP). A further objective was to channel promising new markers into the Pipeline. We have successfully achieved these goals, made some interesting research discoveries, and secured substantial federal funding for the future enhancement and sustainability of this Pipeline for the PNWSCBP.

The third objective was to demonstrate the Pipeline to the sweet cherry producer community. The departure of Dr. Jim Olmstead, MAB outreach coordinator, hampered our activities in this area, but some efforts in 2009 were and will be made nevertheless.

### Summary of Findings

- To integrate the MASS approach into the PNWSCBP, cost-efficient and logistically feasible stages for conducting genetic screening were identified. These are the greenhouse and lath house stages, in the months prior to field-planting. Potential savings with MASS are highest for traits not expressed until trees reach reproductive maturity (such as all fruit quality attributes), and even more so than for the Washington apple breeding program (WABP).

- The genetic markers for fruit size developed at MSU in recent years with federal and WTFRC funding support will indeed be valuable for increasing breeding efficiency for large fruit in the PNWSCBP.

- S-genotyping of 245 ’04 cross seedlings determined that about 70% of seedlings had intended parentage. 104 self-fertile seedlings were identified.

- A genetic marker for self-fertility is available, but its use in early seedling selection is pending investigation of the opportunity cost to other important traits.

### Recommendations

The recommended next breeding step is to incorporate DNA information gained on parents, selections, and seedlings into breeding decisions in the PNWSCBP, and to consider the use of existing genetic tests for reducing the number of inferior seedlings to be field-planted from 2010.

The recommended next research step is to expand analyses from ‘04 seedlings to the ‘05 and ‘06 cross seedlings fruiting in the next couple of years. With fruit quality evaluations, these 5000-6000 seedlings would represent a powerful “training population” for verifying and characterizing utility of fruit size markers, the self-fertility marker, and selfing as a crossing strategy, to deliver efficient MAB schemes.

We recommend support of a dedicated Genetic Screening Technician, using funds from routine breeding program operating costs, to ensure labor availability for conducting the DNA marker screening component of future high-throughput seedlings selection in the WSU sweet cherry and apple breeding programs. We will not implement routine MAB schemes that do not provide a net savings to the breeding program, after accounting for Technician support and other molecular screening costs. Our concept of routine MAB is to reduce traditional operating costs, and recommend reinvestment of savings into breeding operations for increased efficiency of producing superior new cultivars for the PNW sweet cherry industry.