Enabling Marker-Assisted Seedling Selection in the Washington Apple Breeding Program

D.A. Edge-Garza and C.P. Peacea
Department of Horticulture and Landscape Architecture
Washington State University, Pullman, WA
USA

Yanmin Zhu
USDA-ARS Tree Fruit Research Laboratory
Wenatchee, WA
USA

Keywords: MAB Pipeline, Malus × domestica, Md-ACS1, cost-efficiency

Abstract
Breeding new cultivars is a slow and expensive process, but genetic screening tools exist to improve efficiency. We employed the marker-assisted breeding (MAB) Pipeline approach to facilitate routine use of MAB in the Washington apple breeding program. This study traced the journey of one marker-locus-trait (M-L-T) association from publication through high-throughput PCR-based genetic testing to application. The final stages of the Pipeline involve determining cost-efficient high-throughput marker-assisted seedling selection (MASS) schemes with available genetic tests, followed by a trial run on several thousand seedlings. For maximum cost-efficiency in the Washington apple breeding program, genetic screening should be implemented prior to expensive grafting and field planting operations but usually after inexpensive visual culling for traits such as disease susceptibility. Routine MAB is now enabled for this tree fruit breeding program and we estimate that at least 60% of conventional operating costs for first-stage seedling selection could be saved using the available genetic test.

INTRODUCTION
Breeding new cultivars is slow, incurring huge operational costs for growing and maintaining inferior seedlings in the field. Although genetic screening tools exist that might reduce these costs, and are used to this effect in many crop plants, little is being applied to tree fruit breeding. The Washington apple breeding program focuses on better eating quality, where flavor and texture are primary targets for improvement, and is a typical program that would benefit from routine marker-assisted breeding (MAB). An initial requirement for MAB is the availability of trait-locus-marker (T-L-M) associations, which describe specific cases of genetic markers for loci controlling particular traits (Bliss, 2010). A multi-stage pipeline approach has been developed that addresses various considerations in converting reported T-L-M associations into routine marker-assisted breeding tools (www.rosbreed.org).

The eight-stage “MAB Pipeline” is summarized as follows. First, available T-L-M associations are prioritized according to importance to the breeding program’s objectives. Second, efficient genetic screening methods are identified that allow ready access to the DNA information provided by the T-L-M association. Third, the originally reported genetic marker is adapted to available efficient genetic screening methods. Fourth, the T-L-M association is validated in crop-wide germplasm. Fifth, utility of the T-L-M association is evaluated in breeding program-specific germplasm. Sixth, T-L-M association information for parent cultivars is considered for parent crossing decisions. Seventh, cost-efficiency and logistics of MASS in the breeding program is analyzed to identify efficient strategies. Eighth, a MASS trial use is performed in a high-throughput

a cpeace@wsu.edu

Proc. IS on Molecular Markers in Horticulture
Eds.: N.V. Bassil and R. Martin
Acta Hort. 859, ISHS 2010
manner on seedlings in the breeding program. Successful progression through each of these stages is expected to enable implementation of marker-assisted breeding on a routine basis.

The present study traced the journey of one T-L-M association through the MAB Pipeline for the Washington apple breeding program, focusing particularly on the final stages.

**MAB PIPELINE**

**Available Trait-Locus-Marker Associations**

Many genetic markers are reportedly associated with horticulturally important traits in apple. One of these is the Md-ACS1-indel marker associated with a member of the 1-aminocyclopropane-1-carboxylate synthase (ACS) gene family. Genetic variation in the *Md-ACS1* gene, part of the ethylene biosynthesis pathway in ripening apple fruit and located on apple linkage group 16, is associated with postharvest storability, mostly affecting softening by differential allelic effects on natural ethylene production (Costa et al., 2005). Two alleles are common in modern apple cultivars. *Md-ACS1*-1, the ancestral version of the gene, is associated with normal fruit ethylene production, while *Md-ACS1*-2 contains an insertion in the gene’s promoter that is associated with lower ethylene levels (Sunako et al., 1999; Oraguzie et al., 2004). Individuals homozygous for the “1” allele produce fruit with normal (high) ethylene levels during ripening, heterozygous “12” individuals have lower ethylene production, and “22” homozygous individuals have the lowest ethylene production and are thus the most desirable in breeding for longer storage life.

**Prioritization (Stage 1)**

As the goal of Washington apple breeding is to improve the eating quality of apple (primarily flavor, texture, and appearance), the *Md-ACS1*-indel trait-locus-marker association is of high priority, warranting further progression through further MAB Pipeline stages.

**Genetic Screening Efficiency (Stage 2)**

Three methods of DNA extraction that were readily available, the metallic bead method, the silica bead method, and the Theonyx automated system, were compared for start-up equipment costs, running costs, throughput rates, and sampling logistics. The silica bead method was chosen for its relatively low start-up and running costs, high throughput rate, and simple tissue sampling requirements (i.e., freeze-drying not required).

A desktop study was performed to compare efficiency of available genotyping platforms. Comparisons were made of each genotyping technology’s range of genetic markers that can be analyzed, capacity in terms of sample throughput, logistics in terms of labor and time to prepare samples, and costs for each data point at different throughput levels. An efficient genotyping platform for the Washington apple breeding program was identified as fragment analysis on an ABI 3130xl DNA Analyzer.

**Improved Markers (Stage 3)**

The published Md-ACS1-indel marker test was originally developed for agarose gel electrophoresis and thus not immediately compatible with high-throughput genotyping on the ABI 3130xl. Primers were redesigned to amplify fragments within the range of the ladder standard used for the ABI 3130xl.

**Validation (Stage 4)**

Since the original publication of the *Md-ACS1*-indel trait-locus-marker association, results for several hundred cultivars and seedlings have been reported. *Md-ACS1*-indel predicts apple storability more reliably in mid- to late-season cultivars than in
early-season cultivars (Oraguzie et al., 2004, 2007).

Utility (Stage 5)

Fruit of mid- to late rather than early-season tend to be stored long-term for later distribution. As the Washington apple breeding program is interested in improving the storability of mid- to late-season cultivars of apple, a study based on this program’s germplasm determined that the Md-ACS1-indel marker was indeed strongly associated with fruit storability, and could theoretically be used for selecting superior individuals within the breeding program (Zhu and Barritt, 2008).

MAPS (Marker-Assisted Parent Selection) Decisions (Stage 6)

The Md-ACS1 genotypes of parent cultivars used in the Washington apple breeding program have been identified (Zhu and Barritt, 2008), and this information has been used in recent years to select parent combinations that are likely to result in a greater proportion of seedlings with long-storage fruit (i.e. without “11” homozygotes and especially with “22” homozygotes). Prior to availability of Md-ACS1-indel information, a cross made in 2006 between 'Honeycrisp' (“12”) and 'Cripps Pink' (“12”) produced several thousand seedlings, of which only one quarter are predicted to carry the most desired allelic combination (“22”) at the Md-ACS1 locus. Such a cross is not an efficient means of achieving new cultivars with long storage life, and has been avoided since.

MASS Cost-Efficiency and Logistics (Stage 7)

Determination of cost-efficiency and logistics of MASS began with an assessment of costs and time periods associated with conventional breeding operations. The first test stage of the Washington apple breeding program encompasses the first eight years after crosses are made (Fig. 1). This is the stage at which MASS would have impact by reducing seedling numbers. In the first test stage, seedlings numbers are halved by the time of planting (year 5) due to non-germination of seeds, low vigor for some 1-year-old seedlings, and susceptibility to disease for some 2-year-old seedlings (without any associated costs to conduct this culling), and reduced to less than 1% by the end of first test stage of seedling evaluations when only the best few seedlings are propagated for the multi-site second stage testing. Most traits of interest are assessed phenotypically in seedlings once they produce fruit, usually in years 7 and 8. The best periods for genetic marker screening appear to be prior to expensive bud-grafting and field-planting, particularly if markers are used to predict fruit phenotypes. The total cost of conventional operations per initial seed, from crossing to tree removal, was calculated to be approximately $20. However, because only about half of the initial number of seeds are eventually budded, planted, and evaluated in the field (Fig. 1), the average cost per initial seed is close to $10. The three conventional seedling reduction operations prior to budding have no associated costs. With this breeding scheme, several windows were identified for performing genetic screening (given the letters A to E in Fig. 1).

Cost and time parameters for conventional and marker-assisted breeding operations were modeled in a spreadsheet-based decision-support tool to identify MASS opportunities. The spreadsheet calculated savings per original seed theoretically achieved by implementing MASS, and identified the most cost-efficient and logistically feasible window for genetic screening. Parameter value estimates used in spreadsheet calculations for cull levels prior to bud-grafting were estimated at 10, 11, and 37.5% for lack of germination, low vigor, and powdery mildew disease susceptibility, respectively. With a 75% cull level provided by Md-ACS1-indel when used for ‘Honeycrisp’ x ‘Cripps Pink’ seedlings, MASS savings were predicted to be $6.24 per initial seed. The A window was identified as the most cost-efficient in this case. Numerous other hypothetical situations were examined and sensitivity analyses conducted that confirmed robustness of the decision-support tool. The C3 window was often identified as the most efficient, increasingly so when cheap phenotypic culling levels were high, although the A, B, and C windows were calculated to provide similar savings in most cases. We considered 2964
seeds of the ‘Honeycrisp’ x ‘Cripps Pink’ cross made in 2006. By the time we were ready to apply the improved Md-ACS1-indel marker, the window had progressed to C3, for which predicted cost savings were $6.12 per initial seed (or $18,130), only marginally less than for the optimal A window.

**MASS Trial Use (Stage 8)**

Genetic screening was conducted for the ‘Honeycrisp’ x ‘Cripps Pink’ cross, for which progeny size had been reduced to 1886 within the C3 window through normal breeding operations. DNA was extracted with the silica bead method, and the Md-ACS1-indel marker used to genotype seedlings on the ABI 3730xl platform. Realized costs of genetic screening were determined from maintaining records of labor and supplies.

Actual proportions of seedlings culled did not exactly match predictions. Seed germination and low vigor proportions followed predicted levels, but disease susceptibility was only about 20%, which resulted in 400 more surviving seedlings at C3 than predicted. While a cull level of 75% was predicted by the Mendelian inheritance pattern of Md-ACS1 alleles, 89% of the progeny actually showed the inferior genotype. Furthermore, genetic screening reliability was at about 80% (i.e., about 20% of DNA tests were blank due to failed DNA extraction or PCR). These alterations resulted in MASS being even more effective than predicted. Without MASS, an extra $7500 would have been spent on budding, planting, and evaluating additional seedlings not culled by disease susceptibility. The increased cull level due to the higher than expected proportion of seedlings with inferior genotypes saved a further $6000. However, the less-than-perfect genetic screening reliability resulted in almost $2500 of additional costs to re-screen blanks. Overall, MASS implementation saved approximately 60% of conventional costs that would have been associated with maintaining and evaluating seedlings with poor fruit storability due to their Md-ACS1 genotype. With 95% genetic screening reliability, which we are now routinely achieving, 70% of conventional breeding costs could be saved with the one genetic test.

**CONCLUSION**

The Md-ACS1-indel marker for fruit storability was progressed through the MAB Pipeline for the Washington apple breeding program, bridging the gap between a reported T-L-M association and routine marker-assisted breeding. Seedling selection using just this marker was confirmed as cost-efficient for this program: genetic screening of seedlings at the appropriate stage can currently save the program 60% of conventional operating costs. Efforts are currently underway to genetically screen thousands more apple seedlings using the Md-ACS1-indel marker, and to pipeline new T-L-M associations.

**ACKNOWLEDGEMENTS**

The candidness of Dr. Bruce Barritt, WA apple breeder (retired) in aiding scrutiny of conventional breeding operations, and enthusiasm for exploring MASS opportunities, is gratefully acknowledged. The continuing collaboration with Dr. Kate Evans, current WA breeder, is appreciated. We would also like to thank Daniel Serna, Bonnie Konishi, Danielle Baker, Stacey Haendiges, Jeremy Rowland, Terrence Rowland Jr., Vanessa Talbott, and Kylie Seger for technical assistance, and Deven See for critical advice on and access to additional facilities and equipment. This project was funded by the Washington Tree Fruit Research Commission.

**Literature Cited**


Figures

---

Fig. 1. The first seedling test stage of the Washington apple breeding program. Each letter corresponds to a possible window in which genetic screening of individuals could occur (A = prior to seed planting, B = greenhouse seedlings, C = prior to bud-grafting, D = prior to field-planting, and E = after field-planting). Associated costs for conventional breeding operations are included.