FINAL PROJECT REPORT

Project Title: Increasing decision confidence in cultivar development and adoption

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Agency Name: USDA-CSREES, Specialty Crop Research Initiative
Amount awarded: $2,000,000 + same matched by universities and industry (9/09-8/13)
Notes: "Tree Fruit GDR: Translating Genomics into Advances in Horticulture". PI: Dorrie Main. Co-PIs include Evans and Peace. "Breeder's Gateway" and "Grower's Gateway" efforts of that project benefited from specific examples of follow-through such as in this Decision Confidence project, allowing programming resources in the GDR project to be leveraged here.

Total Project Funding: $89,963

Budget History:
Budget 1: Washington State University

Item	2012
Salaries[1]	8,007
Benefits[1]	4,019
Wages[2]	13,000
Benefits[2]	1,937
Equipment	0
Supplies – molecular[3]	12,000
Travel – in-state	2,000
Travel – international[4]	3,000
Plot Fees	
Miscellaneous[5]	0
Total	43,963

[1] Salary for 3 months for genetic screening technician Terrence Rowland, 0.25 FTE, 50.2% benefits.
[2] Timeslip employment for PhD students Sujeet Verma and Yingzhu Guan and field and fruit lab technicians in Wenatchee, with 14.9% benefits for enhanced phenotyping efforts.
[3] $7000 for genome scans (96 WABP individuals to enhance that available from RosBREED data) and $5000 for fill-in data and new markers for specific trait locus genetic tests on WABP individuals in Peace lab, Pullman.
[4] Trip for PI Dr. Peace to Australia for late project discussions with co-PI Dr. Hardner and Australian apple industry reps.
[5] No cost to current project: funds remaining from 2011 project will be used for consultancy fees of co-PI Dr. Bink as in 2011 proposal; funds remaining from 2011 workshop (which did not involve Drs Bink, van de Weg, or Bliss in person due to unavailability) will be used to hold a workshop in summer 2012 (Activity 4).

Budget 2: Australia Crop Genetic Services Pty Ltd

Item	2012
Salaries[1]	40,000
Benefits	
Wages	
Benefits	
Equipment	
Supplies	
Travel[2]	6,000
Plot Fees	
Miscellaneous	
Total	46,000

[1] Consultancy fees for activity 1 and 2 analyses by co-PI Dr. Hardner, 40% time of one year
[2] $6000 for travel Australia to Washington for one week

RECAP ORIGINAL OBJECTIVES

Overall goal: Improve release and adoption decisions about the WABP's new scion cultivars by revealing and communicating genetic potential for commercial performance.

Specific objectives:
1. Improve efficiency and predictability of advanced/elite selection trials by optimizing field experimental design.
2. Put rapidly accumulating DNA information to immediate use by describing genetic potential of new cultivars in the context of commercial production.
3. Demonstrate to the WA apple industry the efficacy and opportunities of a combined field-design and DNA-based approach to decision-making.

SIGNIFICANT FINDINGS

Opportunities for increasing decision confidence in new cultivar development in the WABP and new cultivar adoption by the Washington apple industry were refined and enacted or explored, tested, and adapted to WSU's program for new cultivar development, release, and management.

Trial information: Evaluation and delivery of opportunities for improving efficiency of WABP field trials to accurately predict genetic potential (Objective 1)

- Sensory trait evaluation methods used in WABP P2 trials are robust; selection for any one trait is not likely to lead to detrimental consequences for other traits; and sensory evaluations may be relied on completely and instrumental measures removed
- Eating quality contributed more to overall fruit quality than did appearance quality; the former was highly influenced by crispness and juiciness and not firmness or acidity, while the latter was moderately influenced by russet amount and ground color but not size (P2)
- Doubling the number of individuals assessed in P2 would increase the probability of detecting elite selections; to achieve this for the same resource cost, incomplete designs could be used so that not all entries are assessed at each location
- Fruit only need to be evaluated following storage (or only at harvest) to adequately predict genetic potential; fruit evaluation at any age is suitable for predicting relative genetic potential of selections for most traits providing the average (main) effect of age is taken into account in the analysis; there is little value in increasing number of years of assessment to more than 2 years assuming use of 3 locations and 3 harvests per year for each entry; and there is little value in doubling the number of fruit assessed for each sample from 5 to 10
- Identifying storage disorder fatal flaws prior to P3 would provide a large efficiency gain
- Objective predictions of genetic potential for fruit quality traits based on all available information is recognised as the best approach in WABP trials, but the technology for streamlined delivery of this information each year requires further software development

DNA information: Evaluation and delivery of opportunities for use of DNA information to improve confidence in genetic potential prediction (Objective 2)

- Use of available DNA tests continued for crossing and seedling selection, providing large estimated resource savings.
- Our DNA test for acidity, crispness, and juiciness was used extensively in 2012 for seedling selection and also for crossing decisions.
- Our DNA tests for storability were used primarily in crossing decisions, helping to enrich new generations with superior alleles, which is the most efficient use of a trait-predictive DNA test. In addition to reducing the need for seedling DNA testing for the ethylene genes, generating a higher proportion of seedlings that are genetically superior for storability provides more opportunity to select for superiority in other traits.

- \>4000 seedlings predicted to be inferior for the above traits were eliminated. Their early culling provides an estimated future resource savings of $60K. Such resource savings can be redirected to focus on those plants with the best predicted chance of becoming new cultivars.
- Cultivars and selections have been placed in genetic categories describing predicted performance for bitter pit susceptibility, sunburn susceptibility, skin color, sweetness, and acidity. These advances in DNA information were made possible by the RosBREED project. New DNA tests for these traits are therefore now available for WABP use in 2013 onwards.
- An informal international collaboration was initiated to explore the promising approach of Genomic Selection for the WABP using RosBREED data.

Information to/from industry: Refinement of a WABP–industry information delivery and feedback system for increasing potential for commercial success of new WABP cultivars (Objective 3)
- The useful Trait target checklist approach will be advanced in late January
- The effectiveness of the many avenues used by the WABP for delivering information on genetic potential of new cultivars will be investigated in a dedicated socio-economics study in the planned RosBREED 2 project.

RESULTS & DISCUSSION

1. ***Improve efficiency and predictability of advanced/elite selection trials by optimizing field experimental design***
   = Evaluation and delivery of opportunities for improving efficiency of WABP field trials to accurately predict genetic potential

1a. <u>Adjust for main effects</u>
   Adjustment for main effects of year of assessment, age, and location were routinely included in quantitative analyses of P2 trials in 2012, as recommended from 2011 findings.
1b. <u>Increase replication</u>
   Following indications from initial results suggesting that increased replication of fruit number would increase accuracy of genetic potential of traits with low heritability such as instrumental sweetness, apples were juiced singly and soluble solids were recorded rather than the WABP typical practice of pooling juice from each sample of five apples. However, further analysis of this trait revealed that it was unlikely that this level of increased replication would have the desired effect so sampling reverted to the typical less time-consuming practice after the first few weeks of the 2012 season.
1c. <u>Enhanced fruit quality phenotyping</u>
   Juice samples from some WABP P2 selections and all the RosBREED germplasm from WSU, University of Minnesota, and Cornell University were obtained and will be run through a Gas Chromatograph before the end of this project to obtain profiles for individual sugars and acids by PhD student Yingzhu Guan, breeding trainee in the RosBREED project. Some evaluation of further germplasm of the Apple Germplasm Library at the Sunrise orchard was completed, concentrating on presence/absence of sunburn.
1d. <u>Genetic correlations among traits</u>
   Genetic correlation among most traits is low, which suggests that selection for one trait will not lead to detrimental consequences for other traits. In addition, these results suggest that HARDNESS and CRISPNESS are not being confounded by sensory assessors – they are appropriately being assessed as separate traits. Following are various findings for specific traits (in capitals, as they are labeled in WABP fruit evaluations).
   Genetic potential of entries (P2 selections and standard varieties in the trials) for the composite fruit quality rating of OVERALL fruit quality rating was more highly correlated with EATING QUALITY rating than APPEARANCE QUALITY rating. Therefore, eating quality

contributed more to assessors perception of overall fruit quality than did appearance quality. Genetic potential for the composite fruit quality rating of overall EATING QUALITY was moderately correlated with CRISPNESS and JUICINESS, weakly correlated with SWEETNESS and AROMATIC TASTE, and poorly with HARDNESS and TARTNESS. Therefore, assessor perceptions of eating quality were most influenced by CRISPNESS and JUICINESS, and very little influenced by HARDNESS or TARTNESS. Genetic potential for the composite fruit quality rating of overall APPEARANCE was moderately correlated with RUSSET and GROUNDCOLOR, indicating that both traits contribute equally to the WABP's consideration of overall appearance, but SIZE was not important.

Genetic potentials were similar for SWEETNESS and AROMATIC TASTE, and were also similar for CRISPNESS and JUICINESS; therefore, each trait within the pair could be controlled by similar genes and selection for one positively influences the other. A moderate negative relationship observed between HARDNESS and SIZE means that selections with smaller fruit tended to have firmer fruit or perhaps are more difficult to rate for HARDNESS due to limited flesh amount. Correlation between Digi-Test CN and CRISPNESS may be improved if variability in size is taken into account.

As expected there was a high genetic correlation ($r > 0.8$) between TITRATABLE ACID and TARTNESS, and SUGAR ACID RATIO and TARTNESS; however, the genetic correlation between Digi-Test M1 and HARDNESS, Digi-Test M2 and HARDNESS, Digi-Test CN and CRISPNESS, and BRIX and SWEETNESS was only moderate ($r = 0.5$-0.7). Correlation between Digi-Test CN and CRISP may be improved if variability in size taken into account.

1e. Connectedness among P2 trials

Models of alternative design for P2 trials indicated that doubling the number of individuals assessed in P2 increased the probability of detecting elite selections. Genetic analysis of P2 data indicated that the interaction between genetic potential and location was low. Therefore, for the same resources, incomplete designs could be used so that not all entries are assessed at each location, meaning more entries could be tested for the same resources. However, the risk of losing information from the loss of a location was felt to be too great to implement this design.

1f. Tree age effects

Age did not affect the overall mean of a trait, except Digi-Test FRUITWEIGHT. There is little evidence from the analysis that indicates the *relative* genetic potential of an entry to other entries differs among ages. There were few traits where the genetic potential was significantly affected by tree age (RUSSET and APPEARANCE SUMMARY) but the only a few entries changed rank and the influence was not large and there was no discernible pattern in the results.

1g. P3 sources of variation

A datafile of all variables assessed in P3 has been prepared for future analysis; however, the design of P3 trials is not as robust for statistical analyses compared to P2 trials. The November 2012 workshop concluded that P3 trials provide only informal estimates of mean performance and P3 is best focused on (i) fatal flaw detection, and (ii) providing familiarity with selection to breeder and potential adopters, particularly for traits with large environmental (and perhaps externally manageable) influences, although detection of the genetic potential for these types of traits are difficult without robust designs. However, many selections in P3 have been rejected based on a fatal flaw; therefore, it would be more efficient to accurately identify these fatal flaws earlier in the selection process. Options for improving methods to identify storage disorder fatal flaws prior to P3 were discussed during the November 2012 workshop; however, some participants suggested further research is required to develop strong recommendations for the adoption of these new methods.

1h. Streamlined delivery of quantitative genetic analyses

The mean genetic potential (for a trait) of a selection that is predicted based on all available information is widely accepted as the most objective and best estimate of the genetic potential of an individual. In the November workshop, the value to WABP of objective predictions of the

genetic potential for fruit quality traits was recognised. The accuracy of genetic potential predictions will increase as more data is available from ongoing annual assessments.

This project (and the previous 2011 decision confidence project) has developed the technology required for predicting genetic potential. However, to support adoption of this technology by WABP further work is need to extend software that has been developed for research purposes to operation software that can be routinely implemented by WABP staff and deliver user-friendly informative output.

1i. <u>Sampling assessment</u>

The two major sources of variation for all traits in WABP P2 trials was determined to be (1) genetic variation, i.e., differences among selections due to their differing genetic makeup, and (2) variation among samples after all other effects had been removed. The former indicates that the trials contain material with differences in genetic potential from which superior selections can be selected. The latter indicates that increasing replication will improve accuracy of predicting genetic potential, especially for certain traits like SWEETNESS and AROMA. Sensory trait evaluation methods used in WABP P2 trials are robust and any bias in individual evaluations are removed by taking the entry mean across multiple harvests, years, and locations, as indicated by the strong average effect of an entry across location, ages, years of assessment, and harvests, and the lack of large higher order interactions between entry and any of these factors.

The lack of significance and interaction for any fruit quality trait between entry and *storage condition* (i.e., fresh or two months storage) for most entries suggests that fruit only need to be evaluated following storage (or only at harvest) to adequately predict genetic potential. This conclusion was made because there was no significant difference between the ranking of entries for each trait assessed on fresh fruit or on fruit after two months storage except for HARDNESS and CRISPNESS. Golden Delicious was more susceptible than other cultivars evaluated to loss of CRISPNESS and HARDNESS after two months storage.

The lack of evidence of any significant and meaningful interaction between entry and *age* for any fruit quality trait suggests that evaluation at any age is suitable for predicting genetic potential, providing the average (main) effect of age is taken into account in the analysis.

A model of the accuracy of predicted genetic potential was developed to evaluate the impact of alternative sampling designs for P2 trials. There was little increase in accuracy of predicting genetic potential by increasing number of years of assessment to more than 2 years, assuming use of 3 locations and 3 harvests per year for each entry. Assuming each observation is a sample of 5 fruit, the accuracy of predicting genetic potential was not greatly improved by doubling the number of fruit assessed for each sample.

Use of instrumental measures for indirect prediction of genetic potential for sensory fruit quality traits was less accurate than direct selection on the sensory traits themselves, assuming the sensory trait evaluated is equivalent or closer to the sensory trait used by consumers to determine appeal. As instrumental methods are not available for all sensory traits (hence some sensory evaluation is required) and there is no large cost saving in not assessing some sensory traits if some sensory evaluation is required, there seems no strong logic to replace sensory with instrumental evaluation. This may be revised if the correlation between Digi-Test CN and CRISPNESS can be improved, and an instrumental means of assessing SWEETNESS, GROUNDCOLOR, and RUSSET can be found that has a high genetic correlation with these traits that are the main determinants of OVERALL fruit quality rating.

2. ***Put rapidly accumulating DNA information to immediate use by describing genetic potential of new cultivars in the context of commercial production***
= Evaluation and delivery of opportunities for use of DNA information to improve confidence in genetic potential prediction

2a. _Ma_ locus information – refinement of use in breeding

The WABP's use of the _Ma_ locus DNA test that partially predicts seedling performance for the valuable traits of **crispness**, **juiciness**, and **acidity** (and perhaps also firmness and several other traits) was increased in 2012 over previous years. Large resource savings were calculated to have been made by incorporating this DNA information into parent and seedling selection decisions, assuming that the selections made according to this test will result in superior material in the breeding orchard. Descriptions below exemplify what can be achieved with any DNA test.

In _parent selection_, the DNA test was used in 2012 to enrich for alleles that tend to confer in superior levels in these traits, especially from Honeycrisp, and to avoid the accumulation of inferior alleles in P1 breeding populations. This use of the _Ma_ locus DNA test in supporting crossing decisions helped refine the creation of P1 populations for which a higher proportion of seedlings are expected to perform at and above target levels for these traits. In a few years when the P1 seedlings produce fruit in the breeding orchard and a higher proportion of seedlings achieve selection thresholds used in previous years, the choice can be made to (1) concordantly increase the number of "keepers" entering P2 or (2) to raise the bar for one or more traits thereby maintaining a similar number of "keepers" that have even better genetic potential for the DNA-tested traits than has been possible with conventional parent selection approaches. Note that such DNA-informed parent selection has been performed since ~2006 using the DNA tests of ethylene genes for fruit storability (2b. below), and so the resulting seedling populations that are now beginning to fruit should, on average, have improved genetic potential for storability than in previous years. We have not yet begun after-the-fact formal evaluation of how well DNA-screened seedling populations are performing compared to efforts before this "revolution". Another advantage of DNA testing in parent selection is the reduced need for DNA testing on seedlings for those particular tests used to guide crossing.

In _seedling selection_, more than 7000 seedlings were DNA-tested in 2012 (mostly for the _Ma_ locus and a little for one of the ethylene genes) – approximately double the number screened in 2011for the _Ma_ locus. Almost 60% of seedlings were judged by the DNA tests to be genetically inferior and were culled. This number of strategically screened seedlings represents more than half the young seedlings raised in 2012 and an estimated net resource savings in the next few years of $60,000 by avoiding the costs of raising those suspected inferior seedlings and instead concentrating efforts on those plants with the best predicted chance of becoming new cultivars. Progress in late 2011 and early 2012 in understanding the relative effects of the multitude of _Ma_ locus genotypes present in breeding germplasm increased the number of target desirable and undesirable marker genotypes.

The treasure trove of data from the RosBREED project (obtained from high-resolution genome scanning and multi-year standardized phenotyping at massive, unprecedented scales) is being mined for value to the WABP. For example, predictions for acidity levels according to genotypes at the _Ma_ locus on chromosome 16 have now been refined by consideration of a second major locus (the "_Acidity_" or "_A_" locus) on chromosome 8. When effects on acidity are analyzed separately, both loci are observed to have a low, a medium, and a high acidity genotype (Figure 1), making each DNA test a useful semi-predictor of acidity levels among individuals.

A more informative and detailed picture is achieved when the two loci are examined jointly (Figure 2). While in general the two loci combine additively, the interesting exceptions are the high-low (_Ma-A_) and low-high combinations pertaining to genetic categories 7 and 3, respectively. Genetic category 7 is associated with low acidity (bland-tasting fruit) rather than the expected medium to high acidity considering both loci. Or if only the _Ma_ locus was considered, high acidity would be expected. Similarly, predictions according to the "_A_" locus are highly dependent on the configuration of the _Ma_ locus. Therefore, when breeding for medium- to high-acid new cultivars, either locus alone is not a suitable predictor; a combined test based on both the _Ma_ and "_A_" loci would be preferred. Such a combined test is now available for WABP parents and elite selections.
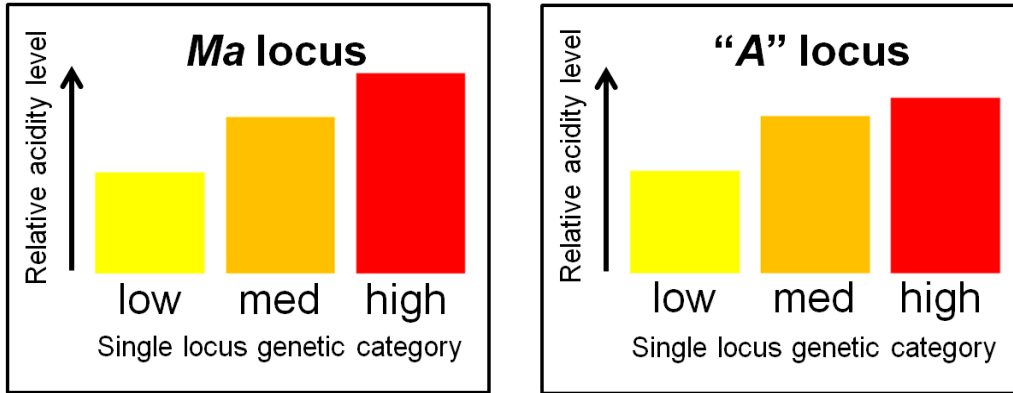
Figure 1: Relative acidity levels according to two regions in the apple genome independently influencing fruit acidity, the Ma *locus and the "A" locus. Effects were determined from a dataset generated in the RosBREED project based on observations on more than 600 individuals (pedigree-linked cultivars, selections, seedlings, and ancestors) representing U.S.-wide apple germplasm, Washington certainly included, that have been (1) evaluated for fruit quality for two years at harvest and after 10 weeks and 20 weeks of cold storage and (2) genome scanned with the high-resolution 8K International RosBREED Consortium Apple SNP array.*
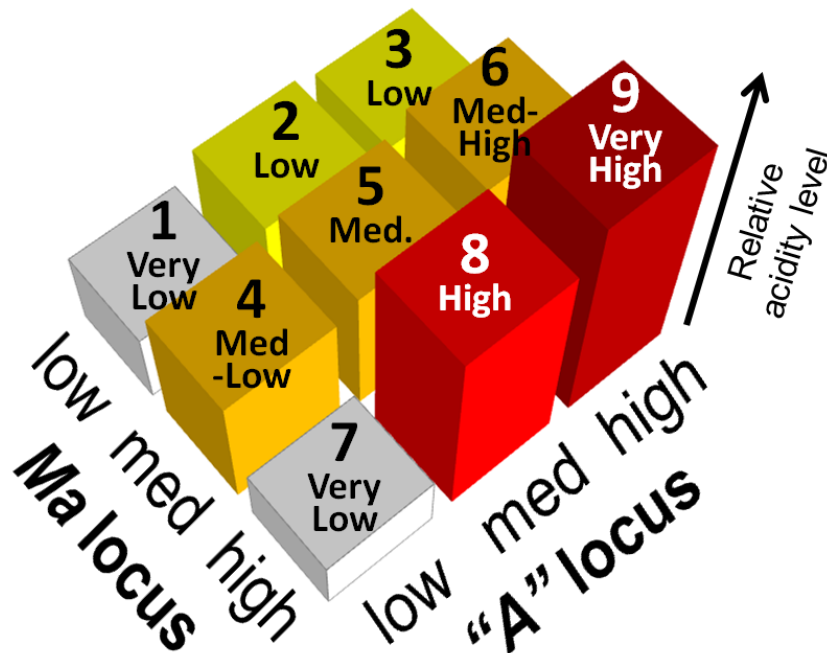

Figure 2: Relative acidity levels of nine genetic categories defined by DNA markers at two regions in the apple genome jointly influencing fruit acidity, the Ma *locus and the "A" locus. Descriptive labels from "Very Low" to "Very High" were given based on quantitative differences that were consistently observed within each year-and-storage-regime dataset and averaged across the entire dataset. The entire dataset was that described for Figure 1. Calculations were made with the simple case of just two contrasting alleles (high acid, low acid) for each locus. In the future, further distinctions could be made within these alleles for sub-types with intermediate and/or more extreme effects, but for now these two-allele models are suitable as they already explain a large degree of genetic variation for acidity in breeding germplasm.*

The nine acidity genetic categories were consistent over storage and over two years, as described below. Specific predictions for titratable acidity (TA) across three storage regimes were calculated based on the available two seasons of data (2010 and 2011) from the RosBREED project. Such predictions can be expressed as the probability that an individual belonging to one of the nine acidity genetic categories is above or below a defined TA threshold (Figure 3). These results are on a finer scale to those of Figure 2: while the latter considered both years and all storage regimes at once, the predictions in Figure 3 separate each year-storage dataset but are still grouped according to the *Ma-A* genetic categories. The Very Low categories (7 and 1) often have probabilities of greater than 50% that fruit will be at least 0.1 units below the average TA observed for a given year and storage regime, and often have probabilities of less than 10% that fruit will be at least 0.1 units above the average (Figure 3) – in other words, they tend to have bland fruit. In contrast, in the Very High category (9), those probabilities are reversed – tart fruit.
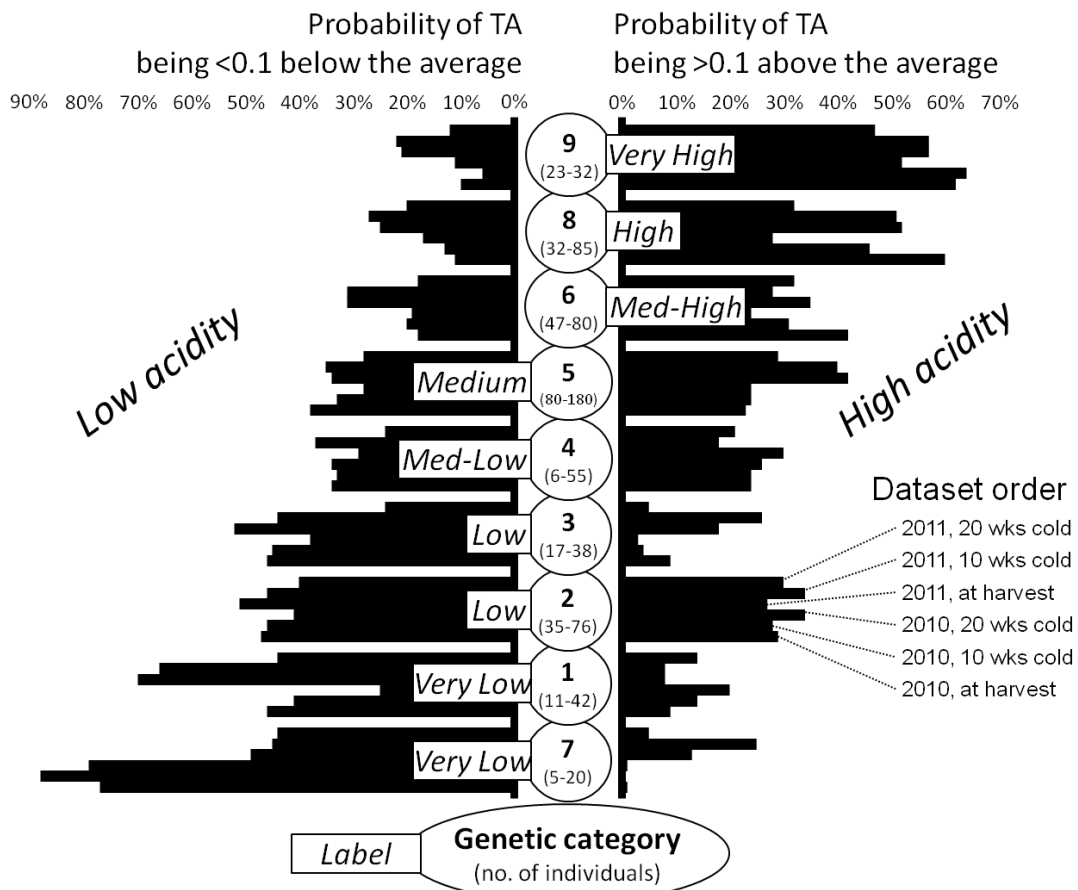

Figure 3: Estimated probability of an apple cultivar (or selection or seedling) achieving thresholds for titratable acidity (TA) before and after cold storage, according to genetic categories defined by two acidity loci. The dataset was that described for Figure 1. The trait acidity loci, Ma *and "A", and their nine genetic categories are those described in Figure 2. Probabilities were calculated by converting all observations in each category into Normal distributions and using the NORMDIST function in Excel with thresholds of 0.1 units (%) of TA above and below the average TA of all individuals in a given year and storage regime. Results for sensory acidity ("tartness", scored on a 1-5 scale) were very similar to TA, as expected because these two measures of acidity were highly positively correlated.*

Interestingly, no commercial cultivars were observed to belong to either acidity extreme (Very Low or Very High, categories 7, 1, or 9), and few belonged to the Low or High categories (2, 3, and 8). The bulk of commercial cultivars were in the Medium acidity genetic categories (4, 5, and 6). However, half the seedlings arising from any random cross will be in the non-Medium categories and therefore could be avoided (or higher acidity purposely targeted) by using the DNA information described here in crossing decisions and/or in greenhouse seedling screening. This DNA information on the *Ma* and "*A*" loci regarding acidity will be incorporated in 2013 breeding decisions.

2b. <u>*ACS* and *ACO* gene information – refinement of use in breeding</u>

As in previous years, the two DNA tests for **fruit storability** based on the ethylene genes, *ACS* and *ACO*, were used in *parent selection* to avoid creating populations with a high degree of seedlings containing alleles for high ethylene levels that tends to be associated with poorer storage potential. Numerous elite WABP selections, genotyped for these genes, are now available that serve as efficient parents. This creation of seedling populations enriched for low ethylene alleles helping to confer extended storability has meant that the use of these DNA tests in *seedling selection* is much reduced from previous years. This outcome highlights the positive connection between these two levels of DNA testing. Another way to look at it is that most DNA tests should have a limited shelf life for seedling selection as long as the DNA information is being even more efficiently used for parent selection. All else being equal, it takes much less effort to make a cross resulting in no inferior seedlings at a particular region in the genome than it does to make a different cross that requires screening and culling of the inferior seedlings. In addition, the efficient crosses result in more seedlings superior at the targeted genomic region, providing greater opportunity to select for superiority in other traits and genomic regions.

2c. <u>Use of further DNA information in the WABP</u>

Further DNA information has been gained from the RosBREED dataset for various fruit quality traits (2d. below). WABP parents and most P2 and P3 selections have been placed into genetic categories determined by genomic regions influencing traits such as susceptibility to bitter pit, susceptibility to sunburn, and amount of blush/stripe cover. In addition to use in *parent selection* beginning in the 2013 crossing season, this DNA information will now advise breeding decisions in *elite selection advancement*.

2d. <u>Predictability of further genetic tests</u>

Important external appearance traits of **susceptibility to bitter pit**, **susceptibility to sunburn**, and **skin color attributes** (amount of blush/stripes, relative degree of blushes vs. stripes, and cover hue) were chosen for in-depth genetic analysis using the RosBREED dataset. The WABP component of the RosBREED dataset was specifically investigated for bitter pit and sunburn susceptibilities to find those genomic regions influencing these traits as they occur in this region. Genomic regions were indeed found, and genetic categories developed for them. For example, two bitter pit loci were identified and combined to produce four informative genetic categories (Figure 4).

For **sweetness**, PhD student Yingzhu Guan found some genomic regions influencing the various measured aspects of sweetness (SSC, sensory 1-5 scores, and amounts of individual sugars) within the RosBREED project, but these regions have yet to be converted into predictive DNA tests. Similarly, PhD student Sujeet Verma found several genomic regions influencing crispness, juiciness, and/or acidity (in addition to the *Ma* locus and "*A*" locus described in 2a.) but DNA tests targeting them have not yet been developed. Many **other fruit quality traits** measured in the RosBREED project on breeding germplasm representative of the WABP, including **susceptibility to various other disorders**, await analytical attention.
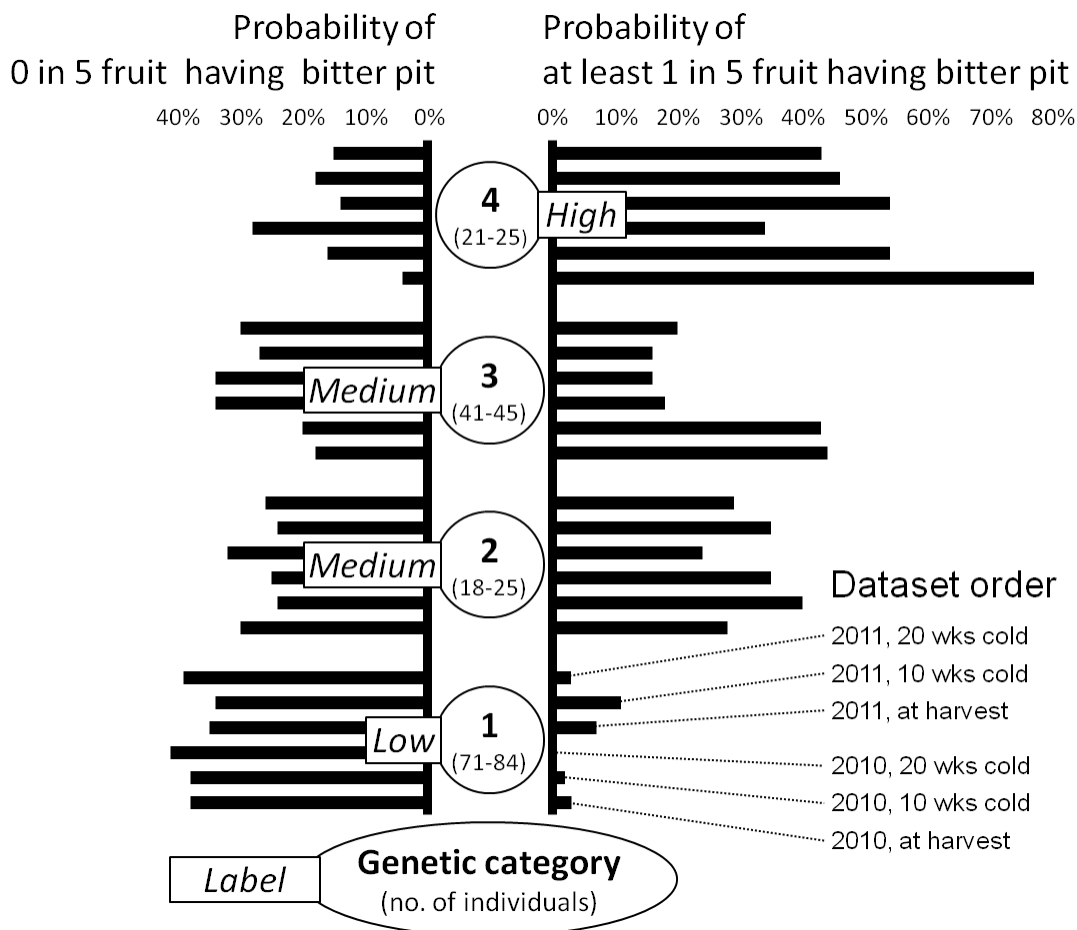
Figure 4: Estimated probability of an apple cultivar (or selection or seedling) achieving thresholds for bitter pit incidence before and after cold storage, according to genetic categories defined by two bitter pit loci, Bp-1 and Bp-2. Four genetic categories were developed jointly for the two bitter pit loci. The dataset was that described for Figure 1; probabilities were calculated as described for Figure 3.

2e. Genetic Selection

The 2012 November workshop extensively discussed the use of thousands of DNA markers across the whole genome for constructing Realized Relationship (RR) coefficients and Genomic Selection methods for improving the accuracy of predicting genetic potential of entries. Preliminary work on the application of RR approaches to RosBREED data was undertaken but hampered by incomplete datasets and inconsistencies in results between pedigree and RR methods. An informal collaboration between RosBREED, Plant & Food Research (New Zealand), and University of Queensland (Australia) was initiated to move this research forward to provide a stronger foundation for the implementation in operational breeding. Genomic selection is being applied operationally in New Zealand apple breeding to greatly reduce the number of entries entering advanced testing field trials. Conditions required for implementation of RR or GS are: technical expertise, dense marker array (further research required on how dense), large training populations with accurate phenotyping, and recalibration of training population after several generations of breeding. Results from the RosBREED analysis will be used to further evaluate the opportunities for WABP.

3. ***Demonstrate to the WA apple industry the efficacy and opportunities of a combined field-design and DNA-based approach to decision-making***
   = Refinement of a WABP–industry information delivery and feedback system for evaluating and increasing potential for commercial success of new WABP cultivars

3a. Target trait checklist

The checklist, with its list of traits, target levels (thresholds), and standard cultivar anchors to transparently align breeding targets, selection thresholds, and new DNA test development, has two main useful features over previously used approaches to prioritizing among traits (1) it considers trait levels rather than traits themselves, and (2) it splits trait levels into two categories: Essential (or Needs) and Bells & Whistles (or Opportunities). Discussions in the November workshop focused on what the numbers really mean, when to best use it, and how it aligns with socio-economics results coming from the RosBREED project. This topic will be discussed further with the Breeding Program Advisory Committee (BPAC) and WTFRC & industry representatives in the Genomics, Genetics, and Breeding meeting in late Jan 2013 immediately prior to the WTFRC Apple Research Review.

3b. Information delivery and feedback

Information about new WABP cultivars was delivered through Good Fruit Grower articles (Hanrahan et al., issue 63[11]; Evans and Barritt, issue 63[14]), storage season presentations in Yakima and Wenatchee which included fruit tasting, field days (with fruit), fact sheets, posters, fruit and presentations at grower meetings, and the delivery of gift boxes to numerous industry contacts throughout the year. The effectiveness of each avenue will be investigated in the planned RosBREED 2 project in a dedicated socio-economics study, with involvement by Dr. Desmond Layne. The industry advice channels for the WABP were restructured during 2012 with the formation of the BPAC and a Cultivar Licensing Committee (CLC) who would take over the different roles of the IAC. BPAC members had their first opportunity to taste some Phase 2 selections at the November meeting. Initial discussions outlining technology transfer options for the WABP have already taken place with Dr. Layne with a view to greatly increasing the web presence of the WABP once Dr. Layne takes up his Extension Leader appointment to WSU in February.

EXECUTIVE SUMMARY

Choice of scion-rootstock combinations is the single most profit-influencing decision that a grower can make. While planting standard scion cultivars is an option to mitigate risk, new cultivars from breeding efforts provide sustained genetic solutions to flaws in standard cultivars or to address market opportunities.

The Washington Apple Breeding Program (WABP) creates new genetic combinations, selects among them to find winners, and then releases superior new cultivars to the Washington apple industry. This program is resource-hungry in running costs, land, expertise, and time, and success is not assured. But the potential payoffs for the Washington apple industry are enormous and justify the huge investment. We sought to increase decision confidence in new cultivar development and adoption by ensuring the breeding program is aiming for the right targets, is accurate and efficient in its execution, and is effective in conveying successful outcomes to industry clients.

Detailed investigation of selection trial design, especially of Phase 2, identified numerous strengths that underscore progress to date in developing superior new cultivars. Some opportunities were identified to consider for further streamlining routine operations. Such opportunities include:
- reducing redundancies on sensory and instrumental measures of the same essential trait
- focusing more on those traits contributing to the decision to move selections forward
- evaluating fruit only after storage
- reducing the number of years of fruit evaluation
- doubling the number of selections in Phase 2 trials, which could be achieved with the same resources by use of incomplete trial designs
- identifying storage disorder fatal flaws prior to Phase 3
- software development to provide breeder access to routine complex analyses of all breeding data so that genetic potential predictions are objectively based on all available information

DNA information was used to improve confidence in genetic potential prediction. The RosBREED project channeled further DNA information into the WABP. Successes include:
- use of increasingly refined DNA tests for storability, crispness, juiciness, and acidity in crossing and seedling selection to provide large estimated resource savings ($60K from marker-assisted seedling selection, probably even more for marker-assisted parent selection)
- genetic categorization of cultivars and selections to describe predicted performance for bitter pit susceptibility, sunburn susceptibility, skin color, sweetness, and acidity
- availability of new DNA tests from 2013 onwards for the above-mentioned traits and others

The WABP–industry information delivery and feedback system for increasing potential for commercial success of new WABP cultivars was addressed in 2012. Advances include:
- the WABP's use of many avenues for delivering information on genetic potential of new cultivars, the effectiveness of which is planned for investigation in a dedicated socio-economics study in RosBREED 2
- Dr. Des Layne being hired into the role of tree fruit extension team leader at WSU

More detailed reports on project components, including a summary of the November workshop, are available to apple industry members on request.