RosBREED
Enabling marker-assisted breeding in Rosaceae
www.rosbreed.org

Dedicated to the genetic improvement of U.S. rosaceous crops

May 31, 2011 **Volume 2 Issue 2**

RosBREED's Second Annual Project Meeting, East Lansing, Michigan, March 2011

The second annual meeting of the RosBREED participants was held in East Lansing, Michigan from March 8 - 11 at the James B. Henry Center and was attended by 47 scientists. The first day and a half of the meeting was a general workshop for the RosBREED group, during which an update on the overall objectives of the project was laid out along with how each of the RosBREED teams were contributing to the overall goal. Breakout sessions were held to focus discussions on upcoming project activities. A highlight of this meeting was the "Show and Tell" session that focused on the attributes of apple, peach, cherry and strawberry fruits – what is available now versus which new fruit quality traits will be available in the near future through RosBREED's interventions (more on page 2).

RosBREED's Executive Committee, from left to right: Gennaro Fazio, Dorrie Main, Cholani Weebadde, Eric van de Weg, Amy Iezzoni, Cameron Peace, Jim Luby, Jim McFerson, Nahla Bassil, and Chengyan Yue

RosBREED participants who attended the 2011 yearly meeting

Inside this Issue

NIFA
USDA
United States Department of Agriculture
National Institute of Food and Agriculture

RosBREED II - "Show and Tell"

As a part of RosBREED's Second Annual Meeting, ten of the apple, peach, cherry and strawberry Demonstration Breeders of RosBREED led a "Show and Tell" session at the James B. Henry Center (East Lansing, MI) on March 8, 2011.

This session focused on the attributes of the currently available fruit in the markets and what is on the horizon through RosBREED's efforts. John Clark spoke about the quality and taste of fresh market peaches that are available to consumers versus a ripe peach that is picked from a tree and eaten. John proclaimed, "Most of you would agree: A good peach is hard to beat. But most of you would agree: A good peach we ain't got."

The session was well received. For more details follow the web links below in "RosBREED in the News".

John Clark (University of Arkansas), Ksenija Gasic (Clemson University) and Dave Byrne (Texas A&M) describing the peaches currently available in the markets

Susan Brown (Cornell University) and Kate Evans (Washington State University) looking at Honeycrisp apples

RosBREED in the news

To read more about RosBREED's "Show and Tell", please visit the following online magazine articles (click on the article title):

Fruit Grower News Magazine
RosBREED bringing fruit breeders together

Good Fruit Magazine
RosBREED collaborators hold planning session

RosBREED by the numbers

2,734,080

Number of SNP markers generated in the last four weeks by the Michigan State University iSCAN facility for 455 tart and sweet cherry cultivars, ancestors, and representative seedlings.

Amy Iezzoni and Dan Zarka (Michigan State University) downloading the barcode information for the SNP arrays

RosBREED's Pedigree-Based Analysis (PBA) Team continues data analysis support through software development, training and hands-on assistance

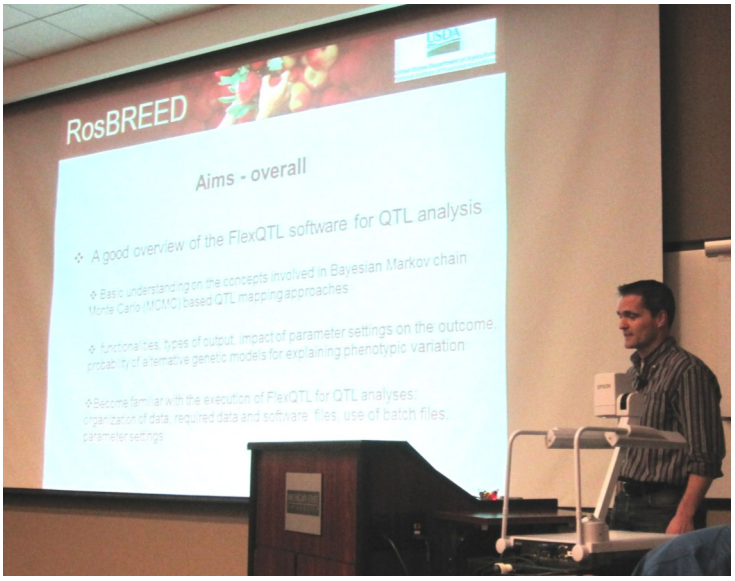
By Cholani Weebadde, Extension Team Leader

RosBREED's PBA Team is responsible for developing and documenting software and training material and training of the project's breeders and their Breeding Trainees in the use of PBA (a statistical framework designed to identify diagnostic genetic tests to inform breeding decision-making) in addition to providing support with their data analyses. The PBA approach helps enhance the efficiency of plant breeding, as its results can be used to predict the genetic potential of breeding parents so that efficient parental combinations can be made.

PediMap and FlexQTL™ are two software programs that RosBREED is establishing for the U.S. Rosaceae community for data analysis and management. The PBA Team conducted its first hands-on training in the use of PediMap software for RosBREED's Demonstration Breeders and their Breeding Trainees at an introductory session in January, 2010 (in San Diego, at RosBREED's First Annual Meeting) and at a two-day workshop in June 2010 at Michigan State University, East Lansing, Michigan. Dr. Roeland Voorrips [Plant Research International (PRI), Netherlands], father of PediMap, conducted the session in San Diego while the PBA Team Leader, Dr. Eric van de Weg (PRI), conducted the PediMap and FlexQTL™ sessions in East Lansing.

As a direct follow-up to these two sessions, a two-hour webinar was conducted by Dr. Umesh Rosyara (Michigan State University) as a refresher to the 2010 training. The refresher was held just prior to the two-and-a-half-day workshop organized in East Lansing from March 9 - 11, 2011 to provide further training in FlexQTL™ software. This workshop was attended by 44 participants and included 10 of the 12 Demonstration Breeders of RosBREED and all of the 12 Breeding Trainees. Drs. Marco Bink (PRI) and Eric van de Weg conducted all the sessions of the workshop. At this workshop, discussions were held about the importance of controlling the quality of data obtained and analyzed. As a result, monthly webinars were initiated between the PBA Team (Drs. Eric van de Weg and Umesh Rosyara) and the project participants so that the PBA Team can provide timely data analysis support.

The next PBA training workshop is scheduled for March 13 - 15, 2012 in East Lansing, Michigan. The focus of the workshop will be on discovery of QTL for fruit quality traits and interpretation and application of FlexQTL™ breeding value output in crop improvement programs.



Marco Bink, PBA Team member, Plant Research International, Netherlands and Eric van de Weg (not pictured) conducted the lectures and software exercises.

PBA Workshop attendees discussing conceptual breakthroughs

Jim McFerson (Washington Tree Fruit Research Commission) and Travis Stegmeir (Michigan State University) working together on PBA workshop exercises

Community Breeders' Page

The Marker-Assisted Breeding Pipeline: Channeling socio-economic and DNA information into routine breeding operations

By Cameron Peace, MAB Pipeline Team Leader

***In this issue*:**
"Fast-tracked" MAB Pipelining

What's in a byline?

Enabling marker-assisted breeding in Rosaceae. What does it mean? "Enabling" means we are not just touting the promise but providing the instructions for implementation. "Marker-assisted breeding" (MAB) means integration of socio-economic and DNA information into routine breeding operations, supporting breeding decisions with supplemental knowledge. "In Rosaceae" means across the crops belonging to this diverse plant family, such as almond, rose, pear, and raspberry, in addition to the crops included for demonstration purposes of peach, cherry, strawberry, and apple. Scion or rootstock breeding, at public or private institutions – all are our target beneficiaries. Knowledge, tools, and skills obtained for one Rosaceae crop breeder are often applicable to others.

The MAB Pipeline in action in 2011

We enable MAB with the MAB Pipeline. The MAB Pipeline is a systematic process for identifying and conducting efficient breeding schemes. Each stage is illuminated to light the way to successful MAB. Over its four-year duration, RosBREED is establishing powerful infrastructure for each stage to enable MAB. But rather than waiting until the whole edifice is built before implementing efficient parent and seedling selection schemes, "fast-tracked pipelining" is underway to bring to fruition several marker-locus-trait associations that are available, very promising, yet unused in Rosaceae breeding… until now. With jewels in the genome that excite our Demonstration Breeders, 2011's goal for the MAB Pipeline Team has been to inform parent selection decisions as soon as possible. Achieving this goal requires rapid progress of promising markers through Pipeline stages 1-6 (Figure 1, next page).

Fast-tracked pipelining is positively benefiting apple breeding *now*. In Minnesota, breeders Jim Luby and David Bedford used DNA information in the form of functional haplotypes for the *Ma* locus (this issue's Jewel in the Genome) to choose exciting cross combinations and avoid certain others. Spin-off benefits of the translated marker data included confirming pedigree records for most Minnesota selections, detecting some incorrect records, deducing likely pedigrees in certain cases, and providing confidence in the genetic potential and identity of elite selections on the path to commercial release or in use as parents. A second targeted trait locus for fast-tracking, *MdExp7* for fruit firmness, also revealed useful functionality for Minnesota apple breeding and was incorporated in parent selection decisions this May. Jim and David, welcome to the rank of DNA-informed breeders!

Jim Luby and David Bedford, University of Minnesota, tasting crisp, delicious apples from their breeding program

Community Breeders' Page cont.

Fast-tracked MAB Pipelining…

RosBREED's MARKER-ASSISTED BREEDING PIPELINE in action!

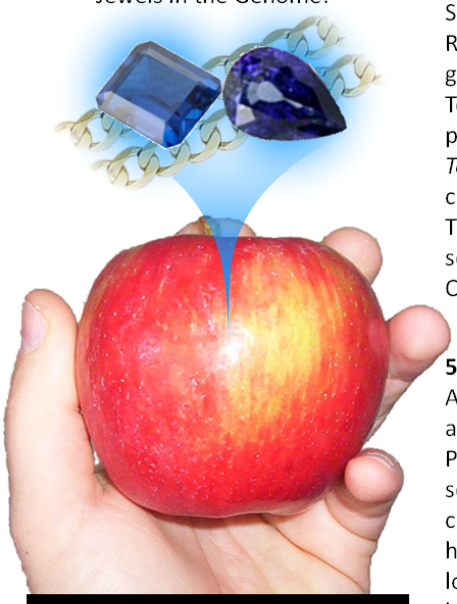
1. Prioritization
Two promising fruit quality trait loci for each crop were chosen from literature and experience (*MAB Pipeline and Breeding Teams*). These targets addressed trait priorities identified in Breeder surveys (*SE Team*). The *Ma* locus* was one of the target trait loci for apple, reportedly influencing acidity, crispness, and juiciness.

2. Genetic Screening Efficiency
For internal project purposes, the reliable, capable, and dedicated lab of Dr. Nahla Bassil at USDA-Corvallis, Oregon, is the default service provider for genetic screening (*Genotyping Team*).

3. Improved Markers
Previously reported markers for the target trait loci were tested, and all worked fine (*Genotyping Team*). For the *Ma* locus, two flanking SSRs were used. Although one is ~14 cM from the locus, it still contributes to informative haplotypes.

David Bedford performs controlled crossing for a new generation of superior apples in the University of Minnesota apple breeding program

* See this issue's Jewels in the Genome!



Honeycrisp: useful alleles for the *Ma* locus and *Md-Exp7*

4. Validation
So far so good. But now came the real work. Crop Reference Set individuals were DNA-extracted and genotyped with the trait locus markers (Genotyping Team). In the meantime, the same germplasm was phenotyped for fruit quality and databased (*Breeding Team*). Finally, the FlexQTL™ program was run to confirm QTL presence (*PBA and MAB Pipeline Teams*). The *Ma* locus markers revealed >20 haplotypes segregating in apple breeding pedigrees. Significant QTLs for several traits were confirmed at this locus.

5. Utility
Analyses were refined (so far only for apple) to specific breeding locations. Performance levels associated with each segregating haplotype were calculated and compared (*MAB Pipeline Team*). Functional haplotypes were detected for the *Ma* locus, some associated with desirable trait levels and others with poor performance.

6. Parent Selection Decisions
The integrated and translated socio-economics and DNA information was offered to RosBREED's Demonstration Breeders – at least to apple breeders who still had time before the pollination season to incorporate the new information on genetic potential of their parents in their crossing plans (*Breeding Team*).

Figure 1: "Fast-tracked" MAB pipelining efforts in 2010 - 2011 have integrated socio-economics and DNA information to support breeding decisions

Surveying growers to address market-driven needs

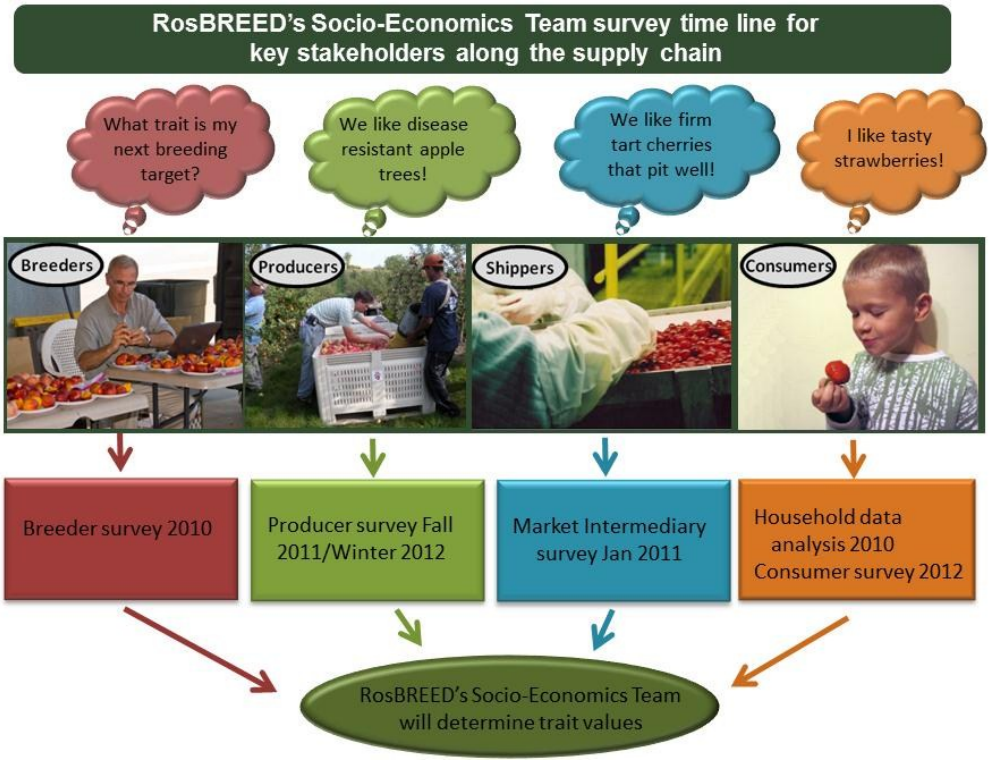
By RosBREED's Socio-Economics Team (Chengyan Yue, Karina Gallardo, and Vicky McCracken).

The introduction of new cultivars guarantees sustainability of the rosaceous fruit industry in a highly competitive business environment, by attracting consumers with innovative and appealing fruit cultivars. But target trait selection for the new cultivars is challenging.

From a marketing perspective, breeding is a process, like many other new product development activities, that creates and delivers value to customers that benefits key stakeholders (i.e., producers, processors, shippers and packers, retailers, and consumers) along the supply chain. Given the goal of fruit breeding is to provide such value, breeders should consider stakeholders' needs and wants in order to increase new cultivars' likelihood of success.

The Socio-Economics (SE) Team of RosBREED has conducted a survey of the North American rosaceous fruit breeders. Survey results show that breeders face socio-economic challenges and these challenges are at least as important as technical challenges. For example, one important socio-economic challenge is "uncertainty if a cultivar would be commercially viable." More frequent communication between breeders and industry stakeholders might help lessen this challenge.

Growers constitute one of the most important stakeholder groups in the supply chain. Their input is crucial in informing breeding decisions. During the winter of 2011/2012 (see diagram right), the SE team will conduct a survey of apple, sweet cherry, tart cherry, peach, and strawberry growers in major producing states. The goal of this effort is to become more knowledgeable about growers' perceptions of the importance of targeted plant and fruit quality traits. The information will be used to objectively estimate economic values for such traits. Growers' input, in combination with feedback received from other supply chain stakeholders, will help guide breeding programs' decisions. This will add to RosBREED's overall goals of having breeding programs targeting the right traits, applying modern genetic tools more efficiently, and speeding the development and release of superior cultivars that meet the expectations of the supply chain and the major stakeholders. We will distribute the grower survey in November 2011 by mail.



In the next Newsletter Issue (August), we will describe clicker surveys that are being developed and will be conducted during this winter's grower meetings.

Advisory Panel Member Profile Page

The success of RosBREED will be determined in large part by the people behind it. Because of this, we wanted to give you some insight into these individuals — whether they represent university extension, the scientific community, or industry — who are at the core of our efforts. Panel members were asked about their background and what they want to accomplish as part of the RosBREED project. Here is what they had to say.

Extension

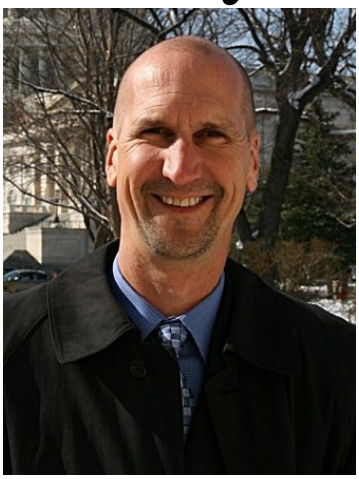
JAMIE SHERMAN
Associate Research Professor, Department of Plant Sciences and Plant Pathology, Montana State University, jsherman@montana.edu

What work do you do? The focus of my research is to identify and utilize markers in support of wheat breeding at Montana State University. Also, I am the principle investigator for the current TriticeaeCAP, and responsible for coordinating the education efforts. See http://wheat.pw.usda.gov/triticeaecap/ for more information about this project. A goal of the project is to establish a Plant Breeder Training Network where content can be shared and collaborations can be built. TCAP students have already begun interacting in this environment. I am also part of the team collaborating with minority serving institutions working to attract a more diverse population to plant breeding.

Why are you interested in RosBREED? I am always looking for opportunities of collaboration.

How do you feel you can contribute to RosBREED? Utilizing my previous and current experiences, I hope to empower RosBREED. I have enjoyed attending the RosBREED Annual Advisory Panel Member meetings. I feel that I gain as much or more than I give and so appreciate the opportunity to participate in RosBREED.

Industry



BILL DODD
Secretary, US Apple Association. Owner of Hillcrest Orchards Inc, bill@ohioapples.com

What work do you do? My main job is to sell apples for Ohio's Fruit Growers Marketing Association to larger wholesale customers, but I am involved in many aspects of the apple industry. The Midwest Apple Improvement Association has several interesting new apple crosses that are being evaluated. I will be involved in patenting and marketing anything that comes out of this breeding program. My family owns a 90 acre farm near Amherst, OH and we grow about 35 acres of apples, mostly for our retail market and pick your own.

Why are you interested in RosBREED? RosBREED has the potential to help develop better apples. Better apples would hopefully translate to increased consumption. Increased consumption is the best thing that could happen to our industry. Providing consumers with wonderful texture, exceptional keeping qualities and disease resistance would benefit the apple industry tremendously.

How do you feel you can contribute to RosBREED? As the RosBREED project progresses and discoveries are made, I am looking forward to discussing and prioritizing RosBREED's progress with the other Advisory Panel members.

Scientific

C. ROBIN BUELL
Associate Professor, Department of Plant Biology, Michigan State University, buell@msu.edu

What work do you do? Genomics, bioinformatics, and computational biology of plants and plant pathogens.

Why are you interested in RosBREED? Translating genomic information into agricultural systems is challenging yet perhaps the most important aspect of genomic research. The Rosaceae are an interesting set of crop species with a unique set of challenges.

How do you feel you can contribute to RosBREED? My experience with the USDA-funded SolCAP (Solanaceae Coordinated Agricultural Project http://solcap.msu.edu) provide insights into the technical and research challenges of applying genomics to a crop that are of benefit to RosBREED.

Breeder profile: Jim Hancock

By Audrey M. Sebolt, Project Assistant

Jim Hancock, Michigan State University

Dr. Jim Hancock, one of the Demonstration Breeders of RosBREED for strawberry, started his career in strawberry and blueberry breeding at Michigan State University in 1979. Realizing the limitations of the narrow germplasm base of the cultivated strawberry, from the onset of his strawberry breeding program, his primary focus was on improving the germplasm. Therefore, he spent the first twelve years of his strawberry breeding program on several germplasm collection trips throughout the United States and South America in collaboration with Chad Finn (USDA-ARS Pacific Northwest berry breeder; RosBREED strawberry Demonstration Breeder) and Jim Luby (University of Minnesota; RosBREED apple Demonstration Breeder), among others.

Jim has also worked with a network of strawberry breeders in North America for evaluating and sharing the germplasm collected across the hemisphere. After characterizing the newly collected germplasm, Jim began to make crosses within the germplasm collection (wild x wild) and wild x cultivated.

Each spring, Jim makes approximately 100 crosses, of which 70% of the parents are ever-bearing or remontant. The ever-bearing trait is desirable for growers because once the strawberry plant flowers, it will continue to flower and produce fruit several times in a growing season as opposed to short day varieties that produce only once per season.

Each spring, Jim Hancock and his crew plant 6,000 to 7,000 first-generation strawberry plants. Jim is currently evaluating approximately 50 advanced selections which are short day and ever-bearing, high yielding, and have excellent fruit quality (see box to right). All of his seedlings are planted and evaluated in non-fumigated soil and therefore any that survive are considered at least moderately tolerant to black root rot, which is common in Michigan soils and most other production regions.

Jim, his Breeding Trainee Sonali Mookerjee (see next page), and Chad Finn have selected cultivars and seedlings that will be part of the RosBREED reference germplasm sets for strawberry. The RosBREED strawberry Crop Reference and Breeding Pedigree Sets (CRS and BPS respectively) will be replicated in Michigan, Oregon, California, Florida, and New Hampshire and therefore additional collaborations have been developed to expand Jim's network. Jim is excited about these new collabora-

Traits of interest for Jim's program:

Ever-bearing cultivars start to fruit and keep fruiting in a growing season. Other traits include

- High commercial yields
- Plant archetype with well exposed fruit and a thrifty canopy
- Fruit that are large and bright red
- Fruit with increased aromatics and are sweet yet have a hint of tart flavor

Below are images of a few key traits that Jim and Sonali are evaluating. Images on the left are of traits that are considered to be undesirable and to the right desirable.

Inflorescence type:

Right: short pedicel; Left: long pedicel. A long pedicel is desirable because of better fruit development and ease of harvest.

Fruit shape:

"Turtle-neck fruit" and "Monkey-face fruit" compared to a Perfect fruit shape. Turtle-neck and Monkey-face fruit are generally considered to be not aesthetically attractive to consumers.

Continued on page 9

Breeder profile continued

Jim Hancock in Ecuador (1996) using highly sophisticated scientific techniques for extracting seeds from fruit

tions and the opportunity to see how his selections will perform in the states of Florida and California, which are warmer climates. All five locations will evaluate the CRS and BPS for the traits indicated in the strawberry standardized phenotyping protocol (www.rosbreed.org/resources/fruit-evaluation).

Recently, Jim's program was visited by Jerry Hill, whose RosBREED Team is developing a breeding information management system. One of its many capabilities is that it manages breeders' trait data and assists with cross planning. Jim is excited about the new system because he believes it will be an excellent tool for designing crosses; he will be able to more easily visualize the pedigree of selections of interest along with their traits. Jim may consider making diverse crosses if the system reveals potentially new and exciting cross combinations. In addition to providing an excellent management tool for parent and seedling selection, the data management system will also serve as a catalog for future breeders that may use his germplasm and trait data. Stay tuned for the next issue of our Newsletter where we will describe this data management system in more detail.

Meet Jim's Breeding Trainee: Sonali Mookerjee

Sonali Mookerjee in East Lansing (2011) using current highly sophisticated scientific techniques

Why did you choose Jim Hancock's program? When I joined the Plant Breeding, Genetics, and Biotechnology Program at MSU, I wanted to get training in plant breeding. I had been working with apples, so I had a preference for continuing to work with a crop in the same family. Dr. Jim Hancock had this project on strawberry breeding that I could work on and that would give me the opportunity to get experience in traditional, as well as molecular breeding. I could also participate in the RosBREED project and gain from all the valuable resources in RosBREED. I am extremely thankful to Dr. Hancock that he let me work with him because this was possibly the best breeding training I could hope for. Dr. Hancock's enthusiasm, optimism, and encouragement, makes research so much fun!

What is your thesis project if it has been determined? I am developing a linkage map for octoploid strawberry using SSR markers. I will be using this map to identify QTL linked to photoperiod and temperature regulation of flowering in strawberry and identify markers linked to these traits.

What benefits have you seen by being part of RosBREED? Being a RosBREED Project Assistant gives me the opportunity to be a part of this unique effort of implementing marker-assisted breeding into traditional breeding in Rosaceous crops. It gives me the opportunity to understand and follow streamlined research efforts involving many branches of science, many institutions, and many researchers with a common goal. I am able to interact with breeders, scientists, and graduate students from so many research institutions. I can contribute to a unique multi-state strawberry pedigree-based analysis project which will produce a lot of valuable information for all strawberry breeders/scientists. I get the opportunity to participate in the workshops on Pedigree-Based Analysis, an area that I was not familiar with.

Jewels in the Genome

By Amy Iezzoni, Project Director

What is a "Jewel in the Genome?"

- *An individual's genome is the full complement of genetic information that it inherited from its parents. Within this vast repertoire of genetic information, individual genes are being discovered that control critical production and fruit quality traits. As these valuable rosaceous gene discoveries are made and put into breeding applications, we will describe them in this column as "Jewels in the Genome."*

Crisp, juicy apples with pleasing acidity are highly desirable for the fresh market. However, obtaining the optimum combination of these desirable textural and taste attributes has been challenging as soft, mealy and poor tasting apples frequently occur in breeding populations. In apple (and many other fruits), malic acid is a major contributor to fruit acidity. The locus that controls malic acid level in apple, named *Ma*, is located near the top of apple linkage group (LG) 16 (Maliepaard et al. 1998). Interestingly, this same region on LG 16 also contains gene(s) that influence apple crispness and juiciness (King et al. 2001).

Alleles for the *Ma* locus in apple are associated with a wide range of eating quality. For example, some types appear to be associated with desirable tart crisp juicy apples, other types with just crisp or tart apples, and some with poor quality for all these traits (Fig. 1). This locus is currently under detailed investigation in RosBREED (Fig. 2), and is already revealing much wider diversity in U.S. breeding germplasm than the alleles originally reported in European apple populations.

With genetic knowledge of *Ma* locus alleles in their germplasm, breeders can harness this wide array of genetic diversity to make desirable parental combinations and select seedlings with superior performance prior to field planting. Using this DNA information is particularly powerful as it simultaneously provides predictions for three high priority traits (acidity, crispness and juiciness). Therefore, because the *Ma* locus will lead to more efficient breeding of apples with desirable eating quality, it is chosen as our sixth featured "Jewel in the Genome."

King GJ, Lynn JR, Dover CJ, Evans KM, and GB Seymour. 2001. Resolution of quantitative trait loci for mechanical measures accounting for genetic variation in fruit texture of apple (*Malus pumila* Mill.). Theor Appl Genet 101: 1227-1235.

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, and GJ King. 1998. Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor Appl Genet 97: 60-73.

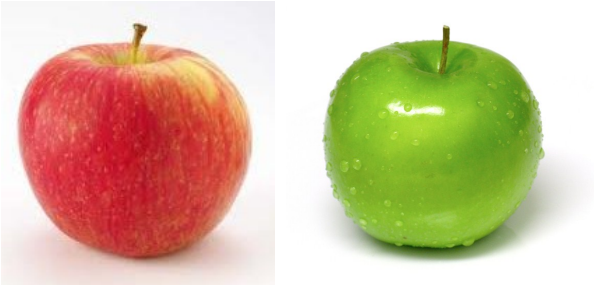


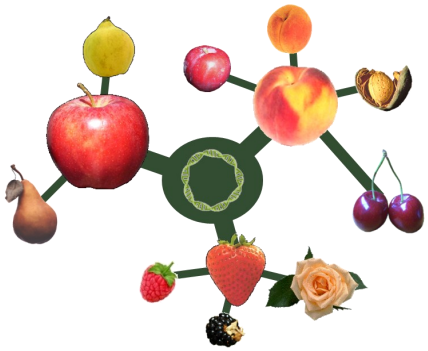
Figure 1. Unusual allelic combinations at the *Ma* locus for Honeycrisp (left) and Granny Smith (right), may contribute to their extreme fruit quality attributes.

Figure 2. Evaluating crispness with a Mohr Digi-Test.

RosBREED: Enabling marker-assisted breeding in Rosaceae

RosBREED headquarters:
Michigan State University
A342 PSSB
East Lansing, MI 48824
Phone: 517-355-5191, x1391
E-mail address: iezzoni@msu.edu

RosBREED Newsletter staff:
Editor-in-chief: Cholani Weebadde, RosBREED Extension Team Leader weebadde@msu.edu
Design: Audrey Sebolt, RosBREED project assistant, grantzau@msu.edu
Contributing editors: David Karp, Cameron Peace, and Brian Sparks



Visit us at
www.rosbreed.org

Contact information

RosBREED project director:
Amy Iezzoni (Michigan State University)
RosBREED Team Leaders:

Name	Team	Organization
Nahla Bassil	Genotyping	USDA-ARS, Corvallis
Gennaro Fazio	Breeding information management system	USDA-ARS, Cornell University
James Luby	Breeding	University of Minnesota
Jim McFerson	Industry	Washington Tree Fruit Research Commission
Dorrie Main	Genomics	Washington State University
Cameron Peace	Marker-assisted breeding pipeline	Washington State University
Eric van de Weg	Pedigree-based analysis	Plant Research International, The Netherlands
Cholani Weebadde	Extension	Michigan State University
Chengyan Yue	Socio-economics	University of Minnesota

Calendar of events

- **September 25-28, 2011: American Society for Horticultural Sciences will meet in Hawaii. For more details, please visit ASHS.**
- **January 12, 2012: Annual RosBREED Advisory Panel Member meeting. San Diego, CA**
- **March 12-15, 2012: Annual RosBREED Project Planning meeting for project participants. East Lansing, MI**