RosBREED

Combining Disease Resistance with Horticultural Quality
in New Rosaceous Cultivars

V9.3 AUG 2019

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RosBREED: Project over but work goes on

Amy Iezzoni, Project Co-Director, Michigan State University and Cameron Peace, Project Co-Director, DNA-Informed Breeding Team Leader, Washington State University

The RosBREED project is officially ending at the end of August. However, you'd never know it by all the energy and pace of achievements that are showing no signs of slowing down! At the recent American Society for Horticultural Science (ASHS) annual conference, not only were the latest results and impacts described in 12 presentations and three posters, but the chatter ranged from discussions of results "hot off the press" to new things that can be learned from the existing data, and the potential parents and advanced selections developed or identified this season.

All this and more is driven by the community's rapidly advancing implementation of DNA-informed breeding. It highlights the legacy of RosBREED: a collaborative community empowered with the ability to apply genetic knowledge for creating and advancing valuable breeding germplasm. Superior new cultivars are definitely in the pipeline. Many of these new cultivars will achieve the RosBREED target of combining superior fruit and floral quality AND disease resistance across the range of crops in the project.

On behalf of the RosBREED team, we gratefully acknowledge the support provided by the NIFA-SCRI program. We are just as grateful to our amazing industry partners, who laid the foundation for the SCRI project, provided the impetus for both RosBREED projects, and kept us constantly focused on deliverables. We also greatly value the wonderful contributions by colleagues on our Scientific and Extension Advisory Panels and our scientifically generous international research partners. Thanks to all of you!!

Fig. 1. Ellie gives her mom, Dorrie Main, a thumbs up as if signifying a positive end to the project.

Fig. 2. The RosBREED project may be over, but planning is underway for numerous spin-off projects.

Jewel in the Genome

Early-Flowering Transgene Locus in Apple

Amy Iezzoni, Project Co-Director, Michigan State University and Cameron Peace, Project Co-Director, DNA-Informed Breeding Team Leader, Washington State University

Breeding new apples is a long and costly process as apples have an extended juvenile period from seed to flowering that can last five or more years. Apple breeders often propagate seedlings on precocious rootstocks to shorten the cycle, but this only reduces the cycle by approximately two years. This long duration from seed to flowering is especially problematic for breeders who wish to introgress alleles from wild apple species or conduct parallel crosses over two or more generations to pyramid and combine multiple alleles for disease resistances.

One relatively fast strategy to reduce the generation time is use of transgenic genetic stocks that carry the early-flowering gene *BpMADS4*, originally identified in birch trees. In apple, T1190 is a widely used *BpMADS4*-trangenic stock developed by work led by Henryk Flachowsky (Julius Kühn-Institut, Germany) that can reduce the juvenility period to less than 10 months (1, 2). In RosBREED, Jay Norelli used T1190 to pyramid and combine fire blight and scab resistance from apple cultivars Splendour and Enterprise into a single individual that can be used as a disease-resistant donor parent.

In each generation, Norelli selected for the resistance alleles and presence of the early-flowering *BpMADS4* transgene. Because the chromosome segment that contains the *BpMADS4* is maintained in plant materials through most of the introgression process, and therefore *BpMADS4* effectively becomes an inherited allele, the genetic content of the flanking region will also be transferred to offspring, bordered by any subsequent cross-over locations. Prior work narrowed the insertion site in T1190 to a region on apple chromosome 4 (2). The 30-cM region spanning this insertion site contains QTLs associated with apple fruit skin and flesh hardness, skin russetting, and flesh mealiness (3). Therefore, it would be important for breeders to have knowledge of the QTL alleles brought along with selection for the transgene or left behind if there is nearby recombination when the transgene is selected against. RosBREED research identified the ancestral origin of the transgene site in T1190 (that resulted from transformation of a 'Pinova' seedling) to be a chromosome segment from Cox's Orange Pippin, thus providing breeders with knowledge of the alleles that could be selected along with the transgene (3; Fig. 3). The other parent of T1190 was not known until RosBREED research deduced it to be the apple cultivar Idared, with T1190's non-transgene chromosome 4 segment originating from the ancestral cultivar Wagener (3).

Because knowledge of the ancestral haplotypes in which an early-flowering transgene is embedded will lead to the more efficient breeding of apple cultivars, it is featured as a RosBREED "Jewel in the Genome".

References

1. Flachowsky H, Peil A, Sopanen T, Elo A, Hanke V. 2007. Overexpression of *BpMADS4* from silver birch (*Betula pendula* Roth.) induces early flowering in apple (*Malus* × *domestica* Borkh.). Plant Breed. 126:137-145.
2. Flachowsky H, Le Roux P, Peil A, Patocchi A, Richter K, Hanke M. 2011. Application of a high-speed breeding technology to apple (*Malus* × *domestica* Borkh.) based on transgenic early flowering plants and marker-assisted selection. New Phytol. 192-364-377.
3. Luo F, van de Weg E, Vanderzande S, Norelli, Flachowsky H, Hanke V, Peace C. 2019. Elucidating the genetic background of the early-flowering transgenic genetic stock T1190 with a high-density SNP array. Mol. Breeding 39:21.

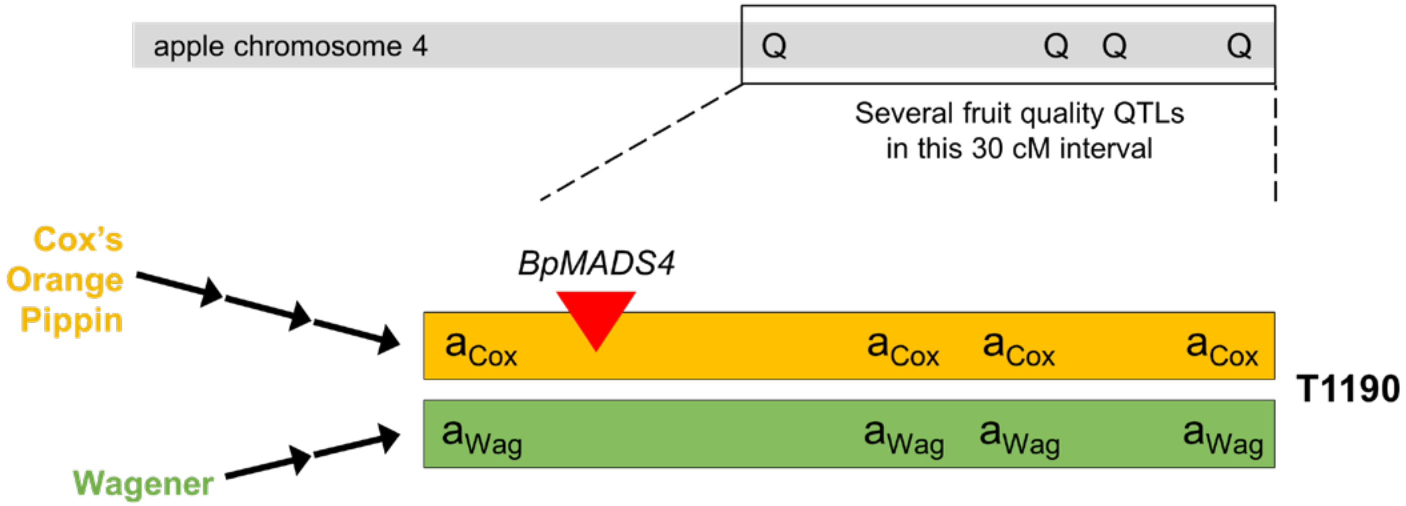


Fig. 3. Genomic position of the early-flowering transgene BpMADS4 in the genetic stock T1190. The two haplotypes in this region inherited by T1190 from its ancestors 'Cox's Orange Pippin' (maternal great-grandparent) and 'Wagener' (paternal grandparent) carry specific alleles (a) for the several QTLs (Q) reported to reside there. Adapted from Ref. 3.

ASHS RosBREED Workshop

Amy Iezzoni, Project Co-Director, Michigan State University and Cameron Peace, Project Co-Director, DNA-Informed Breeding Leader, Washington State University

We hosted an Oral Session at the 2019 ASHS annual conference entitled: *RosBREED: Combining Disease Resistance with Horticultural Quality in New Rosaceous Cultivars*, as a formal culmination to the project for our horticultural science colleagues.

Cameron Peace hosted the session, which began with overview remarks from Project Director Amy Iezzoni, then lead to presentation from seven RosBREED participants summarizing outcomes of major sections of the project. All eight presentations can be found at the ASHS conference web site: https://ashs.confex.com/ashs/2019/meetingapp.cgi/Session/9425.

Some highlights:

- Targeted socio-economics information the revealed that superior flavor was the highest priority for Florida strawberry producers, while peach growers in the Southeastern U.S. generally put a very high value on brown rot resistance.
- Outcomes and impacts in breeding, pathology, and genetic discovery and practical implementation include large-effect locus discovery, validation of their utility in breeding germplasm, DNA test development, and DNA test application. Impacts included examples of breeding efficiencies gained as well as newly created breeding materials combining multiple desirable attributes. These "needle in a haystack" materials would not have been found without the use of DNA-based diagnostics.
- First time reports on successful use of genomic selection for those traits with complex inheritance such as yield and sweetness in strawberry and brown rot resistance in peach.
- Broad, tangible outcomes made possible by an energized collaborative community include the large number of publications generated, graduated RosBREED students who are now in breeding positions, and spin-off projects pursued and funded.

Fig. 4. Jason Zurn, RosBREED Crop Postdoc in Nahla Bassil's program at USDA-ARS Corvallis, presents during the ASHS conference.

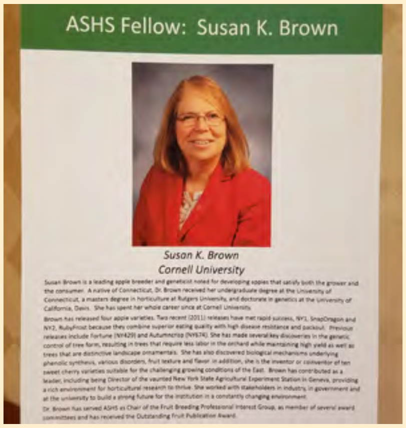
New ASHS Honorees: Chengyan, Nahla & Susan!

Outstanding Graduate Educator
Chengyan Yue

Chengyan Yue
University of Minnesota

ASHS Fellow: Nahla V. Bassil

Nahla V. Bassil
USDA, ARS,
National Clonal Germplasm Repository



ASHS Fellow: Susan K. Brown

Susan K. Brown
Cornell University

RosBREED Delivers

Jim McFerson, Extension Team Leader, Washington State University

"Are they going to accomplish what they promised or are they going to spin wheels and waste money?"

This question from one of our industry stakeholders at the final RosBREED Advisory Panel meeting in January 2019 was entirely typical of the candor and focus on the bottom line that project participants have come to expect from all our stakeholders.

It is also a fair question. After all, our two successive RosBREED SCRI projects have now spent nine years and more than $16 million to improve the effectiveness of U.S. rosaceous crop breeding programs and deliver stakeholders superior new cultivars. That is a lot of time and lot of money invested in two large, complicated projects – stakeholders were right to be healthily skeptical.

The first project, active 2009-2013 was titled "RosBREED: Enabling Marker-Assisted Breeding in Rosaceae". The second phase, active 2014-2019, was titled "RosBREED: Combining Disease Resistance with Horticultural Quality in New Rosaceous Cultivars." They were indeed large and complicated, with lofty aspirations. They were also guided by a large and very active set of nearly 50 stakeholders who formally served on project Advisory Panels.

Advisory stakeholders representing industry, extension, and scientific sectors contributed significantly to the design and approach of both projects. Once the projects were funded, stakeholders then devoted an entire day annually to reviewing overall project progress, challenges, deliverables, and future plans. Project annual reports, which will be available on-line at the Genome Database for Rosaceae web site, attempted to provide solid, compelling technical detail on project objectives, with a minimum of scientific jargon, acronyms, and obscurities. Presentations by project team leaders were informal but complete. Information flowed in multiple directions.

After such active and tiring work, group dinners were restorative and motivating. Then, as stakeholders returned home, RosBREED participants got back to business, adjusting program activities where appropriate and in anticipation that each coming year would deliver scientific breakthroughs and practical outcomes. After nine such meetings and ten years to achieve project objectives, the RosBREED's return on investment is manifested in a range of deliverables and legacies.

Legacies

New Knowledge
- Publications
- Archived data (GDR: Genome Database for Rosaceae)

New Germplasm
- Parents with valuable alleles
- Breeding families, genetically enriched
- Promising new selections

Diagnostic Tools
- DNA tests
- SNP arrays

Education, Training & Professional Development
- Graduate degrees
- Demonstration & community breeders

Stakeholder & Community Development
- Rosaceae community & spin-off projects
- Industry stakeholder engagement

Fig. 8. Some legacy products of RosBREED.

New Scientific Knowledge

Publications

Fig. 9. Peer-reviewed scientific publication output of RosBREED.

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Archived Data (GDR)

Fig. 10. The Genome Database for Rosaceae is the archival repository for genetic data discovered in part by the RosBREED projects (www.rosaceae.org).

Germplasm

Fig. 11. Jay Norelli, USDA-ARS Kearneysville, working with a fast-flowering transgenic genetic stock that aids introgression of disease resistance. Photo credit: Jay Norelli.

Diagnostic Tools

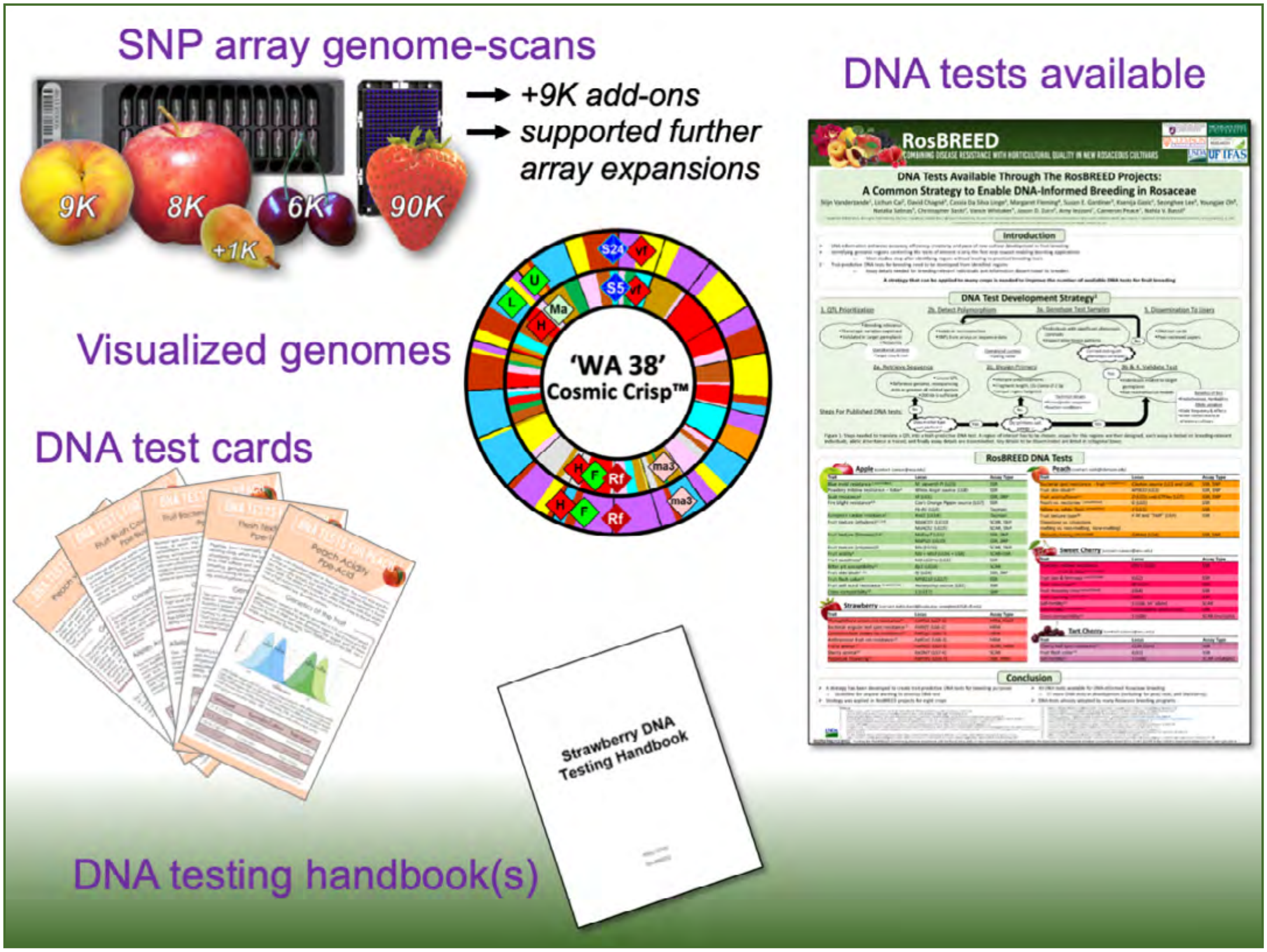

Fig. 12. Graphical representation of the types of diagnostic tools available as a result of RosBREED.

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Education, Training, and Professional Development

Graduate Students

Fig. 13. Current cohort of graduate students involved in RosBREED (photo taken during the Participnat Meeting in Mar 2019, East Lansing, MI).

Training/Workshops

Fig. 14. Students, postdocs, and senior scientists deep in conversation after a workshop presentation at the Participant Meeting in Mar 2018, East Lansing, MI.

Professional Development

Professional development through RosBREED has resulted in a marked increase of breeders utilizing DNA-informed methodologies, according to surveys and interviews of all 100 U.S. Rosaceae breeding programs (Peace and Coe, unpublished data).

Breeders using any DNA info by late 2018 = 73%

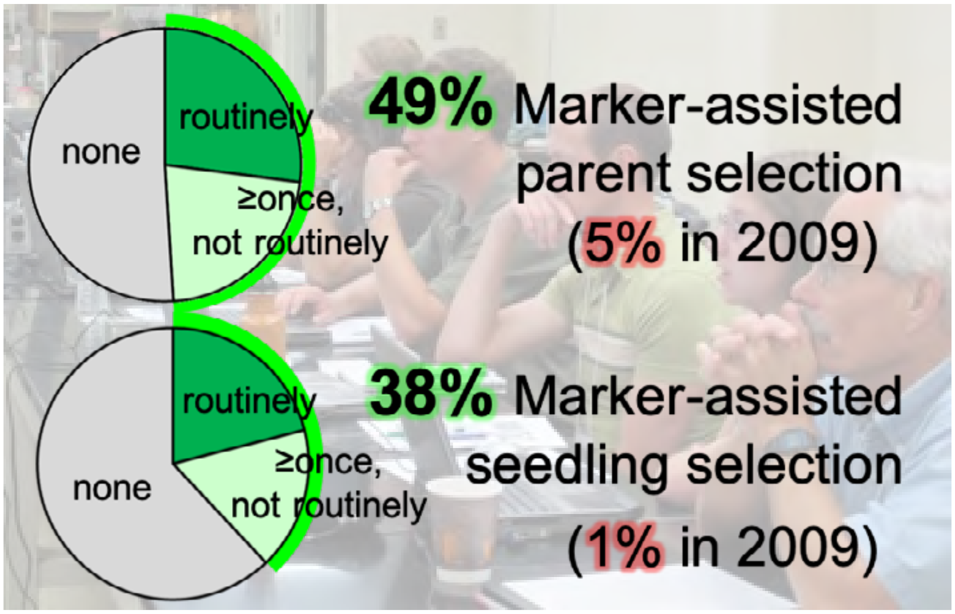

Fig. 15. Increased use of DNA-informed methodologies by the breeding community as a result of RosBREED.

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Community Development


Fig. 16. RosBREED participants, Mar 2018, East Lansing, MI.


Fig. 17. International partners, Mar 2018, East Lansing, MI.


Fig. 18. Project stakeholders, Jan 2017, San Diego, CA.


Fig. 19. Mandie Driskill, Caneberry Field Day, Aug 2019, Aurora, OR.

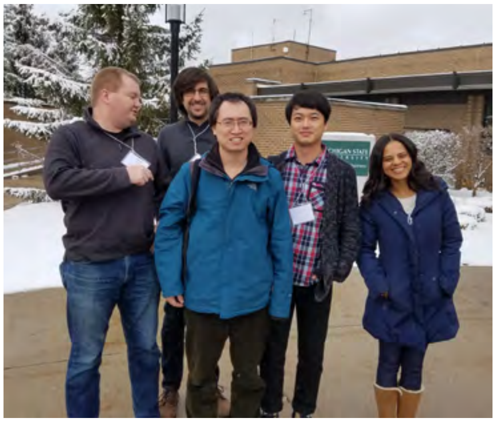


Fig. 20. Crop postdocs, Mar 2018, East Lansing, MI.

Spin-off Projects

USDA-NIFA SCRI Project – Strawberry

Next-generation disease resistance breeding and management solutions for strawberry

PI: Steve Knapp (UC Davis)

USDA-NIFA OREI Project – Strawberry

Advancing the development of seed-propagated hybrid varieties in strawberry for organic agriculture

PD: Lise Mahoney (Univ New Hampshire)

USDA-AMS SCBGP Project – Prunus

Short and long-term solutions for Armillaria root rot in *Prunus*

PD: Ksenija Gasic (Clemson Univ)

Resource Links

RosBREED Newsletters
www.rosbreed.org/industrymedia/newsletters

RosBRIEFs
www.rosbreed.org/industrymedia

DNA Tests
www.rosbreed.org/breeding/dna-tests

GDR Database
www.rosaceae.org

Community Events

10th International Rosaceae Genomics Conference (RGC10)
Mar 31 - Apr 3, 2020
Barcelona, Spain
http://rosaceaegenomics.com/index.php?go=inicio

Where Are They Now?

Matt Clark
Univ. of Minnesota

Terrence Frett
Sun World International

Megan Mathey
Spring Meadow Nursery

Travis Stegmeir
Lasson Canyon Nursery

Sujeet Verma
Univ. of Florida

Jonathan Fresnedo
Ohio State Univ.

Paul Sandefur
Fall Creek Farm & Nursery

Lise Mahoney
Univ. of New Hampshire

Congratulations, Texanna & Wanfang!

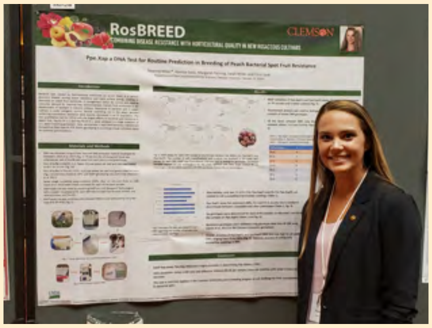


Fig. 21. Texanna Miller, an undergraduate student in Ksenija Gasic's program at Clemson University, won 3rd place in the undergraduate poster competition at ASHS with a poster titled: Ppe-Xap a DNA test for routine prediction in breeding of peach bacterial spot fruit resistance. Co-authors Margaret Fleming, Chris Saski, Sarah B. Miller, and Ksenija Gasic. Photo credit: Kseniji Gasic.

Fig. 22. Wanfang Fu, a graduate student in Ksenija Gasic's program at Clemson University, received the Shepherd award from American Pomological Society (APS) for best research paper published in the Journal of APS in 2018: Breeding for tolerance to brown rot in Clemson University peach breeding program. Co-authors Ralph Burrell, Cassia De Silva Linge, Guido Schnabel, and Ksenija Gasic. Photo credit: Kseniji Gasic.

Community Breeders' Page

Ten Tips for DNA-informed Breeding

Cameron Peace, Project Co-Director, DNA-Informed Breeding Leader, Washington State University

The art of Rosaceae crop breeding is changing. While we can still develop an industry-changer from a chance cross, greater objectivity and technology is being applied than in the days when only phenotypic and pedigree records inferred the genetic worth of each individual. DNA information provides us with an unprecedented window on and access to valuable genetic factors. With DNA-informed breeding, we can target and achieve desired outcomes more efficiently, accurately, and creatively than ever before.

Such was stated in RosBREED 2's first Community Breeders' Page article in December 2014. This column aimed to apprise you of the latest knowledge, tools, and concepts and to provide a reference resource for scientific advances in rosaceous DNA-informed breeding. These pages have covered:

- **Upstream research approaches** (articles: *A culture of breeding impact from genomics research, DNA test conversion*)
- **Technology interfacing** (articles: *Parentage & pedigree, Breeding creativity, DNA test cards, Jewels in parents*)
- **New DNA information** (article: *Functional genotype lists*)
- **Events** (articles: *Where is RosBREED?, Assessing your program's opportunities, Experiencing "the genotype"*)

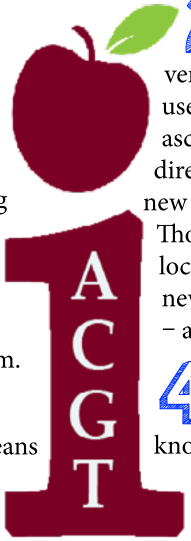
Where are we now with DNA-informed breeding in Rosaceae? Enjoying a bridged chasm. Most rosaceous crop breeders are using DNA-based diagnostic genetic information one way or another, as described in these pages last Newsletter (Winter/Spring 2019). DNA information now applied in breeding has arisen from fundamental genomics research advances – such as whole genome sequences, transcriptome characterization, targeted resequencing, genome-scanning assays for genetic polymorphism, chromosomal maps, QTL discoveries, and gene functional validation – that have been translated to crop genetic improvement by focusing on breed-relevant germplasm, commercially valuable traits, affordable tools, and accessible services. And the kinds and degrees to which such information is mobilized has increased, as described in the previous Community Breeders' Page article. The use of DNA-based diagnostics to supplement or satisfy informational needs during breeding planning and operational decisions is now clearly *conventional* (while the *traditional* approach means relying entirely on phenotypic and pedigree records).

Ten years of the RosBREED projects have revealed numerous needs and opportunities for DNA-informed breeding that are common across rosaceous crops as well as countless specific needs of just one or two breeding programs. I've assembled ten tips (next pages) for adopting and expanding DNA-based diagnostic information use on Rosaceae breeding germplasm, so that your program can efficiently benefit from strategies, tools, and knowledge now available. Good luck – with good information!

Ten tips for DNA-informed Breeding

1 Much more than MASS

So many practical applications are possible using DNA information in a breeding program, as highlighted in these pages since 2010. There are characterization applications (identity and relatedness) and evaluation applications (performance). Then there are various germplasm "levels" — from wild sources of valuable attributes through pre-breeding generations to parents expected to lead to new cultivars, seedling families, advanced selections of increasing eliteness, and to-be-released or already commercialized cultivars. Marker-assisted seedling selection (MASS) is just evaluation on seedling families — a small slice of the spectrum.



2 Be sure of what you have

As germplasm is the basis of a breeding program, verifying the identity of what you have is the most critical use of DNA information. "Elites" are where it matters to ascertain the correct identity, as it connects the individual directly to all the information already known about it. The new parent you obtained – is it really what you asked for? Those replicated selections entering the multi-year, multi-location field trial – are they all the right individuals? That new cultivar being propagated up to commercial numbers – are the clones really true-to-type?

3 Who's the father?

Knowing both parents of each breeding individual means that decisions are correctly informed about the sources and inheritance likelihood of desirable attributes. Successful controlled crossing means that you've chosen both sets of alleles, maximizing your ability to combine desirable alleles – and so verifying success of pollination methods is more than helpful. SNP arrays are effective here for your elite germplasm (and simultaneously reveal further insights), but smaller sets of markers readily suffice. For paternity-testing of many offspring, small sets of generic DNA markers are efficient, and can be the same markers as used for trait-predictive DNA testing (#7).

4 Jewels in parents' genomes

For each parent you're thinking of using, you'll want to know their alleles at trait-influencing loci – see the twinkles in each parent's eyes. Besides indicating useful crosses, the information will also reveal which families would benefit from DNA testing (#7). For detecting jewels, you can run a battery of all available DNA tests across your parent pool. Or you can have a DNA profiling conducted via SNP arrays, which you might have already done for identity/relatedness purposes – trait locus allele information can be extracted from SNP array data. With this genotypic insight, you can then identify pairwise cross combinations that will give all or a high proportion of offspring with target allelic combinations.

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Cont'd: Ten tips for DNA-informed breeding

5 Noble pedigrees

Knowing how much and in what ways your germplasm is different from others is fundamental to your program's distinctiveness and potential for future success. Just because something is said to have arisen from a distinct region doesn't mean it is genetically distinct. SNP arrays are highly recommended tools for determining relatedness among your elite germplasm individuals, to verify or deduce the pedigrees of parents, selections, and cultivar releases as far back as possible. Aim for ascertaining qualitative genetic relationships rather than quantitative−shared alles across each chromosome rather than an overall similarity coefficient or relative positioning on a PCA plot or dendogram. In that way, you'll obtain specific information to guide decisions, rather than general.

6 All you can know

Run SNP arrays on your elite germplasm. Such germplasm is limited in number, so genotyping costs are kept low. SNP arrays provide consistent DNA profiles, so that all individuals are compared on the same basis. Many questions can be answered with simple manipulations of the raw data. But we have a community-developed data curation workflow to clean up the data further, visualization software, and a public database of SNP-array DNA profiles for comparing new profiles to. Update your own database each year for new parents and selections that you've created or obtained. If you're feeling particularly creative, you could identify pairs of parents with elite performance but contrasting ancestry (i.e., different haplotypes) and exploit maximized complementarity.

7 Onto MASS

With applications of #2-#6 in routine operation under your belt, you might as well jump into DNA testing of your greenhouse-growing seedling families. First (and perhaps only), choose and use DNA tests that separate *essential* trait levels. These are DNA tests for which the results reveal certain allelic combinations that you know cannot exist in a would-be cultivar from your program. Use all such available DNA tests rigorously! Once DNA is extracted and DNA tests for essential contrasts are in use, it's easy to add less critical DNA tests. These are DNA tests predicting contrasting phenotypes for which the desirable type would be only an *enhancing* feature to a new cultivar. Remember that the most informative DNA tests are those that explain all or most of the heritability (even if the trait has low heritability), not necessarily most of the phenotypic variation. An example would be a DNA test for disease resistance in which symptoms are elicited with difficulty and inconsistency in susceptible individuals, but the test distinguishes resistant individuals.

8 Your local expert

Your program will need a dedicated expert to consider opportunities, interact with service providers, interpret, and enact outcomes of DNA information-gathering. My estimate is no more than 5% of someone's time across any given year to benefit from applications of #2-#6, to take advantage of low-hanging fruit. To succeed in #7, I suggest you'll need about 10% time of the primary breeder* plus 10% of a technician, or 50% of a technician, or (for public institutions) a full-time graduate student who understands the breeding program well and collaborates with DNA-informed breeding experts in other programs (*or 10% of a DNA-informed breeding expert's time; my shameless plug for job security). There are genetics hotshots out there now − graduated "RosBREEDlings" and postdocs itching to apply their practical DNA-informed skills to your program. Many are already employed as such in public and private breeding programs − snap them up while you can!

9 Get value for your money

For each service provider of DNA-based diagnostics, there are pros and cons in physical accessibility, sampling ease, DNA extraction speed and reliability, genotyping technologies available, genotyping speed and reliability, customer support, and price points throughout that often depend on the scale of your jobs. Try out two or more service providers to gain experience in doing it and compare value for money.

10 Meeting your specific needs

You have specific traits and/or germplasm sources for which discoveries or tests have not yet been made. To get practical outcomes for these, you'll need to partner up. If you need QTLs discovered through to DNA tests to be developed, collaborate with a research group. If DNA test development is all you need, a commercial service provider should do the trick. Many trained professionals are now out there who appreciate the need to target practical breeding application.

In Our Stakeholders' Words

Advisory Panel Roundtable Jan 2019

> *Definitely an SCRI project where translation from science to application is incorporated – extension is not an add-on.*

> *Jewels in the Genome, GWS and pyramiding are proud achievements.*

> *Excited about the future and what's next – build on the momentum for spin-off projects.*

> *Taxpayer funds have been effectively used.*

> *There will be a lasting impact seen in breeding rosaceous crops around the world.*

> *Students and trained professionals are a great product.*

> *DNA-enhanced breeding is now functional and effective, finally!*

> *Project has been awesome – up to us to go back home and talk to our industry to reinforce the value of project outcomes.*

> *Great successes: Collaborative spirit in the research community (including transdisciplinary) and with industry stakeholders.*

> *Bummer! It's ending just as I am beginning to understand what it's all about!*

Special thanks to all of our partners!

It's been a great ten years.

CAL POLY SAN LUIS OBISPO

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