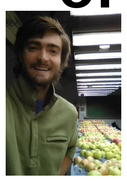
RosBREED

Enabling marker-assisted breeding in Rosaceae

www.rosbreed.org

Harnessing the power of RosBREED: Development, validation, and application of DNA tests for predicting peach flavor and other valuable rosaceous tree fruit traits



Presenter: Paul Sandefur [1]

Co-Authors: Cameron Peace [1], Terrence Frett [2], Alejandra Salgado [2], John R. Clark [2], and Ksenija Gasic[3]

[1]Washington State University, Pullman, WA 99164 USA ; [2]University of Arkansas, Fayetteville, AR 72701 USA ; [3]Clemson University, Clemson, SC 29634 USA

Peach fruit quality DNA tests – *Development* to *Deployment*

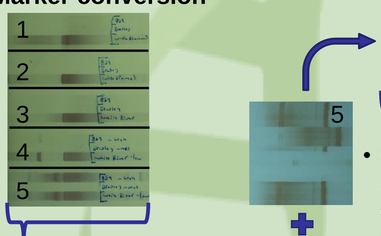
DNA tests that predict valuable trait levels are essential for widespread adoption of marker-assisted breeding (MAB) of rosaceous tree fruit. The RosBREED project has facilitated development of DNA tests for important traits in peach, apple, and cherry, including peach sweetness and acidity. Based on a quantitative trait locus discovered on peach chromosome 7 by RosBREED collaborators explaining ~20% of phenotypic variation for titratable acidity (TA) in normal acid peaches and ~10% of variation in soluble solids content (SSC), a DNA test ("**G7Flav-SSR**") was developed at Washington State University (WSU). The G7Flav-SSR DNA test was converted from a SNP array-based marker with six diplotypes to a PCR-based SSR marker with three functional genotypes whose effects were confirmed within RosBREED Peach Demonstration Breeding Programs and validated within the University of Arkansas Peach Breeding Program (UA). Although this DNA test can be used alone, when combined with the *D* locus DNA test (CPPCT040b)[1] the predictive power of the combined DNA test makes it a valuable tool for MAB. This combined DNA test can be used to enhance breeder confidence in selecting parents and seedlings with specific, valuable TA and SSC levels, thereby increasing overall breeding efficiency.

Follow the glowing peach from development to deployment of the new peach flavor DNA test G7Flav-SSR.

Development

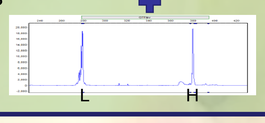
The G7Flav-SSR DNA test was developed through marker conversion from the RosBREED 9K SNP array with 6 diplotypes to a PCR-based SSR marker with 6 SSR genotypes (1), converted to a high-throughput format (2), and combined with the *D* locus CPPCT040b[1] DNA test (3).

1. Marker conversion



- Although low acidity could be differentiated from high acid, no test alone could differentiate medium – Therefore, multiplexing was required
- Once multiplexed, all SNP diplotypes were represented by SSR alleles, **But,** for MAB utility high-throughput conversion was required

2.



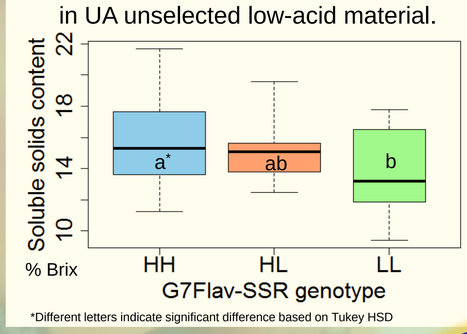
3. To maximize MAB utility, G7Flav-SSR was combined with the acidity CPPCT040b DNA test (L-low acid alleles; H-high acid alleles)

Combined DNA test for maximum predictability ready for validation

Validation

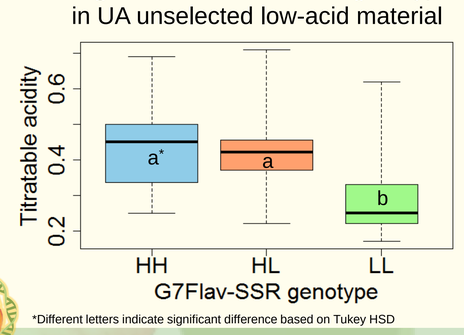
Validation of G7Flav-SSR was conducted on unselected families of UA germplasm (n= 4). Within UA seedlings grouped as low acid by CPPCT040b, the G7Flav-SSR "LL" genotypes (homozygous for the low acid allele) had a significantly lower SSC than the "HH" genotypes (homozygous for the high acid allele) (Fig. 1). Within the same group the G7Flav-SSR "LL" genotypes had a significantly lower TA than both the "HH" and "HL" genotypes (Fig. 2).

Fig. 1. SSC of G7Flav-SSR genotypes in UA unselected low-acid material.



*Different letters indicate significant difference based on Tukey HSD

Fig. 2. TA of G7Flav-SSR genotypes in UA unselected low-acid material



*Different letters indicate significant difference based on Tukey HSD

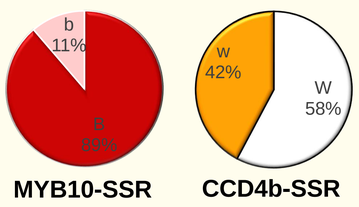
New tests

Additional peach DNA-tests developed

Flesh color + blush

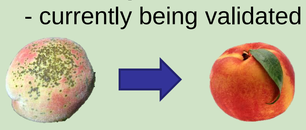
MYB10-SSR combined with **CCD4b-SSR[4]** to predict flesh color (white vs. yellow) and degree of blush

MYB10-SSR allele (B: high blush; b: low blush) and CCD4b-SSR allele (W-white; w-yellow) frequencies in UA selected material



Bacterial spot

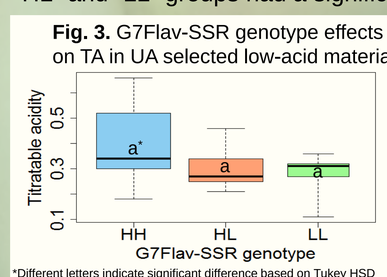
Xap-SSR
- currently being validated



Deployment

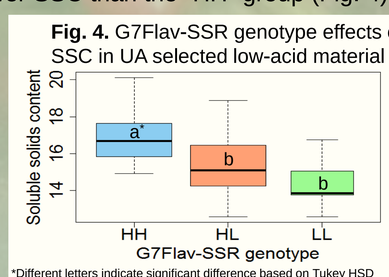
Although differences between the genotype groups "HH", "HL", and "LL" in UA selected low-acid material (the parent pool) were not significantly different for TA (Fig. 3), the "HL" and "LL" groups had a significantly lower SSC than the "HH" group (Fig. 4).

Fig. 3. G7Flav-SSR genotype effects on TA in UA selected low-acid material



*Different letters indicate significant difference based on Tukey HSD

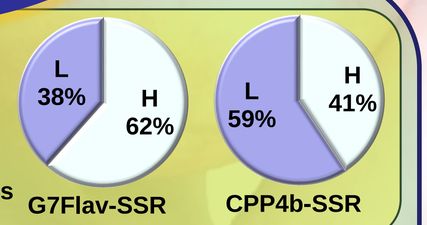
Fig. 4. G7Flav-SSR genotype effects on SSC in UA selected low-acid material



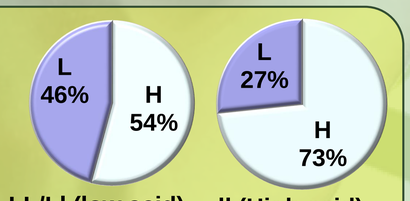
*Different letters indicate significant difference based on Tukey HSD

The genotype effects showed G7Flav-SSR + CPPCT040b-SSR was ready for MAB
- G7Flav-SSR + CPPCT040b were screened on UA parent pool in summer 2014 to inform spring 2015 parent and cross selection
- G7Flav-SSR + CPPCT040b will be screened on 2015 seedlings

G7Flav-SSR and CPPCT040b allele frequencies in Arkansas selections



G7Flav-SSR allele frequencies in Arkansas selections grouped by CPPCT040b genotype



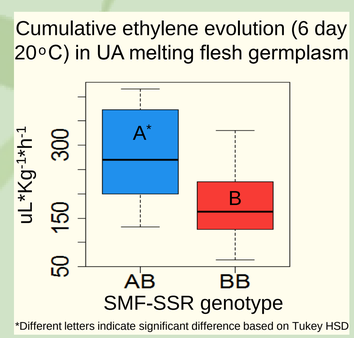
New tests

Additional peach DNA-tests developed

Flesh type + ethylene evolution

SMF-SSR combined with **endoPG-6[3]** to predict flesh type (melting flesh, non-melting flesh, non-softening flesh) and rate of ethylene evolution

Cumulative ethylene evolution (6 day 20°C) in UA melting flesh germplasm

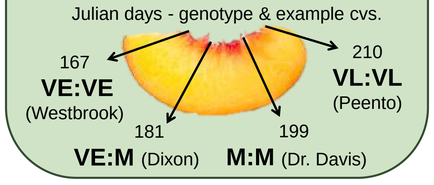


*Different letters indicate significant difference based on Tukey HSD

Maturity date

G4Mat-SSR
– currently being validated

Julian days - genotype & example cvs.



Other crops

Apple

Additional DNA-tests are being developed for apple:
- Lg8A-SSR[2]: acidity
- Lg1Fru-SSR: sweetness

Sweet cherry

Additional DNA-tests are being developed for sweet cherry:
- G4Mat: maturity date
- Firmness

CONCLUSIONS

By harnessing the power of collaboration, specifically the integration of pedigree, phenotypic, and genotypic data generated by RosBREED team members for QTL discovery, the development of these DNA tests was possible. Tools such as G7Flav-SSR are now available to make DNA-based predictions a routine part of tree fruit breeding. This advance in DNA-informed breeding represents an example of successful collaboration among institutions and across disciplines and sets the stage for the development of more DNA tests. Future work will broaden the adoption of DNA-informed breeding across Rosaceae tree fruit crops through the advancement of these and other DNA tests.

[1]Boudehri K, Bendahmane A, Cardinet G, Troadec C, Moing A, Dirlewanger E. 2009. Phenotypic and fine genetic characterization of the *D* locus controlling fruit acidity in peach. BMC Plant Biology 9:59.
[2]Powell A, Sandefur P, Verma S, Evans K, and Peace C. 2014. The power of two: Maximizing predictive strength in breeding for apple acidity by combining DNA tests. RGC7 poster.
[3] Peace C, Crisosto C, and Gradziel T. 2005. Endopolygalacturonase: a candidate gene for *Freestone* and *Melting* flesh in peach. Molecular Breeding 16:21-31.
[4]Adami Y et al. 2013. Identifying a carotenoid cleavage dioxygenase (*ccd4*) gene controlling yellow/white fruit flesh color of peach. Plant Molecular Biology Reporter 31:1166-1175.

Funding for *RosBREED: Enabling marker-assisted breeding in Rosaceae* is provided by the Specialty Crop Research Initiative Competitive Grant 2009-51181-05808 of the USDA's National Institute of Food and Agriculture.