Final Technical Report - MDARD/Craft Beverage Council
Development of Red-Juiced Apple Cultivars for Michigan Hard Cider
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Abstract: Hard cider is a rapidly growing sector of the Michigan beverage industry, and novelty/specialty ciders are in demand. Some non-commercial apple cultivars produce red juice, and can be used to make excellent hard cider with an appearance similar to rose or red wine. However, none have been optimal for production in Michigan. This grant supported *Michigan PureRed*, a long-term project that uses traditional plant breeding to develop a collection of hardy, vigorous and disease-resistant apple cultivars that produce fruit with strongly red-colored juice and optimal characteristics for use in hard ciders.

Original goals and objectives. The goal of the Michigan PureRed apple breeding project is to develop a collection of hardy, vigorous and disease-resistant apple cultivars that produce fruit with strongly colored, red juice optimal for hard cider production. The specific objectives for this MCBC-funded period (2020-2021) were:
1) To obtain phenotypic information for populations of apple plants generated in 2016 and 2017 from crossing a variety of red-juiced parents. This includes plant vigor, disease resistance, bloom and fruit maturity time, and fruit size, juice yield, and juice biochemistry (sugar/soluble solids, pH, acidity, total phenolic content, and subjective taste and flavor).
2) To make further crosses using the identified superior, elite individuals from this collection as parents, creating new and potentially even better cultivars.
3) To map genetic loci that are important for intense red juice color, and genetic alleles associated with this trait.

Results and conclusions (according to objectives)
1) A small subset (<5%) of evaluated lines exhibited dwarfism and aberrant development, likely resulting from cytogenetic problems. This is apparently common in apple breeding projects. The majority of the remaining plants have shown the expected degree of vigor as grafts to dwarfing rootstock (the use of dwarfing rootstocks permits more efficient cultivation and evalution, and is consistent with modern apple production). However, we noted that plants with the most highly pigmented foliage (dark purple) generally grew slowly, and this was attributed to decreased photosynthetic efficiency. Regarding disease resistance, the populations that were evaluated were selected the previous year(s) for growth under low-maintenance conditions. The remaining progeny evaluated during this period were essentially free of fire blight and scab, two of the most serious Michigan apple afflictions. In general, we also noted that the progeny displayed similar or greater range in spring bloom time and fruit maturity date as other cider apple cultivars. The subset of very early blooming progeny were eliminated from further consideration as these may be subject to frost damage in some years. We prioritized representatives of early-, mid-, and late-season maturing progeny as a range in harvest time is desired by growers. However, we found that, in general, very early maturation was associated with ‘dry fruit’ and poor juice extraction, and such progeny were also eliminated. Of the remaining lines, most (70-85%) were eliminated

from further use (direct use and use as parents in further crosses) due to two traits: small fruit size and/or high astringency. Only a small fraction of progeny produced fruit of acceptable size (>2.5" diameter). Concerning juice color, we found that a high proportion (at least 15%) of the populations produced fruit with juice color that was more intensely red than any apple cultivar currently characterized, some with intensity similar to beet juice. As anticipated, all of these traits segregate independently in populations, and progeny with optimal combinations of these traits are rare. However, because we have evaluated so many, we have so far identified at least 10. The top achievement was the identification of progeny that are better than 'Otterson' and 'Cranberry', two red-juiced cultivars currently in commercial use. Potentially superior subsets of progeny are being re-evaluated currently (Aug-Oct 2021). One of these has already been distributed to a grower for field testing under commercial conditions.
2) We carried out genetic crosses in spring of 2020 and 2021 at the Clarksville Research Station. These included crosses between PureRed progeny, as well as between PureRed progeny and selected parents of the PureRed families. Progeny from crosses made in 2019 are now in the field. For crosses made in 2020, fruit was harvested in Aug-Oct 2020 and resulting seedlings were germinated and grown in controlled environment rooms at MSU. We extended the scope of work for crosses made in 2021 to include crosses to many domestic apple cultivars. The fruit will be harvested in Aug-Oct 2021 and progeny generated will be cultivated (potentially) in contract with a commercial nursery.
(3) Red juice color is controlled primarily by a single genetic allele, called MYB10-R6, which was previously identified by a research group in New Zealand. However, the genetic bases of *highly-colored, intense* red juice is more complicated. We approached this by creating several populations that are isogenic (all the same) for the MYB10-R6 allele, but highly genetically diverse otherwise. Because tracking juice-color genes requires evaluation of fruit, and because progeny are expected to produce fruit only after several (3-5) years, we initially attempted to utilize seedling foliage color as a proxy for fruit color in order to select (and therefore map) for this trait. This should have greatly reduced the number of progeny to be evaluated, and effort. However, we have now found that seedling color is influenced too much by germination conditions, and so all of the progeny (instead of just a subset) were planted at the MSU Clarksville site for future evaluation. During this past grant period, a research group in China identified an additional genetic allele that appears to strongly contribute to the intensity of red juice color, and we will use this information to guide our further research. In spring of 2021, we generated an additional seven isogenic populations for this purpose.

Timeline. Apple breeding programs such as PureRed are long-term efforts. For new cultivar development, at least 10 years is needed between the time a cross is made, and when the evaluations of the fruit are completed. *Michigan PureRed* is expected to be an ongoing project, with this two-year funding from MCBC instigating continuous research and development to be supported in the future by plant royalties from the cultivars that are generated. During the funding period for this report (April 1, 2020 - July 31, 2021), we continued to evaluate phenotypic traits of PureRed progeny generated previously, focused evaluation efforts on potential superior progeny, and performed additional crosses to generate more progeny populations for evaluation. In addition, we developed genetic tools to enable more precise selection in the future. We have now released one line for testing to a grower, and hope to release additional lines this coming year. If these perform well over the next few years, they will be made available to Michigan growers immediately.

Work accomplished (by objective).

1) Phenotyping of existing PureRed selections:

The Michigan PureRed collection now comprises nearly 350 individuals growing at the MSU Clarksville Research Station. Most (>90%) have flowered and produced fruit sufficient for analyses. Two representatives are propagated from each selection as grafts to dwarfing rootstock. At least 10 fruit were evaluated from each of the two replicates for each of >300 progeny, yielding two biological replicates each. For each juice analysis listed, two technical replicates were performed. Selections were evaluated according to the following criteria:

• Fruit size. Selections with fruit < 1.75 in average diameter, or with >10 % < 1.75 in diameter, were immediately culled. We found that this is the minimal fruit size to enable efficient processing with existing apple equipment.

• Juice color was assessed for two parameters, color density and hue. Juice was measured for absorbance in the wavelength range of 400 to 600 nm, using a spectrophotometer. Color density was determined by the summation of absorbance measurements at 420 and 520 nm, while hue was determined by ratio of absorbance at 420/520 nm. Selections with color density less than 75 % that of the strongly-pigmented 'Otterson' were culled.

• Juice yield was determined by measuring total extractable juice after shredding and pressing. Those selections that passed these three criteria were subjected to additional analyses of fruit juice traits:

• Sugar/soluble solids content were determined using a digital refractometer.

• PH was determined with a pH meter.

• Titratable acidity (mostly malic acid) was measured using an autotitrater.

• Total phenolic content was determined using the potassium permanganate titration method.

Following selections made in 2020 and previously, 60 progeny with the most desirable traits have now been propagated, with 5 individuals each established on dwarfing rootstock at the CRC site. The first of these should bear fruit in 2023, and all plants will be observed and analyzed for four years following development to full production.

2. Further improvements to Michigan PureRed selections:

The anticipated phenotypes of the initial PureRed selections reflect those of the parents used in the crosses. Due to the nature of genetics, otherwise superior selections will likely have distinct trait flaws - for example, susceptibility to fire blight, or small fruit size. The initial PureRed population provides an excellent opportunity to target these undesirable traits for elimination. Additional rounds of crossing were carried out in spring of 2020 and 2021 to combine desirable traits in the progeny plants. The expected result is a defect-free selection with highly desirable fruit juice traits. A total of 35 progeny were grafted to dwarfing rootstock at the MSU Clarksville site during late winter. The number of plants was less than anticipated, because MSU COVID policies impeded frequent access to the growth facilities.

3. Mapping genetic loci for intense pigmentation

In Spring 2020 and 2021 we carried out controlled crosses between heterozygous and homozygous, MYB10-R6 genotypes including Cranberry, Otterson, and several Michigan PureRed lines, with many non-MYB10-R6 genotypes including Fuji, Honeycrisp, and Gala.

Progeny from 2020 crosses were germinated and cultivated in controlled environment rooms at MSU, and grafted to dwarfing rootstocks in late winter (2020-2021). Fruit from crosses made in spring 2021 is now maturing (fall 2021) and seed will be germinated over the coming winter.

Communication activities, accomplishments and impacts

Results of this two-year (spring 2019-summer 2021) study have been communicated to peer, professional and industry audiences through the following items (most recent listed first):

- Popular press: "Red-fleshed apples could keep Michigan cider makers in the black" by Kyle Davidson. Great Lakes Echo, March 30, 2021.
- Popular press: "MSU professor changing cider's taste, breeding red-fleshed apples for juice". wzzm13.com, March 5, 2021.
- Popular press: "An MSU research wants to get red-fleshed apples to grow". Lansing State Journal, Feb 24, 2021.
- Distributed scionwood of a PureRed line to Robinette's Apple Hause and Winery, Grand Rapids MI for field testing.
- Trade article: "Red flesh for red ciders" by Matt Milkovich. Good Fruit Grower, June 24, 2020.
- We assisted Left Foot Charlie Winery in development of red cider from apples grown at MSU ('Otterson'). https://untappd.com/b/left-foot-charley-otterson/2630597 and provided several hundred pounds of fruit for evaluation in 2019 and 2020.
- Trade article: "Testing cider cultivars" by Stephen Kloosterman. Fruit Grower News, Sparta MI October 2019.
- Presentation: "Red-juiced apple cultivars for hard cider production. Empire State Producers Expo, Syracuse NY, 2019
- Presentation: "Cider Varieties and PureRed Breeding Program". December 2019 GLEXPO, Grand Rapids, MI.
- Presentation: Red-juiced apples and their use in rose cider. CiderCon 2019, Chicago, IL.
- Social Media (YouTube): from MSU Communications: https://youtu.be/eomIHxaLKdc
- Collaboration with Robinette's Apple Hause and Winery, Grand Rapids MI to produce red sweet cider and hard cider
- Presentation to MI apple growers: 'Evaluation and Development of Red-Juiced Apple Cultivars for Michigan Production'. MSU-CRC Field Day, 2019, Clarksville, MI.
- Presentation to Great Lakes-area apple growers: 'Red Fleshed and Other Special Varieties for Sweet Cider'. GLEXPO Sweet Cider session, December 2019.
- Trade article: 'Researcher's quest for a red-juiced apple pays off'by Stephen Kloosterman. Fruit Grower News, Sparta, MI. October 23, 2019. https://fruitgrowersnews.com/article/researchers-quest-for-a-red-juiced-apple-pays-off/
- As a result of this work, we have been regularly distributing cuttings from the cultivars Otterson, Cranberry, and Irene for apple growers in Michigan and elsewhere to propagate, in order to establish their own orchards. More than 50 growers have received or inquired about receiving scion wood from us. We have also supplied nurseries with cuttings for propagation. These nurseries included Hostetler in Indiana, Fedco Seeds in Maine, Schlabach's and Cummins in New York, and Adams County in Pennsylvania.

Budget narrative

The project was conducted consistent with the proposed budget by the principle investigator, Steve van Nocker and approved by the State of Michigan. There was no alternative funding used for this project during this past funding period. There was no in-kind support for this project by private business during this period.

Figure 1. Two progeny lines from the Michigan PureRed project available for immediate release to growers MPR2016-00104 (left) and MPR2016-109 (right). Both were produced using 'Otterson' as a paternal parent. 'Otterson' is used for red cider production in Michigan, but suffers from poor juice taste. Both MPR lines are disease-free, vigorous, and produce Otterson-like fruit with enhanced taste and flavor.