Final Technical Report - MDARD/Craft Beverage Council

Michigan Pure-Red: Development of Red-Juiced Apple Cultivars for Michigan Hard Cider Production

May 1 2019 - May 31 2020

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Abstract (88 words). 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 are optimal for production in Michigan. This project uses traditional plant breeding to develop a collection of hardy, vigorous and disease-resistant apple cultivars that produce strongly colored juice optimal for hard cider production. This was the first year of an anticipated two-year project.

Original goals and objectives. The goal of the Michigan PureRed project is to develop a collection of hardy, vigorous and disease-resistant apple cultivars that produce strongly colored, red juice optimal for hard cider production. The specific objectives for this MCBC-funded period were:
1) To collect phenotypic information, including fruit and juice traits, on a population of plants generated in 2016 from crossing a variety of red-juiced parents and propagate selections
2) To create further crosses of superior, elite individuals for future evaluations
3) To initiate experiments to locate genetic alleles conferring strong red juice color on the existing genetic map of apple

Results and conclusions (according to objectives)
1) We evaluated the PureRed breeding lines for phenotypes relating to hard cider production and obtained quantitative information on fruit size, juice color, and juice yield. Breeding lines were also observed and indexed for apparent resistance to the two most serious apple diseases in Michigan - fire blight and scab. Desirable selections were propagated for further observations.
2) In early May in both 2019 and 2020, crosses were carried out to attempt to combine desirable traits of the parental lines. For crosses made in spring of 2019, seeds were harvested in October 2019, germinated, and seedlings were selected based on apparent presence of the major red-juice locus. These are being grown and will be planted in the field in Spring 2021.
(3) In spring of both 2019 and 2020, we carried out controlled crosses between isogenic cultivars varying in pigmentation. For crosses made in 2019, seeds were harvested in fall 2019 and germinated, and seedlings will be planted in the field in Spring 2021. Currently, molecular markers are being used to define a genetic map position for the enhancer.

Project scope and timeline. Apple breeding programs such as PureRed are a long-term effort. 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. This project to evaluate the first PureRed progeny and generate additional progeny is limited to two years. During the funding period for this report (May 1, 2019 - May 31, 2020), we evaluated phenotypic traits of the first PureRed progeny and performed additional crosses to generate more progeny populations for evaluation. The second year of the project will be focused on better evaluation of production and fruit quality characteristics of progeny deemed superior in Year 1, propagating progeny selected in Year 1, and generating even more progeny for evaluation.

Work accomplished (by objective).

1) Further phenotyping of existing PureRed selections:

During this reporting period we could not evaluate all PureRed progeny, because only ~80% of the individuals (now 332) flowered and produced fruit. Another ~60 plants grew poorly (due to disease or innate developmental defects) and were culled. Plants were maintained at the Clarksville Research Center (CRC), with two representatives propagated from each selection and grafted to dwarfing rootstock. At least 10 fruit were evaluated from each of the two replicates for each of 270 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 (Zoecklein et al 1995). 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 (Löwenthal method; Zoecklein et al 1995; Lea 2008).

Following this intial selection, 55 progeny with the most desirable traits were propagated, and 5 individuals each were established on dwarfing rootstock at the CRC site. Additional selections will be made in 2020, and all propagated plants will be observed and analyzed for four years following development to full production (eight years total).

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. A second round of crossing was carried out in Spring 2019 and 2020 to combine desirable traits in the progeny plants. The expected result is a defect-free selection with highly desirable fruit juice traits. All seedlings will be grafted to dwarfing rootstocks in Feb. 2021 for further growth and field evaluation.

3. Mapping the enhancer of pigmentation

The existence of a genetic enhancer of the hyperactive MYB10 allele is hypothesized based on the fact that genotypes isogenic for MYB10 (i.e., heterozygous or homozygous) show varying degrees of pigmentation. Our limited knowledge of this hypothetical enhancer has impeded our development of superior selections. In this step, we are using traditional genetic techniques to map the enhancer to a small area of the apple genome. This will allow the development of a molecular marker. Consequently, this will allow selection of seedlings that will (eventually) produce highly pigmented fruit. To accomplish this, in Spring 2019 and 2020 we carried out controlled crosses between isogenic cultivars varying in pigmentation (Otterson, Fuji, Honeycrisp). In each year, four series of crosses were carried out. In 2019, we generated two populations, Otterson x Fuji with 125 individuals, and Cranberry x Honeycrisp, with 88 individuals. Progeny were evaluated for cotyledon color, as a proxy for fruit color, and genotyped using molecular technologies. Unfortunately, both populations became infected with a disease (powdery mildew) while being grown in a controlled environment chamber, and had to be discarded. The crosses were repeated in Spring 2020 and were successful.

Communication activities, accomplishments and impacts

Although this is a long-term project and we cannot yet demonstrate impact, we communicated out ongoing work to peer, professional and industry audiences through the following items:

- 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
- Presentation: "Cider Varieties and PureRed Breeding Program. GLEXPO, Grand Rapids, MI
- Presentation: Red-juiced apples and their use in rose cider. CiderCon, Chicago, IL.
- Social Media (YouTube): from MSU Communications: https://youtu.be/eomIHxaLKdc
- We worked with Robinette's Apple Hause and Winery, Grand Rapids MI to produce red sweet cider (entered in sweet cider competition at GLEXPO, 12/19).
- 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
- Presentation to MI apple growers: 'Evaluation and Development of Red-Juiced Apple Cultivars for Michigan Production'. MSU-CRC Field Day, 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. To date, 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. A small amount of funding for this project was obtained from Michigan State Horticulture Society (Van Nocker S. Development of Red-juiced apple cultivars for Michigan hard cider production. Michigan State Horticulture Society, 6/1/19-5/30/20 $3,788), and there was no overlap in proposed objectives with this MDARD MCBC project.

Fruit from Michigan PureRed breeding line MPR19-114. This ~2" diameter fruit produces abundant juice with at least four times the color intensity of any known red-juiced apple. This and other promising selections will be propagated in 2020 for production trials.