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Fruit and Nut Germplasm Collections: Treasuries of Genetic Diversity:

Preserving the future with the USDA Plant Genetic Resource Unit tart cherry, grape, and apple germplasm collections

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Abstract

Preservation of crop genetic diversity is critical for the future of American agriculture. The USDA-ARS Plant Genetic Resources Unit (PGRU) at Cornell AgriTech, Geneva, NY preserves collections of apples, cold-hardy grapes, tart cherries, and their wild relatives. These collections hold vast potential to improve our horticultural crops through environmental adaptations, pest and disease resistance, and novel fruit qualities. PGRU's mission is to maintain, acquire, distribute, and conduct research to support global conservation efforts and promote the use of these resources. Over the past decade, PGRU has distributed nearly 65,000 samples from its collections, representing cuttings for propagation, pollen, seeds, vegetative tissues, and DNA. Clonal propagation is used to preserve cultivar and type specimens. The aim of this article is to highlight the value of PGRU collections, describe how they can promote sustainability for the US apple, grape, and tart cherry industries, and address some of the maintenance challenges of preserving clonally propagated crops.

Visitors come from all over the world to see the USDA Plant Genetic Resources Unit's (PGRU) tart cherry, grape, and apple collections. National Plant Germplasm System (NPGS) clonal genebanks like the one in Geneva, NY are remarkable to visit, with acres of genetically distinct trees and vines grown together on one site and accessible for evaluation and utilization. Many visit because they are interested in the history of the accessions represented, such as 'Roxbury Russet', the oldest named American apple cultivar (Bussey and Whealy 2016). These collections represent the past and present of horticulture, but most importantly they represent its future. Plant breeding is as much preparing for the future as it is creating it, and diverse germplasm collections provide potential for long-term genetic improvement. For perennial crops, success and future durability of a cultivar is dependent on current use of quality germplasm. Development and adoption of new specialty crop cultivars can take decades; this process should begin today, founded on broad genetic diversity.

The PGRU clonal collection is co-located with the USDA Northeast Regional Plant Introduction Station on the Cornell AgriTech (New York State Agricultural Experiment Station) campus in Geneva, NY. The apple and grape collections began in 1983 and have deep ties to Cornell University's apple and grape breeding programs, with much of the founding material originating from their genetic resources. The tart cherry collection began in 1995 in collaboration with Michigan State University's cherry program (Iezzoni 2005). PGRU functions through the National Program of Crop Production and Protection, and through the multi-state Regional Research Project NE-9.

Science and technology continue to advance American horticulture and the future is bright. The PGRU collections hold tremen-

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dous potential to improve the apple, grape, and tart cherry industries, including more nutritionally diverse crops, adaptation to shifting climates, and resistance to pathogens. However, future impact depends on present utilization of crop genetic diversity. The intention of this summary is to highlight PGRU's clonal plant genetic resources and our services provided to research communities.

Collections

Tart Cherry

Prunus species provide an assortment of valuable horticultural crops, including cherries, plums, apricots, peaches, and nectarines. PGRU is responsible for tart cherry (*Prunus cerasus*) and its relatives and maintains 131 accessions representing 11 species and hybrids. *P. cerasus* (2n=32) is a tetraploid hybrid of *Prunus avium* (2n=16) (sweet cherry) × *Prunus fruticosa* (2n=32) (European dwarf cherry). Fruit quality, including fruit firmness, acidity, final use, and fruit chemistry distinguish tart cherries from sweet cherries (Serradilla et al. 2016).

Tart cherry is a prime example of the need to utilize broad genetic diversity. U.S. tart cherry production was valued at $56.6M in 2018 (USDA/NASS 2019), with production based on a centuries-old French cultivar, 'Montmorency'. PGRU research is focusing on tart cherry fruit quality, aromatics, and anthocyanin content. Anthocyanin content is of interest because of its contribution to fruit color and nutritional quality of tart cherries (Serradilla et al. 2016). Fruit samples were collected across five growing seasons and measured for different fruit quality traits. Results show total soluble solids (sweetness) ranges from 11.4 to 22.63 °Brix, and malic acid content ranges from 5.3 to 34.7 $g \cdot L^{-1}$. Total anthocyanin content ranges from 72.9 to 2640.7 $\mu g \cdot g^{-1}$ in fresh fruit, with 'Montmorency' on the low end (110.1 $\mu g \cdot g^{-1}$). Cultivars 'Stevensbar', NY 13664, 'Tamaris', and a *Prunus* hybrid PI 657708 had high total anthocyanin content with 1466.4, 1575.6, 2038.0, and 2640.7 $\mu g \cdot g^{-1}$, respectively (unpublished data).

While 'Montmorency' remains fixed in time through propagation, climate conditions and horticultural practices have changed around it. Events such as late frost, higher than normal temperatures, or rainfall significantly impact tart cherry production in the United States (USDA/NASS 2019). Additionally, the cherry industry is moving to primarily mechanical harvest techniques. Cultivars with increased quality, local adaptations, and increased yield will ensure the continued success of the U.S. tart cherry industry (Iezzoni 2008).

Grape

One of the greatest germplasm success stories took place during the 19th and 20th centuries in Europe where vineyards were confronting severe loss in quality and production following introduction of grapevine pests and pathogens from North America. North American *Vitis* species provided genetic resistance to these diseases, sparking an interest in hybrids as table or wine grapes and rootstocks. Despite the success of interspecific grapevines, global production favors *V. vinifera* cultivars for wine and table grapes (Reisch et al. 2012).

Cultivated grapevines, including *Vitis vinifera* and *Vitis* interspecific hybrids, are among the most culturally and economically valuable fruit crops worldwide. Annual global production averages 73.9M metric tons (FAO 2019). Although *V. vinifera* was domesticated near the Black and Caspian Seas, North America and Asia are centers for *Vitis* diversity and targets for conservation (Reisch et al. 2012). The genus *Vitis* is highly diverse, with an estimated 60 species worldwide. The PGRU collection maintains 1,416 accessions with approximately 800 *Vitis* hybrids, 27 species and cultivated hybrids, with an emphasis on cold-hardy grapevines.

Modern breeding continues to utilize wild relatives for disease resistance and climate adaptations. Regional grape production is predicted to shift in response to climate change (Alikadic et al. 2019). Grapevine development is primarily controlled by temperature, measured as chilling hours and growing degree days (Martínez-Lüscher et al. 2016). Sensitivity to extreme cold temperatures limits where *V. vinifera* cultivars are grown. However, hybridization of *V. vinifera* with cold-hardy *Vitis* species can expand the range of grape cultivation (Londo and Kovaleski 2017). Although many grape cultivars, particularly wine grapes, are grown for their distinctive qualities and cultural acceptance, utilization of broader genetic diversity will sustain grape production in the future despite shifting climates.

Apple

The domesticated apple, *Malus domestica*, easily wins in a popularity contest. Unlike most other fresh produce, apples are marketed by name and many consumers are familiar with several cultivars. As one of the world's largest and most diverse apple collections, the apple collection is the center piece of the PGRU clonal repository. PGRU maintains 6,079 unique accessions, representing 55 species and cultivated hybrids, including 2,937 permanent accessions, research populations, seedlings under evaluation, and seed lots.

Apples and wild apple relatives are largely self-incompatible and can hybridize between species, creating a tremendous reservoir of genetic diversity. Despite the diversity of apple genetic resources, analysis suggests that modern apple breeding narrowly relied on a limited number of founding parents (Noiton and Alspach 1996). *M. domestica* is primarily an interspecific hybrid between progenitor species *Malus sieversii* and the European crabapple, *Malus sylvestris* (Velasco et al. 2010; Cornille et al. 2012). Additional species also contributed to apple domestication and more modern breeding included further hybridization to bring key traits from wild relatives into a domesticated background (Brown 2012). As such, *in situ* and *ex situ* preservation of wild *Malus* are a critical focus (Bramel and Volk 2019). Loss of habitat and gene flow of *M. domestica* into wild *Malus* populations are concerning (Cornille et al. 2013).

From 1989 to 1996, PGRU led explorations for wild *Malus* in Central Asia, of which a crowning achievement was the collection of *M. sieversii* from Kazakhstan (Forsline et al. 2003). Resistance for fire blight, apple scab, and blue mold were identified in these resources (Forsline and Aldwinckle 2004; Norelli et al. 2017). More recently, PGRU has been active to conserve wild germplasm of *Malus angustifolia*, *Malus coronaria*, and *Malus ioensis* from North America, and *Malus doumeri* from Vietnam.

Maintenance

As part of the NPGS, PGRU follows the mission to acquire, preserve, characterize, and distribute genetic resources (Bretting 2018). Maintenance is our most significant challenge, particularly for cultivars. Tart cherries, grapes, and apples, like other fruit crops, are propagated clonally to preserve a cultivar's unique identity, which is lost through sexual reproduction. To perpetuate cultivars such as 'Montmorency', 'Concord', or 'Golden Delicious' into the future, they must be clonally propagated; however, these cultivars likely have a secure future independent of the NPGS. Lesser known but equally unique cultivars are at risk for horticultural extinction without conservation programs. Therefore, conservation of these crops is heavily focused on preservation of clonal lineages, rather than allelic diversity and if Curators are not diligent, collections can quickly fill with distinct cultivars of low genetic diversity. For example, PGRU preserves grape cultivar 'Steuben' and both its parents, 'Sheridan' and 'Wayne'. Crop wild relatives can also be utilized for genetic improvement and are a critical focus for conservation and can be preserved as clones or seeds (Volk et al. 2016).

Fire blight is the greatest challenge for the apple collection. PGRU is evaluating Geneva® series apple rootstocks to better manage fire blight. Cold stress is the primary challenge for the *Vitis* collection. Although most of the vines are winter hardy, early or late frosts can damage vines during acclimation or de-acclimation. PGRU *Vitis* accessions are propagated on their own roots to promote re-growth. To prevent accession loss, grapevines and tart cherries are planted in duplicate. Apples are initially planted in duplicate but reduced to single trees once mature to maximize accession density.

Alternatives to Field Maintenance of Clonal Crops

While field maintenance provides immediate access for evaluation and breeding, accessions are continually under biotic and abiotic pressure. PGRU follows conventional practices to maintain apples, grapes, and tart cherries. However, practices that rely on developmental timing (e.g. fire blight management) fall short due to phenological diversity among accessions. Long-term storage of biological material in liquid nitrogen through cryopreservation provides a way to preserve clonal material free from environmental pressures and serves as a backup to replace field accessions lost to disease or catastrophic events. Currently, 70% of the permanent apple accessions are successfully cryopreserved as budwood. As field accessions are lost due to environmental pressures, they can be repropagated from cryopreserved budwood. Challenges for grapevine and *Prunus* cryopreservation protocols, including labor intensive micropropagation techniques, are limiting factors for these crops (Bettoni et al. 2019). Alternatively, genetic resources can be preserved as pollen or seeds, which is an ideal approach for wild accessions for which allelic combinations do not need to be clonally maintained (Volk et al. 2016). PGRU is currently evaluating seed preservation of apomictic apple accessions, which preserves allele combinations. However, apomixis is generally found only in wild, not cultivated *Malus* (Qu et al. 2008).

Distribution and Utilization

Access to germplasm is critical for utilization. Therefore, aside from maintenance, distribution of plant material, including their associated information is one of our highest priorities, and the largest service we provide to our stakeholders. PGRU routinely distributes cuttings, fruit, pollen, vegetative tissues, and DNA. Postman et al. (2006) described an anticipated future era with 'enhanced utilization' of NPGS clonal collections. Worldwide, the NPGS is the largest germplasm provider, distributing approximately 250,000 samples annually (Byrne et al. 2018). From 2009 to 2018, PGRU distributed 65,000 samples representing 4,679 genotypes (USDA 2014). However, this only represents approximately 60% of available PGRU accessions. Distribution gap analysis could identify causes and remedies for underutilized accessions, and lead to enhanced use of PGRU collections.

Characterization

Accessibility and utilization of plant genetic resources are enhanced by characterization. Annually, PGRU images and characterizes 50 to 100 accessions for descriptors defined by Crop Germplasm Committees. This effort provides a base record of the phenotypic variation to facilitate selection of accessions for further research or breeding. Whenever possible, specific traits are targeted for more detailed evaluations across seasons. For example, PGRU evaluated the entire grapevine collection for phenological traits, including budburst, bloom, and veraison over a three-year period. Metabolite variation, particularly when associated with fruit quality, has become an important phenotype to measure. PGRU supported several projects including aroma analysis of apple (Sugimoto et al. 2015), phenolics in grapevines (Liang et al. 2012), and dihydrochalcones in apple (Gutierrez et al. 2018).

Genetics and Genomics

Genetic markers are an indispensable tool for germplasm curation. Distinctive genetic markers can identify duplicated accessions, mistakes in propagation, or misclassified species (Gross et al. 2012; Klein et al. 2018). PGRU utilizes several platforms to genotype its collections, including SSR, SNP arrays, and Genotyping-by-Sequencing (Gutierrez et al. 2018). Although each platform has inherent strengths and weaknesses, no single platform adequately captures the genetic diversity. For example, SNP arrays can exclude crop wild relatives, whereas others, such as GBS, are not well suited for highly heterozygous crops (Hyma et al. 2015). Advances in both genotyping and statistical approaches are having profound effects on genetic studies in perennial fruit crops. Many key historical studies relied on developed populations, generally F_1 crosses using a pseudo-testcross approach. However, association studies can leverage trait diversity observed in both structured and unstructured germplasm; Migicovsky et al. (2016) used historic data from the GRIN-Global database to identify QTL in the PGRU apple collection.

New Approaches

Digital agriculture, including artificial intelligence and machine learning that provides accurate, precise, and efficiently collected data, is the future of agriculture. Phenotyping is the limiting factor in genetic studies, particularly for association studies requiring large population sizes. Technology allows researchers to track growth, planting patterns, diseases and infestations, water needs, soil fertilization, as well as many other traits necessary for successful plantings and growing seasons. PGRU is utilizing aerial imaging and network technology to capture phenological events in apple and tart cherry. Phenological traits, such as timing of budbreak and bloom, are important for understanding adaptability of our genetic resources. However, these traits are challenging to score due to the size of the collections and broad genetic variation associated with each event requiring repeated observations. This technology, if successful, will save time and resources, and more accurately capture trait diversity in large germplasm collections.

Conclusions

Many people visit the Louvre with the set goal of seeing the Mona Lisa, having no further expectations. In order to get to the Mona Lisa, tourists walk through the previous paintings and artwork that had led to such a well-known masterpiece. Similarly, visitors of NPGS plant genetic resource collections come with their eyes set narrowly on specific cultivars, and through time, they learn about the vast genetic history and diversity that has resulted from evolution, domestication, and modern breeding. Museums serve to both preserve culture and inspire future innovation. Likewise, PGRU's mission and vision is to preserve diverse genetic resources and advance plant breeding in the future.

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