Journal of the American Pomological Society 76(2): 50-58 2022

Long Term Preservation of Genetic Resources: The USDA National Plant Germplasm System Apple Collection within the Context of Global Crop Conservation Strategies

Gayle M. Volk[1], Hannes Dempewolf[2], Paula Bramel[2], Victoria Meakem[3], Benjamin Gutierrez[3]

Additional index words: crop, genotype, international, *Malus*, phenotype, preservation

Abstract

Global crop conservation strategies have been written for many crops that are maintained in genebanks around the world. These strategies describe the status of crop collections with regard to collection holdings, management practices, and accessibility. The status of global apple collections was summarized in "A Global Strategy for the Conservation and Use of Apple Genetic Resources", which was published by Volk and Bramel in 2019. The USDA National Plant Germplasm System apple collection is a large, diverse collection of apple cultivars and species that provides plant genetic resources to scientists within the United States and internationally. Plant materials in this collection have been used for a wide range of research including species diversity, phenotypic characteristics (fruit quality, tree traits, disease resistance, etc.), genetics/genomics, domestication, breeding, and more. Therefore, it is particularly important that this collection be well documented, providing critical passport and accession-related data to the user community. New molecular technologies will facilitate cross-collection comparisons, which will better inform collection management practices and the global community.

Global Crop Conservation Strategies

International, national, state, and regional plant collections conserve genetic resources that may provide resistance to abiotic and biotic threats as well as traits that offer higher quality and yields for improved food, beverage, fiber, animal feed, ornamental and industrial crops. Often, popular and economically important plant varieties are conserved in many collections and are relatively secure due their availability, but heirloom, local, and rare varieties may be maintained in one or few collections, if they are conserved at all. In addition, species progenitors and other crop wild relatives may be key to future crop improvements, yet are not adequately preserved and protected in either *ex situ* or *in situ* conditions. It is important to understand the extent of conservation efforts for varieties and crop wild relatives in plant collections to assess their security on a global scale.

Global crop conservation strategies have been developed to assess the status of crop conservation efforts on a worldwide scale, often with an emphasis on national and international genebank collections, particularly for those crops listed in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (Hummer et al., 2015; ITPGRFA, 2009). Forty-three global crop conservation strategies have been developed or are being developed under the coordination of the The Global Crop Diversity Trust, written by crop expert community members, and made publicly available over the past 15 years (Table 1). These conservation strategies usually provide information about the crop and its genetic resources, re-

---

[1] USDA-ARS National Laboratory for Genetic Resources Preservation, 1111 S. Mason St., Fort Collins, CO 80521 USA, corresponding author Gayle.Volk@usda.gov

[2] The Crop Trust, Platz Der Vereinten Nationen 7, 53113 Bonn, Germany

[3] USDA-ARS Plant Genetic Resources Unit, Geneva, NY 14456 USA

Table 1. List of global crop conservation strategies that have been released by the Global Crop Diversity Trust. (https://www.croptrust.org/resources/#ex-situ-conservation-strategies).

Crop	Year	Title of strategy
Apple	2019	A global strategy for the conservation and use of apple genetic resources
Banana	2007	Global conservation strategy for *Musa* (Banana and Plantain)
Banana	2016	Global strategy for the conservation and use of *Musa* (banana) genetic resources
Barley	2008	Global strategy for the ex situ conservation and use of barley germplasm
Beans	2014	Conservation of *Phaseolus* beans genetic resources
Brassica	underway	
Breadfruit	2007	Breadfruit conservation strategy
Capsicum	underway	
Cassava	2010	A global conservation strategy for cassava (*Manihot esculenta*) and wild *Manihot*
Chickpea	2008	Global strategy for the ex situ conservation of chickpea (*Cicer* L.)
Citrus	underway	
Coconut	2008	Global conservation strategy for *Cocos nucifera*
Coffee	2017	Global conservation strategy for coffee genetic resources
Cowpea	2010	Global strategy for the conservation of cowpea (*Vigna unguiculata* subsp. *unguiculata*)
Cucurbits	2021	A global conservation strategy for crops in the Cucurbitaceae family
Edible aroids	2010	Edible aroid conservation strategies
Eggplant	underway	
Faba bean	2009	Global strategy for the ex situ conservation of faba bean (*Vicia faba* L.)
Finger millet	2012	Global strategy for the ex situ conservation of finger millet and its wild relatives
Grass pea	2007	Strategy for the ex situ conservation of *Lathyrus* (grass pea), with special reference to *Lathyrus sativus* , *L. cicera* , *L. ochrus*
Groundnut	underway	Global strategy for ther conservation and use of peanut genetic resources
Lentil	2008	Global strategy for the ex situ conservation of lentil (*Lens* Miller)
Maize	2007	Global strategy for the ex situ conservation and utilization of maize germplasm
Millets	underway	
Oat	2008	Global strategy for the ex situ conservation of oats (*Avena* spp.)
Pea	underway	Global strategy for the ex situ conservation of pea genetic resources
Pearl millet	2012	Global strategy for the ex situ conservation of pearl millet and its wild relatives
Potato	2006	Global strategy for the ex situ conservation of potato
Potato (update)	underway	Global strategy for the conservation of potato
Rice	2010	Development of a global strategy for the ex situ conservation of rice
Sorghum	2007	Strategy for the global ex situ conservation of sorghum genetic diversity
Sorghum (update)	underway	Global strategy for the conservation and use of genetic resources for Sorghum
Strawberry	2008	Global conservation strategy for *Fragaria* (strawberry)
Sunflower	underway	Global sunflower conservation strategy
Sweet potato	2007	Global strategy for ex-situ conservation of sweetpotato genetic resources
Tea	2019	A global strategy for the conservation and use of tea genetic resources
Temperate forages	2021	Global strategy for the ex situ conservation of temperate forages
Tropical forages	2015	A global strategy for the conservation and utilisation of tropical and subtropical forage genetic resources
Vanilla	2021	Global strategy for the conservation and use of vanilla genetic resources
Vigna	underway	
Wheat	2007	Global strategy for the ex situ conservation with enhanced access to wheat, rye and triticale genetic resources
Yam	2010	Towards a global strategy for the conservation and use of yam
Yam (update)	2021	Global strategy for the conservation and use of yam genetic resources

sults of a survey summarizing the status of *ex situ* collections around the world, conclusions from meetings identifying needs within the crop conservation and use communities, and an assessment of the current global conservation systems with a set of priority actions to enhance the security and use for the future. In most cases, these crop strategies are the first comprehensive reviews of collection holdings, management practices, and accessibility that are available for specific crops that focus on developing global systems for the long-term conservation of the crop and its wild relatives.

"A Global Strategy for the Conservation and Use of Apple Genetic Resources" was published in 2019 (Bramel and Volk, 2019) and provides compiled information from 38 apple collections that conserved international (those that are or used to be produced on a large scale) and local apple cultivars as well as breeding lines and wild species. It discusses the urgent need to secure the conservation of accessions within a genebank with safety duplication at multiple locations, cryopreservation, and/or conserve with other collection holders to safeguard against losses due to abiotic or biotic threats.

At the time of the Apple Strategy publication, there were very few genotypic and phenotypic assessments that facilitated collection comparisons because there was an inconsistent use of methodologies. Sharing genetic resources across international borders can be particularly difficult because apple cultivars are vegetatively propagated, most often by grafting, and must undergo stringent phytosanitary testing and treatments to ensure that diseases are not inadvertently spread. Without standard methods and shared germplasm, species and cultivar identifications are challenging, although individual collection genotypic analyses can reveal information about wild species diversity and relationships, duplicates within collections, and gaps. Application of standardized genetic, genomic, phenotypic, and phenomic technologies will improve across collection documentation, comparisons, and use.

The global conservation strategy for apple proposed several priority actions that would lead to a more cohesive international conservation effort (Bramel and Volk, 2019). This included the building of a global apple diversity platform to unite conservation and use community members. This platform could include an international registry and database (or linkages among existing databases) to share tree- and accession-level phenotype, passport, and genotypic information. Working groups established within the platform could focus on best practices for conservation, development of standard operating procedures, coordination of strategies for safety duplication, and share information about new threats that could emerge. A global genotyping effort could use a single genetic platform to compare collections, confirm cultivar identities, and identify gaps at a global scale. In addition, this platform would serve as a mechanism by which the community could share methods, news and information, as well as facilitate training opportunities and build collaborations. The subsequent sections of this paper will review the current information available for the USDA NPGS apple collection and how ongoing and future collaborations with other collections will improve conservation efforts.

USDA National Plant Germplasm System (NPGS) Apple Collection

The NPGS apple collection is maintained in Geneva, New York as trees in the field and seeds in cold storage (Gutierrez et al., 2020). The apple collection was established in 1983 from apple cultivar contributions from breeding and research programs as well as other apple collections both within the United States and internationally. The collection currently has 1,427 *Malus domestica* accessions as single, as duplicate, or even as triplicate field trees. In addition, plant exploration and exchange efforts have established it as one of the largest *Malus* species collections in the world, with 3,433 individuals representing

53 taxa as field trees. Ongoing collaborations have resulted in the development and implementation of methods to cryopreserve apple accessions as dormant buds for long-term secure back-ups at the USDA National Laboratory for Genetic Resources Preservation in Fort Collins, Colorado (Towill et al., 2004; Volk et al., 2017). These cryopreservation technologies have been used at other national genebanks (Höfer, 2015).

In the 1980s and 1990s, the NPGS apple curation efforts focused on building and expanding the collection (Luby et al. 2000; Volk et al., 2015). Genetic marker systems were developed (such as microsatellites; Hokanson et al., 1998; 2001), which were used to confirm and identify core subsets of the NPGS *Malus* collections (Gross et al., 2013; Richards et al., 2009b; Volk et al., 2005; 2009). The same markers were used to genotype the diploid cultivars to identify duplicates and sport families, as well as trees that were misidentified (i.e. rootstock escapes that were labeled as cultivars) (Gross et al., 2012b; Hokanson et al., 1998).

Assessments of NPGS apple collection materials using microsatellite markers as well as chloroplast sequencing have provided a better understanding of the diversity and taxonomic relationships among *Malus* species (Richards et al. 2009a; Volk et al., 2008; 2015). Microsatellite markers and single nucleotide polymorphisms (SNP) have also been used to identify relationships among cultivars, landraces, and wild species (Cornille et al., 2012; Gharghani et al., 2009; Gross et al., 2014; Khan et al., 2014). In addition, the SSR datasets for the apple cultivars served as a reference set to identify heirloom apple trees in the landscape (Routson et al., 2009; Magby et al., 2019; Volk et al., 2021a,b).

Phenotypic data, including images, have been collected for the collection over the past 30 years, and much of that information has been made available in the public database GRIN-Global (Germplasm Resources Information Network, 2022). In many cases, data were collected for a single tree in a single year, although there are some exceptions whereby more comprehensive assessments were performed and published. Access to these data is important, particularly for those who are looking for accessions that have specific traits or to confirm cultivar or taxonomic identities with images. Studies that assessed cider characteristics (Al-Turki and Stushnoff, 2007; Kumar et al., 2021), abscission (Sun et al., 2009), bloom date (Gottschalk and van Nocker, 2013), drought tolerance (Bassett et al. 2011), red flesh fruit (Rupasinghe et al., 2010; van Nocker et al., 2012), dihydrochalcone metabolites (Gutierrez et al., 2018a,b), root architecture (Fazio et al., 2014), fruit ethylene production (Dougherty et al., 2016), fruit firmness (Migicovsky et al., 2021b), fruit softening (Dougherty et al., 2016), and other traits have improved understanding and utilization of the NPGS apple collection. Recently, an extensive phenotyping effort was performed at Canada's Apple Biodiversity Collection, using plant materials that were obtained in part from the NPGS apple collection (Watts et al., 2021).

Phenotypic data, in combination with genetic and genomic data, have recently been used to identify relationships between apple cultivars, crop wild relatives and processes of domestication as well as genes that underly traits. Genotyping by sequencing analyses revealed duplicates and first degree relatives through identity by descent (Migicovsky et al. 2021a). Whole genome sequences of accessions from the NPGS apple collection clarified the domestication of modern apple through hybridization of *M. sieversii* and *M. sylvestris* (Velasco et al. 2010; Duan et al. 2017; Sun et al. 2020). Migicovsky et al. (2021a) highlighted the contributions of *M. sieversii* and *M. sylvestris* during domestication, with increasing genomic contributions of *M. sylvestris* in cider apples and genome-wide association studies (GWAS) using historic data from GRIN-Global described a gene controlling fruit firmness (Migicovsky et al. 2016; Migicovsky et al. 2021b). Quantitative trait loci for fruit size and flavor were identified using genome-wide association mapping, and pan-genome assemblies identified thousands of new genes, many of which were selected throughout apple domestication (Sun et al. 2020). Fruit quality candidate gene diversity was assessed in apple cultivars and crop wild relatives by Wedger et al. (2021).

Disease resistance evaluations using the NPGS apple collection have revealed accessions of *Malus* species with resistance to *Erwinia amylovora* (fire blight; Dougherty et al., 2021; Harshman et al., 2017), *Penicillium expansum* (blue mold; Janisiewicz et al., 2008; Jurick et al., 2011; Norelli et al., 2014), *Colletotrichum acutatum* (bitter rot; Jurick et al., 2011), *Venturia inaequalis* (apple scab; Bus et al., 2012; Fazio et al., 2009; Wang et al., 2012) and others (see Volk et al., 2015).

These evaluations, particularly using a series of seedlings derived from crosses between 'Royal Gala' × *Malus sieversii* accessions, have led to the identification of quantitative trait loci for fire blight resistance (Thapa et al. 2021) and blue mold resistance (Norelli et al., 2017) as well as new genes for scab resistance (Wang et al., 2012). The same set of crosses have been valuable for characterizing the *Ma* locus with pH and titratable acidity in apple (Bai et al., 2012; Xu et al., 2012).

Breeders have access to the large, diverse NPGS apple collection and increasingly to corresponding phenotypic/phenomic and genotypic/genomic data to inform their breeding decisions. New genomic tools are making it easier to include diverse germplasm in breeding programs (Duan et al., 2017; Peace et al., 2019; Wisniewski et al., 2020). Novel alleles, particularly in crop wild relatives, may offer necessary diversity to overcome current and future abiotic and biotic threats, as well as improve quality and yield (Fazio et al., 2008; Harshman et al., 2017; Volk et al., 2015).

Improved management and coordination based on the recommendations of the "Global Strategy for the Conservation and Use of Apple Genetic Resources"

It is critical to have accessions within genebank collections identified correctly, because research programs worldwide depend on these materials. Phenotypic and genotypic data have improved collection management by identifying gaps in species coverage, misnamed accessions, species-domestic hybrids, and duplicates. Correct identification is particularly critical for the apple crop wild relative progenitor species *M. sieversii*, *M. orientalis*, and *M. sylvestris*. It is known that the wild apple (*M. sieversii*) forests of Kazakhstan are genetically admixed with *M. domestica* (Ha et al., 2021; Omasheva et al., 2017). Some of the *M. sieversii* accessions introduced into the NPGS apple collection from Kazakhstan were shown to be hybrids (Gross et al., 2012a). Additional research is needed to definitively identify true species representatives of *M. sieversii*, *M. orientalis*, and *M. sylvestris* in the NPGS apple collection. This information is critical for research programs assessing genetic diversity in wild populations as well as cultivar-crop wild relative relationships and domestication.

European apple collection comparisons have been performed using microsatellite markers (Urrestarazu et al., 2016). New analyses using the 20K SNP array platform, performed in a coordinated manner among many international collections (including the NPGS apple collection), will reveal if cultivars are named consistently across collections, confirm suspected synonyms, and identify species hybrids (Bianco et al., 2014; Buiteveld et al., 2021). They will also increase the utility of genotype information for identifying unknown apple cultivars and their pedigree relationships, thus aiding historic preservation and restoration efforts (Howard et al., 2017). Furthermore, genotypic data can be used to identify unique, desirable cultivars that are not yet available within

US collections, and thus target these novel accessions for streamlined import through quarantine processes.

Improved coordination among international collections, as well as other collections, such as those maintained in botanic gardens, may lead to the use of standardized phenotypic descriptors so that data for collections can be compiled and compared, thus revealing genotype × environment relationships, heritable traits, and genetic resources that may be of interest to breeding and research programs. Importantly, standardized phenotyping efforts will allow for data to be compared among collections and encourage future collaborations. Increased collaboration may also lead to training opportunities for laboratories to adopt improved field management practices to minimize losses due to biotic and abiotic threats and to increase the security of collection through coordinated duplication efforts (i.e. duplicating materials that are unavailable in other collections). In summary, improved standardization and the use of common platforms for collection genotypic, genomic, phenotypic, and phenomic evaluation and characterization will improve the conservation of apple collections on both a national and global scale (Engels and Ebert, 2021).

Acknowledgments. We thank Peter Giovannini for providing up to date lists of Global Crop Conservation Strategies. USDA is an equal opportunity employer and provider.

Literature Cited

Al-Turki, S., and C. Stushnoff. 2007. Total phenolics and antioxidant properties of cider apple cultivars. J. Amer. Pom. Soc. 61(3):131-134.

Bai, Y., L. Dougherty, M. Li, G. Fazio, L. Cheng, and K. Xu. 2012. A natural mutation-led truncation in one of the two aluminum-activated malate transporter-like genes at the *Ma* locus is associated with low fruit acidity in apple. Mol. Genet. Genomics. 287:663-678.

Bassett, C.L., D.M. Glenn, P.L. Forsline, M.E. Wisniewski, and R.E. Farrell Jr. 2011. Characterizing water use efficiency and water deficit responses in apple (*Malus* × *domestica* Borkh. and *Malus sieversii* Ledeb.) M. Roem. HortScience 46:1079-1084.

Bianco, L., A. Cestaro, D.J. Sargent, E. Banchi, S. Derdak, M. Di Guardo et al. 2014. Development and validation of a 20K single nucleotide polymorphism (SNP) whole genome genotyping array for apple (*Malus* × *domestica* Borkh.). PLoS ONE 9(10):e110377.

Bramel, P.J. and G. Volk. 2019. A global strategy for the conservation and use of apple genetic resources. Global Crop Diversity Trust. Bonn, Germany. DOI: 10.13140/RG.2.2.34072.34562

Buiteveld, J., H.J.J. Koehorst-van Putten, L. Kodde, I. Laros, G. Tumino, N.P. Howard, E. van de Weg, and M.J.M. Smulders. 2021. Advanced genebank management of genetic resources of European wild apple, *Malus sylvestris*, using genome-wide SNP array data. Tree Genet. Genomes 17:32 https://doi.org/10.1007/s11295-021-01513-y

Bus, V.G.M., W.E. van de Weg, A. Peil, F. Dunemann, E. Zini, F.N.D. Laurens, J. Blažek, V. Hanke, and P.L. Forsline. 2012. The role of Schmidt 'Antonovka' in apple scab resistance breeding. Tree Genet. Genomes 8:627-642.

Cornille, A., P. Gladieux, M.J.M. Smulders, I.Roldán-Ruiz, F. Laurens, B. Le Cam, A. Nersesyan, J. Clavel, M. Olonova, L. Feugey, I. Gabrielyan, X.-G. Zhang, M.I. Tenaillon, and T. Giraud. 2012. New insight into the history of domesticated apple: Secondary contribution of the European wild apple to the genome of cultivated varieties. PLoS Genetics 5:e1002703

Dougherty, L., A. Wallis, K. Cox, G.-Y. Zhong, and B. Gutierrez. 2021. Phenotypic evaluation of fire blight outbreak in the USDA *Malus* collection. Agronomy 11:144 https://doi.org/10.3390/agronomy11010144

Dougherty, L., Y. Zhu, and K. Xu. 2016. Assessing the allelotypic effect of two aminocyclopropane carboxylic acid synthase-encoding genes *MdACS1* and *MdACS3a* on fruit ethylene production and softening in *Malus*. Hort. Res. 3:16024.

Duan, N., Y. Bai, H. Sun, N. Wang, Y. Ma, M. Li, et al. 2017. Genome re-sequencing reveals the history of apple and supports a two-stage model for fruit enlargement. Nat. Commun. 8. https://doi.org/10.1038/s41467-017-00336-7

Engels, J.M.M., and A.W. Ebert. 2021. A critical review of the current global ex situ conservation system for plant agrobiodiversity. II. Strengths and weaknesses of the current system and recommendations for its improvement. Plants 10, 1904. https://doi.org/10.3390/plants10091904

Fazio, G., H.S. Aldwinckle, G.M. Volk, C.M. Richards, W.J. Janisiewicz, and P.L. Forsline. 2009. Progress in evaluating *Malus sieversii* for disease resistance and horticultural traits. Acta Hort. 814:59-66.

Fazio, G., C.T. Chao, P.L. Forsline, C. Richards, and G. Volk. 2014. Tree and root architecture of *Malus sieversii* seedlings for rootstock breeding. Acta Hort. 1058:585-593.

Fazio, G., P. Forsline, H. Aldwinckle, and L. Pons. 2008. The apple collection in Geneva, NY: A resource for the apple industry today and for generations to come. New York Fruit Quarterly 16(1):5-8.

Germplasm Resources Information Network [Internet]. Beltsville (MD): United States Department of Agriculture, Agricultural Research Service. 8 Jan 2022. Available from: http://www.ars-grin.gov/

Gharghani, A., Z. Zamani, A. Talaie, NC. Oraguzie, R. Fatahi, H. Hajnajari, C. Wiedow, and S.E. Gardiner. 2009. Genetic identity and relationships of Iranian apple (*Malus* × *domestica* Borkh.) cultivars and landraces, wild *Malus* species and representative old apple cultivars based on simple sequence repeat (SSR) marker analysis. Genet. Resour. Crop Evol. 56:829-842.

Gottschalk, C., and S. van Nocker. 2013. Diversity in seasonal bloom time and floral development among apple species and hybrids. J. Amer. Soc. Hort. Sci. 138:367-374.

Gross, B.L., A.D. Henk, P.L. Forsline, C.M. Richards, and G.M. Volk. 2012a. Identification of interspecific hybrids among domesticated apple and its wild relatives. Tree Genet. Genomes 8:1223-1235.

Gross, B.L., A.D. Henk, C.M. Richards, G. Fazio, and G.M. Volk. 2014. Genetic diversity in *Malus* × *domestica* (Rosaceae) through time in response to domestication. Amer. J. Bot. 101:1770-1779.

Gross, B.L., G.M. Volk, C.M. Richards, P.L. Forsline, G. Fazio, and C.T. Chao. 2012b. Identification of "duplicate" accessions in the USDA-ARS National Plant Germplasm System *Malus* collection. J. Amer. Soc. Hort. Sci. 137:333-342.

Gross, B.L., G.M. Volk, C.M. Richards, P.A. Reeves, A.D. Henk, P.L Forsline, A. Szewc-McFadden, G. Fazio, and C.T. Chao. 2013. Diversity captured in the USDA-ARS National Plant Germplasm System apple core collection. J. Amer. Soc. Hort. Sci. 138:375-381.

Gutierrez, B., K. Battaglia, and G.-Y. Zhong. 2020. Preserving the future with the USDA Plant Genetic Resource unit tart cherry, grape, and apple germplasm collections. J. Amer. Pomol. Soc. 74:97-103.

Gutierrez, B.L., J. Arro, G.-Y. Zhong, and S.K. Brown. 2018a. Linkage and association analysis of dihydrochalcones phloridzin, sieboldin, and trilobatin in *Malus*. Tree Genet. Genomes 14:91. https://doi.org/10.1007/s11295-018-1304-7

Gutierrez, B.L., G.-Y. Zhong, S.K. Brown. 2018b. Genetic diversity of dihydrochalcone content in *Malus* germplasm. Genetic Resour. Crop Evol. 65:1485–1502.

Ha, Y.-H., S.-H. Oh, and S.-R. Lee. 2021. Genetic admixture in the population of wild apple (*Malus sieversii*) from the Tien Shan Mountains, Kazakhstan. Genes 12:104. https://doi.org/10.3390/genes12010104

Harshman, J.M., K.M. Evans, H. Allen, R. Potts, J. Flamenco, H.S. Aldwinckle, M.E. Wisniewski, and J.J. Norelli. 2017. Fire blight resistance in wild accessions of *Malus sieversii*. Plant Disease 101:1738-1745.

Höfer, M. 2015. Cryopreservation of winter-dormant apple buds: establishment of a duplicate collection of *Malus* germplasm. Plant Cell Tissue Organ Culture 121:647-656.

Hokanson, S.C., A.K. Szewc-McFadden, W.F. Lamboy, and J.R. McFerson. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus* × *domestica* borkh. core subset collection. Theor. Appl. Genet. 97:671-683.

Hokanson, S.C., W.F. Lamboy, A.K. Szewc-McFadden, and J.R. McFerson. 2001. Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids. Euphytica 119:281-294.

Howard, N.P., E. van de Weg, D.S. Bedford, C.P. Peace, S. Vandersande, M.D. Clark, S.L. The, L. Cai, and J.J. Luby. 2017. Elucidation of the 'Honeycrisp' pedigree through haplotype analysis with a multi-family integrated SNP linkage map and a large apple (*Malus* × *domestica*) pedigree-connected SNP data set. Hort. Res. 4:17003 doi:10.1038/hortres.2017.3

Hummer, K.E., H. Dempewolf, P. Bramel, R. Markham, and E. Stover. 2015. Status of global strategies for horticultural fruit crops. Acta Hortic. 1101: 147-152.

International Treaty on Plant Genetic Resources for Food and Agriculture. 2009. Food and Agriculture Organization of the United Nations. Rome, Italy. https://www.fao.org/3/i0510e/i0510e.pdf

Janisiewicz, W.J., R.A. Saftner, W.S. Conway, and P.L. Forsline. 2008. Preliminary evaluation of apple germplasm from Kazakhstan for resistance to postharvest blue mold in fruit caused by *Penicillium expansum*. HortScience 43:420-426.

Jurick, W.M., W.J. Janisiewicz, R.A. Saftner, I. Vico, V.L. Gaskins, E. Park, P.L. Forsline, G. Fazio, and W.S. Conway. 2011. Identification of wild apple germplasm (*Malus* spp.) accessions with resistance to the postharvest decay pathogens *Penicillium expansum* and *Collectotrichum acutatum*. Plant Breed. 130:481-486.

Khan, M.A., K.M. Olsen, V. Sovero, M.M. Kushad, and S.S. Korban. 2014. Fruit quality traits have played critical roles in domestication of the apple. Plant Genome 7:1-18.

Kumar, S.K., N. Wojtyna, L. Dougherty, K. Xu, and G. Peck. 2021. Classifying cider apple germplasm using genetic markers for fruit acidity. J. Amer. Soc. Hort. Sci. 1:1–16.

Luby, J., P. Forsline, H. Aldwinckle, V. Bus, and M. Geibel. 2001. Silk road apples-Collection, evaluation, and utilization of *Malus sieversii* from Central Asia. HortScience 36(2):225-231.

Magby, J., G.M. Volk, A. Henk, and S. Miller. 2019. Identification of historic homestead and orchard apple cultivars in Wyoming. HortScience 54:8-16.

Migicovsky, Z., K.M. Gardner, D. Money, J. Sawler, J.S. Bloom, P. Moffet, et al. 2016. Genome to phenome mapping in apple using historical data. Plant Genome 9:1–15. https://doi.org/10.3835/plantgenome2015.11.0113

Migicovsky, Z., K.M. Gardner, C. Richards, C.T. Chao, H.R. Schwaninger, G. Fazio, et al. 2021a. Genomic consequences of apple improvement. Hort. Res. 8:1–13. https://doi.org/10.1038/s41438-020-00441-7

Migicovsky, Z., T.H. Yeats, S. Watts, J. Song, C.F. Forney, K. Burgher-MacLellan, et al. 2021b. Apple ripening is controlled by a NAC transcription factor. Frontiers in Genetics 12:671300. Doi: 10.3389/fgene.2021.671300

Norelli, J.L., M. Wisniewski, and S. Droby. 2014. Identification of a QTL for postharvest disease resistance to *Penicillium expansum* in *Malus sieversii*. Acta Hort. 1053:199-203.

Norelli, J.L., M. Wisniewski, G. Fazio, E. Burchard, B. Gutierrez, E. Levin, et al. 2017. Genotyping-by-sequencing markers facilitate the identification of quantitative trait loci controlling resistance to *Penicillium expansum* in *Malus sieversii*. PLoS ONE 12:e0172949. https://doi.org/10.1371/journal.pone.0172949

Omasheva, M.Y., H. Flachowsky, NA. Ryabushkina, A.S. Pozharskiy, N.N. Galiakparov, and M.-V. Hanke. 2017. To what extent to wild apples in Kazakhstan retain their genetic integrity? Tree Genet. Genomes 13:52 DOI 10.1007/s11295-017-1134-z

Peace, C.P., L. Bianco, M. Troggio, E. van de Weg, N.P. Howard, A. Cornille, etc. 2019. Apple whole genome sequences: recent advances and new prospects. Hort. Res. 6:59 https://doi.org/10.1038/s41438-019-014107

Richards, C.M, G.M. Volk, A.A. Reilley, A.D. Henk, D.R. Lockwood, P.A. Reeves, and P.L. Forsline. 2009a. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. Tree Genet. Genomes. 5:339-347.

Richards, C.M., G.M. Volk, P.A. Reeves, A.A. Reilley, A.D. Henk, P.L. Forsline, and H.S. Aldwinckle. 2009b. Selection of stratified core sets representing wild apple (*Malus sieversii*). J. Amer. Soc. Hort. Sci. 134:228-235.

Routson, K.J., A.A. Reilley, A.D. Henk, and G.M. Volk. 2009. Identification of historic apple trees in the southwestern United States and implications for conservation. HortScience 44:589-594.

Rupasinghe, H.P.V., G.M. Huber, C. Embree, and P.L. Forsline 2010. Red-fleshed apple as a source for functional beverages. Can. J. Plant Sci. 90:95-100.

Sun, L., M.J. Bukovac, P.L. Forsline, and S. van Nocker. 2009. Natural variation in fruit abscission-related traits in apple (*Malus*). Euphytica 165:55-67.

Sun, X., C. Jiao, H. Schwaninger, C.T. Chao, Y. Ma, N. Duan, et al. 2020. Phased diploid genome assemblies and pan-genomes provide insights into the genetic history of apple domestication. Nat. Genet. 52:1423–1432. https://doi.org/10.1038/s41588-020-00723-9

Thapa, R., J. Singh, B. Gutierrez, J. Arro, and A. Khan. 2021. Genome-wide association mapping identifies novel loci underlying fire blight resistance in apple. Plant Genome n/a:e20087. https://doi.org/10.1002/tpg2.20087

Towill, L.E., P.L. Forsline, C. Walters, J.W. Waddell, and J. Laufmann. 2004. Cryopreservation of *Malus* germplasm using a winter vegetative bud method: Results from 1915 accessions. CryoLetters 25:323-334.

Urrestarazu, J., C. Denancé, E. Ravon, A. Guyader, R. Guisnel, L. Feugey, et al. 2016. Analysis of the genetic diversity and structure across a wide range of germplasm reveals prominent gene flow in apple at the European level. BMC Plant Biol. 16:130 DOI 10.1186/s12870-016-0818-0

van Nocker, S., G. Berry, J. Najdowski, R. Michelutti, M. Luffman, P. Forsline, N. Alsmairat, R. Beaudry, M.G. Nair, and M. Ordidge. 2012. Genetic diversity of red-fleshed apples (*Malus*). Euphytica 185:281-293.

Velasco, R., A. Zharkikh, J. Affourtit, A. Dhingra, A. Cestaro, A. Kalyanaraman, et al. 2010. The genome of the domesticated apple (*Malus × domestica* Borkh.). Nat. Genet 42:833–839.

Volk, G.M., A.D. Henk, A. Baldo, G. Fazio, C.T. Chao, and C. Richards. 2015. Chloroplast heterogeneity and historical admixture within the genus *Malus*. Amer. J. Bot. 102:1198-1208.

Volk, G.M., M. Jenderek, and C.T. Chao. 2017. Prioritization of Malus accessions for collection cryopreservation at the USDA-ARS National Center for Genetic Resources Preservation. Acta Hort. 1172:267-272.

Volk, G.M., C.M. Richards, A.A. Reilley, A.D. Henk, P.A. Reeves, P.L. Forsline, and H.S. Aldwinckle. 2008. Genetic diversity and disease resistance of wild *Malus orientalis* from Turkey and Southern Russia. J. Amer. Soc. Hort. Sci. 133:383-389.

Volk, G.M., C.M. Richards, A.A. Reilley, A.D. Henk, P.L. Forsline, and H.S. Aldwinckle. 2005. Ex situ conservation of vegetative propagated species: Development of a seed-based core collection for *Malus sieversii*. J. Amer. Soc. Hort. Sci. 130:203-210.

Volk, G.M., C.M. Richards, A.D. Henk, A.A. Reilley, and P.A. Reeves. 2009. Capturing the diversity of wild *Malus orientalis* from Georgia, Armenia, Russia and Turkey. J. Amer. Soc. Hort. Sci. 134:453-459.

Volk, G.M., C.T. Chao, J. Norelli, S.K. Brown, G. Fazio, C. Peace, J. McFerson, G.-Y. Zhong, and P. Bretting. 2015. The vulnerability of US apple (*Malus*) genetic resources. Genetic Resour. and Crop Evol. 62:765-794.

Volk, G.M., J. Magby, A. Henk, S. Miller, and R. Mazur. 2021a. Genetic data inform Yosemite National Park orchard management guidelines. Plants, People, Planet 3:142-154. doi: 10.1002/ppp3.10152

Volk, G.M., K. McCardle, J. Serin, J. Magby, and R. Mazur 2021b. Genetic fingerprinting identifies apple (*Malus domestica* Borkh.) fruit cultivars in historic orchards on public lands. Genetic Resour. Crop Evol. 68:3133–3144.

Wang, A., H. Aldwinckle, P. Forsline, D. Main, G. Fazio, S. Brown, and K. Xu. 2012. EST contig-based SSR linkage maps for *Malus* × *domestica* cv Royal Gala and an apple scab resistant accession of *M. sieversii*, the progenitor species of domesticated apple. Mol. Breed. 29:379-397.

Watts, S., Z. Migicovsky, K.A. McClure, C.H.J Yu, B. Amyotte, T. Baker, D. Bowlby, K. Burgher-MacLellan, et al. 2021. Quantifying apple diversity: A phenomic characterization of Canada's Apple Biodiversity Collection. Plants, People, Planet 3:747-760.

Wedger, M.J., A.C. Schumann, and B.L. Gross. 2021. Candidate genes and signatures of directional selection on fruit quality traits during apple domestication. Amer. J. Bot. 103:616-627.

Wisniewski, M., T. Artlip, J. Liu, J. Ma, E. Burchard, J. Norelli, and C. Dardick. 2020. Fox hunting in wild apples: Searching for novel genes in *Malus sieversii*. Int. J. Mol. Sci. 21:9516. doi:10.3390/ijms21249516.

Xu, K., A. Wang, and S. Brown. 2012. Genetic characterization of the *Ma* locus with pH and titratable acidity in apple. Mol. Breeding 30:899-912.