



# A GLOBAL STRATEGY FOR THE CONSERVATION AND USE OF APPLE GENETIC RESOURCES

Paula Bramel and Gayle M. Volk



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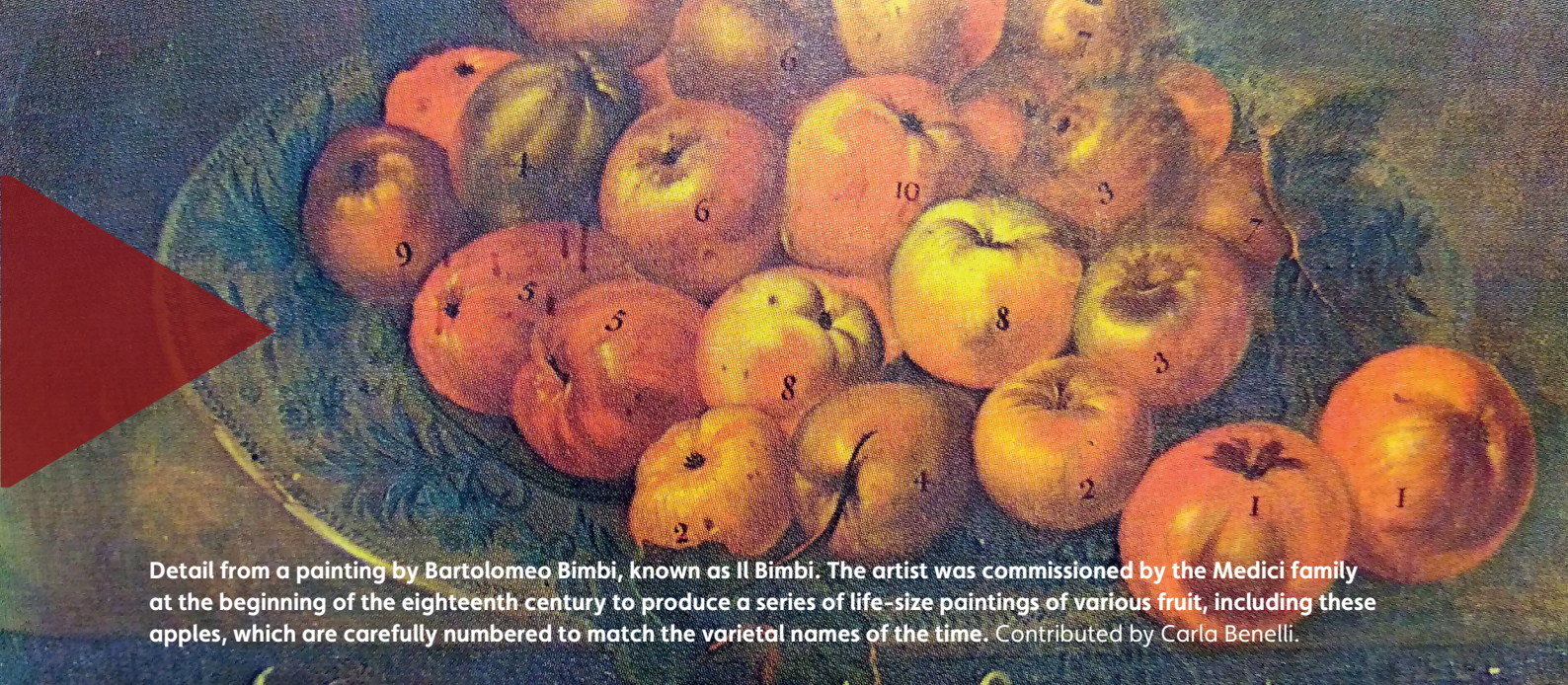
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Detail from a painting by Bartolomeo Bimbi, known as Il Bimbi. The artist was commissioned by the Medici family at the beginning of the eighteenth century to produce a series of life-size paintings of various fruit, including these apples, which are carefully numbered to match the varietal names of the time. Contributed by Carla Benelli.

## EXECUTIVE SUMMARY

Worldwide, apples are amongst the most popular fruits, particularly in temperate climates. They are consumed fresh, baked, and processed as juice, alcoholic cider, cider vinegar, sauce, and fillings. Apples are often low in calories and usually are rich in phytonutrients, flavonoids, antioxidants, and polyphenolics. With their ability to retain quality after storage and their ease of shipping, they are produced in many temperate regions and shipped throughout the world. Mainland China is the world's largest producer of apples while Europe and the Americas are the primary apple fruit exporters (as measured by export value). Europe and Asia are the primary importers (as measured by import value). Some countries produce and consume primarily their own fruit, whereas others are either primarily producers or consumers.

The development of new apple cultivars with a wider genetic base is dependent upon breeders having access to a diverse array of cultivars and germplasm. Breeding programs may maintain their own collections, but often they acquire new materials from public apple genebanks. Traditional apple breeding is a complex process that involves many years of crossing, multi-site field evaluation trials, and consumer testing. Changing climatic conditions, new disease pressures, and the need to produce fruit with fewer chemical inputs to meet consumer demand will result in the need for new apple cultivars with higher levels of resistance or tolerance to abiotic and biotic stresses in the future. Meeting these challenges will depend on having sufficient genetic diversity conserved in genebanks and in the wild.

Taxonomically, the *Malus* genus is a member of the kingdom Plantae, phylum Magnoliophyta, class Magnoliopsida, order Rosales, and family Rosaceae. *Malus* species are native to many countries in the Northern Hemisphere. *Malus* currently includes 144 taxa according to the GRIN Taxonomy for Plants; however, only 59 of these are officially recognized unique species. There are four *Malus* species native to North America, at least two *Malus* species native to Europe, and at least 31 *Malus* species native to Asia, with China as the center of diversity for *Malus*. *Malus* × *domestica* is believed to have been domesticated thousands of years ago in Central Asia. *M. sieversii* is likely the main progenitor. Other species have been introgressed into *M. sieversii* as it moved east and west. Europeans then took apple seeds and scions to the Americas, and a secondary center of diversity developed in North America.

Apples are traditionally conserved in field collections as grafted cultivars. These collections are expensive, long-term investments that have been established for a variety of different purposes that include genetic studies, breeding, conservation, distribution, and public interest. National, state, provincial, and local genebanks, botanical gardens, arboreta, private companies, and NGOs all host collections. Collections of apple genetic resources provide access to living materials (in either tree or seed form) for use in taxonomy, physiology, molecular and evolutionary biology, horticulture, and breeding programs. Apple breeders, orchardists, and nurseries have maintained germplasm collections for many years.

Apples are amenable to *in vitro* culture as well as cryopreservation as dormant buds or shoot tips. Seed from diploids can also be stored for the long term. Pollen can be desiccated and cryopreserved. All of these methods are used to complement field collections. Key wild species are also conserved in nature reserves and seed gardens.

There are a number of sources of information on conserved apple genetic resources within institutions and they are shared at national, regional, and international levels. Information for many apple collections around the world is available through Genesys, with data on 36,654 accessions from 91 institutions. Under the umbrella of the European Cooperative Program for Genetic Resources (ECPGR), efforts are being made to coordinate conservation institutions in Europe and globally, but results are being achieved slowly. Increased utilization of conserved genetic resources is now possible with the identification of a number of core subsets, the development of genomic tools, and large-scale phenotyping efforts. However, the sharing of relevant data globally is still limited. Apple genetic resources face significant risks from climate change, land use changes, and biotic threats. These risks need to be managed through local, national, and international cooperation. Managing quarantine pest and disease risks in phytosanitary regulations significantly hinders international germplasm exchange.

Germplasm exchange is being facilitated by the Multilateral System of the International Treaty on Plant Genetic Resources for Food and Agriculture, with apple being included in its Annex I. In 2006, the Global Crop Diversity Trust started to work with crop conservation and use specialists to develop global *ex situ* conservation strategies for key crops

listed in Annex I of the Treaty. The aim of this Global Strategy for the Conservation and Use of Apple Genetic Resources is to secure the long-term conservation and use of apple genetic resources. The strategy will serve as a framework to bring together stakeholders at all levels to build long-term support through greater awareness, increased capacity, greater community engagement, and sustained funding.

The development of the global conservation strategy involved an initial background study, a survey of the status of major collections, several expert consultations, and some site visits. The survey respondents indicated that the current system for *ex situ* conservation includes a large number of collections that are held by a wide range of governmental organizations, universities, private companies, botanical gardens, foundations, public parks, and individuals. Apple cultivars (including internationally recognized cultivars, landraces, and rootstocks) as well as wild species must be conserved for the long term. Collection surveys and consultations concluded that the current conservation system for *Malus* has a focus on both common and novel diversity, with only limited sharing of accessions and their associated data for research. Overall, the genetic resources conserved within *ex situ* collections are not very secure. To manage these constraints for the long term, greater global collaboration among apple collections is needed. A Global Apple Diversity Platform is proposed to secure the long-term conservation and use of apple genetic resources. This platform will not only link collections and users into a global community but facilitate community actions that have been identified as key needs for a secure, cost-effective, rational global system for the conservation and use of apple genetic resources for the long term.



An apple grower near Manali, Himachal Pradesh, India. Photo by Neil Palmer/CIAT.

## BACKGROUND

Worldwide, apples are amongst the most popular fruits, particularly in temperate climates. They are consumed fresh, processed as juice or puree, and, increasingly, as alcoholic cider. In the USA the average individual eats 7.8 kilograms of fresh apples and drinks 8.4 kilograms of juice a year (Perez and Ferreira, 2017). Consumption is more than twice as high in some European countries. The apple, however, is also suitable for countless other purposes, such as the production of beauty creams, seed oil (obtained as a by-product from the production of juice and cider), distilled alcohol, and fermented products.

Apple consumption has been linked to a reduced risk for many major diseases (Boyer and Liu, 2004; Hyson, 2011). Apples are low in calories: 100 grams of fresh fruit slices provide just 50 calories and contain no saturated fats or cholesterol. They are also high in malic acid, which provides their tart flavor. Apples are rich in antioxidants, phytonutrients, flavonoids, and polyphenolics. Altogether, these compounds help the human body protect itself from the harmful effects of free radicals and assist in the creation of a healthy gut microbiome. Apple fruits contain high levels of vitamin C and beta-carotene. Further, they are an ideal source of B-complex vitamins such as riboflavin, thiamin, and pyridoxine (vitamin B-6). Apples also contain small quantities of minerals like potassium, phosphorus, and calcium. The fruit is rich in dietary fiber, with two-thirds of the fiber and high levels of antioxidants in the peel.

With their ability to retain quality after storage and their ease of shipping, apples are produced in many temperate regions and shipped throughout

the world. China is the world's largest producer of apples, with 2,410,000 hectares harvested annually (FAO, 2017). Overall, China accounts for 49% of global apple production on 46% of the global harvested areas. In fact, eight countries (China, India, Poland, Russia, Turkey, the USA, Iran, and Pakistan) account for about 69% of the global harvested area. Globally, ten countries account for 75% of the total quantity of apples produced: China, the USA, Turkey, Poland, Italy, India, France, Chile, Iran, and Russia. The largest exporters of apples are Poland, China, and the USA, but ten countries account for 75% of global exports. Russia, Germany, the United Kingdom, the Netherlands, and Mexico are the largest importers of apples globally, with ten countries accounting for 50% of global imports. On a regional basis, Europe and the Americas are the primary apple fruit exporters (as measured by export value), and Europe and Asia are the primary apple fruit importers (as measured by import value). Some countries produce and consume primarily their own fruit, whereas others are primary producers or consumers. This strong interdependence of countries with respect to apple production and consumption has led to its high value in the international marketplace, and to significant investment in breeding.

The development of new apple cultivars with desirable characteristics and a wide genetic base is dependent upon breeders having access to as much genetic diversity as possible. Breeding programs may maintain their own collections, but often they acquire new materials from public apple collections or genebanks. Traditional apple

breeding is a complex process that involves many years of crossing, multi-site field evaluation trials, and consumer testing (Evans, 2013).

Changing climatic conditions, new pest and disease pressures, and the production of fruit with fewer chemical inputs to meet consumer demand will result in the need for new apple cultivars with higher levels of resistance to abiotic and biotic stresses. Meeting these future challenges will depend on the genetic diversity that is conserved in genebanks, gardens, abandoned fields, and in the wild being available for use in the long term.

## Malus genetic resources

Taxonomically, the *Malus* genus is a member of the kingdom Plantae, phylum Magnoliophyta, class Magnoliopsida, order Rosales, and family Rosaceae. *Malus* currently includes 144 taxa according to the GRIN Taxonomy for Plants; however, only 59 of these are officially recognized unique species (USDA, 2016). The taxonomy of *Malus* is not completely understood due to a lack of access to germplasm, high levels of diversity within species, interspecific fertility, high levels of hybridization, inadequate specimens, and a lack of genomic data. Several *Malus* phylogenies have been proposed (Forte et al., 2002; Lingdi et al., 2003; Nikiforova et al., 2013; Potter et al., 2007, 2002; Robinson et al., 2001; Volk et al., 2015a). The *Malus* taxa reported in apple collections and Genesys (2017) are listed in Annex I.

Phenotypic traits have been used to describe sections of the genus *Malus* (Phipps et al., 1990; Robinson et al., 2001; Yu, 1988). Genotyping with molecular marker and sequence data has also been used for species differentiation (Forte et al., 2002; Nikiforova et al., 2013; Robinson et al., 2001; Velasco et al., 2010; Volk et al., 2015a). Chloroplast genome sequence analyses have been recently performed to clarify taxonomic relationships. Nikiforova et al. (2013) identified five clades based on a comparison of 47 chloroplast genome sequences. Volk et al. (2015a) identified only three clades based on four regions of the chloroplast genome from 412 individuals representing 30 *Malus* species. Additional research is needed to clarify the taxonomy, genomic relationships, and phenotypic descriptions of *Malus* species. The success of these endeavors is dependent upon having access to a wide range of true-to-type specimens.

*Malus* species are native to many countries in the Northern Hemisphere. There are four named *Malus* species native to North America and at least two native to Europe (Annex I). China is thought to be

the center of diversity of *Malus*, with at least 31 taxa (Zhou, 1999) identified in the wild (Vavilov, 1992). As a primarily temperate crop, most *Malus* species have winter chilling requirements that must be satisfied for bud break. However, *M. doumeri* is a non-winter hardy species that is native to Taiwan and Southeast Asia. The diversity within *Malus* species in the wild is not well known, but Cornille et al. (2013b) concluded that the species *M. sylvestris* has a weak spatial genetic structure, likely due to long-distance dispersal of pollen and seeds. Richards et al. (2009) and Volk et al. (2008) identified localized, regional genetic structures among some subsets of *M. orientalis* and *M. sieversii* populations.

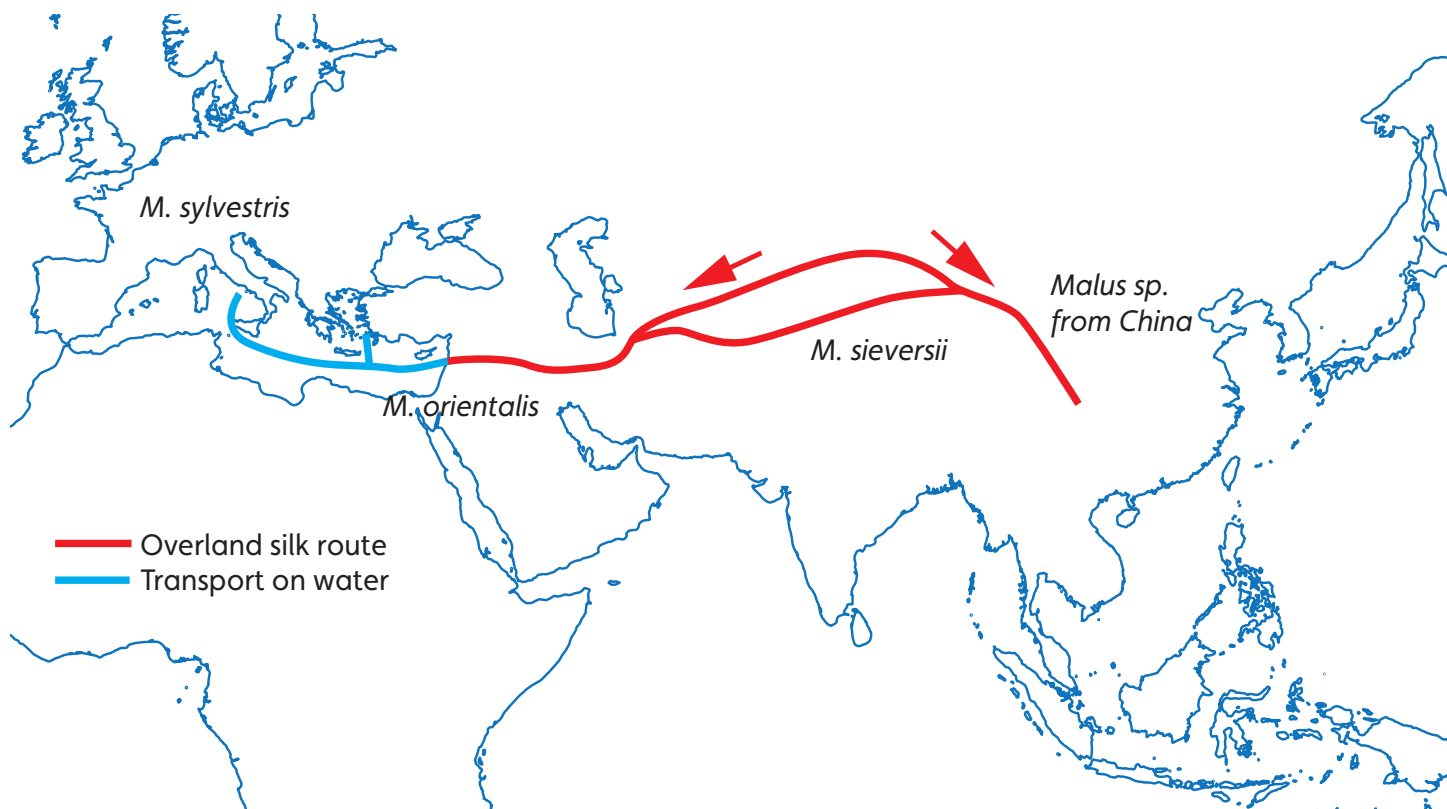
## Malus × domestica

*M. × domestica* was domesticated thousands of years ago. Molecular evidence has supported the primary contribution of *M. sieversii* as a progenitor of the domesticated apple in Europe, with *M. sylvestris* serving as a secondary contributor (Cornille et al., 2014, 2013b, 2012; Jacques et al., 2009). Some western species, including *M. orientalis* and *M. sylvestris*, may have been introgressed into *M. sieversii* as seedlings that were transported from the Tien Shan Mountains in Central Asia through the Middle East and into Europe (Fig. 1; Höfer et al., 2013; Juniper and Mabberley, 2006; Volk et al., 2015b). Alternative domestication processes may have occurred as *M. sieversii* was transported across China, where it could have hybridized with *M. baccata* and *M. prunifolia* (Fig. 1; Luby, 2003).

Fruits, seeds, and scions of *M. × domestica* were taken to the Americas as Europeans migrated, settled, and planted trees on homesteads and in backyard gardens. Seeds and nursery-bought cultivars were planted throughout the American West, particularly in the 1800s. The diversity of *M. × domestica* expressed in millions of seedling trees resulted in a secondary center of diversity in North America. Bussey (2017) developed a catalog of information on apple cultivars that have been or are still grown in the USA and Canada based upon information compiled from almost 1,700 publications from the last 200 years.

In apple production, genotypes are asexually propagated by way of grafting. Grafting technologies were developed and used thousands of years ago in China, Greece, and throughout Europe (Mudge et al., 2009). Through this vegetative propagation technique, ancient historical cultivars remain available today (Gross et al., 2014). For example, 'Api' seems to be as old as Roman times, while 'Court-Pendu' (known from the 1550s), 'Autumn Pearmain' (1590s), 'Borsdorf'





**Figure 1.** Map illustrating eastern and western routes of *Malus* domestication from *M. sieversii* in Central Asia.

(1500s), 'London Pippin' (1580s), 'Herefordshire Pearmain' (1200s), 'Red Calville' (1500s) and 'Pearmain' (1200s) are all currently available in the UK National Fruit Collection in Brogdale, England.

Historical apple cultivars often have cultural significance and also may offer novel diversity, local adaptation, and other traits useful to breeders (Dolan, 2009; Holland et al., 2006; Routson et al., 2009). Comparisons among and within apple genebank collections have revealed local varieties with novel traits (Gao et al., 2015; Maghradze et al., 2012; Patzak et al., 2012a, 2012b; Zhang et al., 2012) that are not commonly available in the cultivated genepools (Leforestier et al., 2015; Marić et al., 2013; Tartarini et al., 2004). They may have some shared ancestry with local wild species, and thus contain interesting alleles that are introgressed into a genetic background more desirable than that of wild species. Long-lived historical cultivars may also have traits that have allowed them to survive without intensive management (Routson et al., 2009). In Spain, local apple cultivars from an Iberian genepool could be genetically differentiated from a wide set of foreign cultivars (Pereira-Lorenzo et al., 2017, 2007).

Archaeologists, historians, as well as members of the public are often interested in famous cultivars from past eras that are referred to in historical sources. Assessments of historic nursery catalogs, literature,

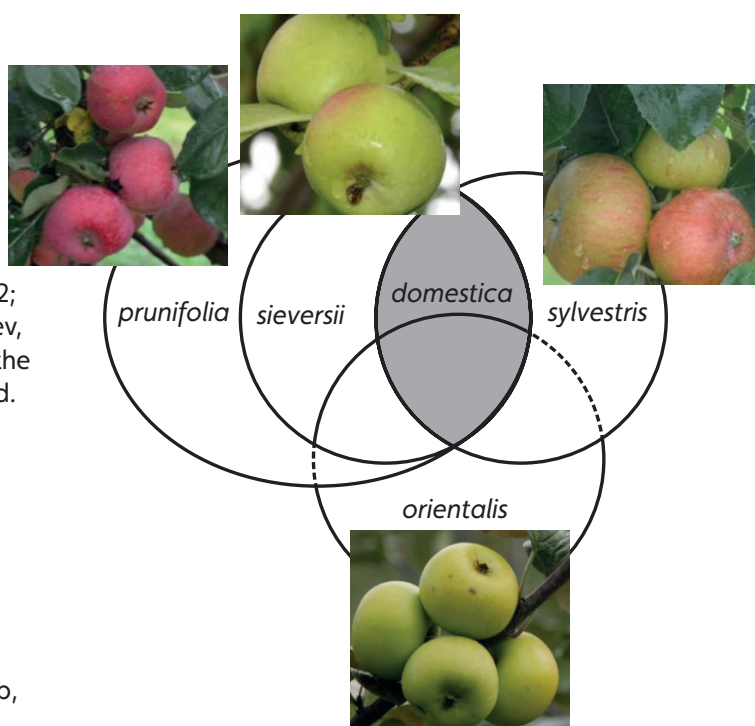
and archived documents provide insights into key cultivars from past eras, which may guide apple conservation efforts (Volk and Henk, 2016; Bussey, 2017; Hennerty, 2014). Because apple trees live for decades, or even centuries, historic cultivars may still be available, even if neglected, from local sources (Guarino et al., 2005; Hennerty, 2014; Pereira-Lorenzo et al., 2007; Pina et al., 2014; Urrestarazu et al., 2012). For example, the apple tree that inspired Sir Isaac Newton's law of universal gravitation in 1666 is still alive; cuttings have been taken, and trees are even for sale (Orange Pippin, 2019).

There is a growing effort being made to rediscover the history of apple cultivars locally or nationally; facilitate efforts to explore locally and identify these historical cultivars; catalog the location and description of rare, uncommon, or heritage cultivars; and make the planting material available for conservation and use. Examples of these efforts are Belgium's Genetic Resources and Disease Resistance Project (Keulemans et al., 2006); the Montezuma Orchard Restoration Project (2017); Maine Heritage Orchard (MOFGA, 2017); People's Trust for Endangered Species (2019); Orange Pippin (2019); the European Cooperative Program for Genetic Resources (ECPGR, 2017); and Le Jardin de Luxemburg in Paris (actually a property of the Senate of the French Republic, which offers likely the oldest collection of apple in the world, as it dates back to 1612 and was never cleared).

## Malus wild species

There are at least four primary wild relatives of *M. × domestica*: *M. sieversii*, *M. orientalis*, *M. sylvestris*, and *M. prunifolia*. Chloroplast and nuclear genomic data provide evidence for close genetic relationships among *M. sieversii*, *M. sylvestris*, *M. orientalis*, and *M. prunifolia*. *M. sieversii* likely served as the source species (Fig. 2; Volk et al., 2015a; Cornille et al., 2013a; Dzhangaliev, 2003). Additional research is needed to elucidate the process whereby *M. × domestica* was domesticated.

The close genomic relationship between the four putative progenitor species and the cultivated apple makes these species more readily used in traditional apple breeding than *Malus* species that are more distantly related. Desirable phenotypes have been identified in these species, for example resistance and low susceptibility to key diseases, including apple scab, fire blight, powdery mildew, and others (Table 1; Volk et al., 2015b). The progenitor species also have traits that may be useful as scientists search for new ways to adapt to climate change, including



**Figure 2. Relationship between *Malus × domestica* and its primary wild relatives based on chloroplast genome sequence data (modified from Volk et al., 2015a).**

**Table 1. Primary wild relatives of *Malus × domestica* and reported resistance/tolerance to abiotic and biotic stresses.**

Common name	Taxon	<i>sieversii</i>	<i>sylvestris</i>	<i>orientalis</i>	<i>prunifolia</i>
Apple scab	<i>Venturia inaequalis</i> (Cooke) G. Winter	x		x	x
Bot canker	<i>Botryosphaeria</i> (Sawada) Shang	x			x
European canker	<i>Neonectria ditissima</i>		x		
Fire blight	<i>Erwinia amylovora</i> (Burrill) Winslow et al.	x		x	x
Powdery mildew	<i>Podosphaera leucotricha</i> (Ellis & Everh.) E.S. Salmon	x			
Woolly apple aphid	<i>Eriosoma lanigerum</i> Hausmann	x			
Light brown apple moth	<i>Epiphyas postvittana</i> Walker	x			
Apple leaf curling midge	<i>Dasineura mali</i> Kieff.	x			
<i>Phytophthora</i>	<i>Phytophthora</i>	x			
Western white root rot	<i>Rosellinia natrix</i> Prill.	x			
Violet root rot	<i>Helicobasidium mompa</i> Tanaka	x			
Apple maggot	<i>Rhagoletis pomonella</i> Walsh	x			
Blue mold	<i>Penicillium expansum</i> Link	x	x		
Apple replant disease	Various	x			
Cedar apple rust	<i>Gymnosporangium juniperi-virginianae</i> Schwein.			x	
Cold hardiness		x			
Late flowering		x		x	
Water use efficiency		x			
Short juvenility		x			
Sunburn resistance		x			
Drought tolerance		x			

Sources: Bassett et al., 2011; Bus et al., 2005; Forsline and Aldwinckle, 2004; Harshman et al., 2017; Hokanson et al., 2001; Isutsa and Merwin, 2000; Khan et al., 2014; Kumar et al., 2010; Luby et al., 2002, 2001; Maguylo and Bassett, 2014; Norelli et al., 2017, 2014.

cold hardiness, late flowering, variation in water use efficiency, resistance to sunburn, and drought tolerance (Table 1; Campoy et al., 2011; Eccel et al., 2009; Luedeling, 2012; Olesen et al., 2011; Petri et al., 2012; Volk et al., 2015b). The use of these species in breeding programs may reduce the time needed to develop new cultivars (Cornille et al., 2012).

Ornamental crabapples are *Malus* species valued for their landscape and wildlife properties, in addition to being used as pollinators in commercial orchards. Crabapples are often selections from wild species or from interspecific hybrids that are desirable from an ornamental perspective (USDA, 2016). Some of these hybrids are valued for their bloom, foliage, and colorful fruit. Crabapples offer a unique form of genetic diversity for conservation. Although usually not desired for their fruit (for human consumption), crabapples available in gardens and arboreta may provide access to wild *Malus* species that may not otherwise be available to breeding programs. The economic value of crabapples as ornamentals and as pollinators is significant (Romer et al., 2003), but herein we focus primarily on the importance of apple germplasm for food and agriculture. This strategy recognizes the value of the crabapples and hybrids to the worldwide community; however, it does not (at this time) identify or fully explore the possible inclusion of crabapple collections in the proposed global conservation system, except as sources of specific traits for apple improvement.

## Ex situ conservation

Collections of apple genetic resources are relatively long-term investments that have been established for a variety of different purposes, including research, breeding, conservation, distribution, and public interest. National, sub-national, or local genebanks, botanical gardens, arboreta, private companies, NGOs, and private individuals all maintain significant apple collections. Two examples of extinct (or recently recreated) collections include Wøldikes Frugthave in Denmark, which contained 278 apple cultivars in the 1870s, and the Alnarp collection in Sweden, which formed the basis for Dahl's pomology, written in 1905-1929. A few of the still existing collections were established as early as the 1920s, including the UK National Fruit Collection in Brogdale, which is currently maintained by the University of Reading, and the collection of the N.I. Vavilov Research Institute of Plant Industry in Russia (Loskutov, 1999). The Pillnitz collection in Germany was started around 1930, and the Balsgård collection in Sweden was formed in the late 1940s. The collection at the Chinese Academy of Agricultural Sciences was established in the 1950s, and the French collection was initiated by the National Institute for Agricultural Research (INRA) in 1946. Most of the other apple genebanks were established more recently; in the USA, for example, the National Plant Germplasm System apple collection was established as part of a national network of clonal repositories in the 1980s (Postman et al., 2006).



*Malus* field genebank collections. USDA-ARS National Plant Germplasm Apple Collection in Geneva, New York.

*Malus* field genebank collections. Chinese apple field collection in Liaoning Province, China.



As a clonally propagated crop, genebank conservation of apple differs greatly from traditional methods used for the conservation of annual seed crops (Volk and Walters, 2003). Apple cultivars are clonally propagated, so each tree of an accession is an exact replica of the mother tree, usually produced from a graft. The seeds of a specific cultivar were created through a cross-pollination event and are therefore not genetically identical to the mother tree, making it impossible to propagate specific cultivars as seeds. Field collections of *M. × domestica* are primarily maintained as grafted trees, in an orchard, which allows for the conservation of specific cultivars or genotypes. This facilitates the use of collection materials for bud distribution, breeding, and phenotypic evaluations.

Some apple genebanks have plantings of seedlings or grafted trees that represent wild species populations. These accessions offer the community access to flowers and fruits of wild species that can immediately be used in breeding programs or for phenotypic and genotypic evaluations.

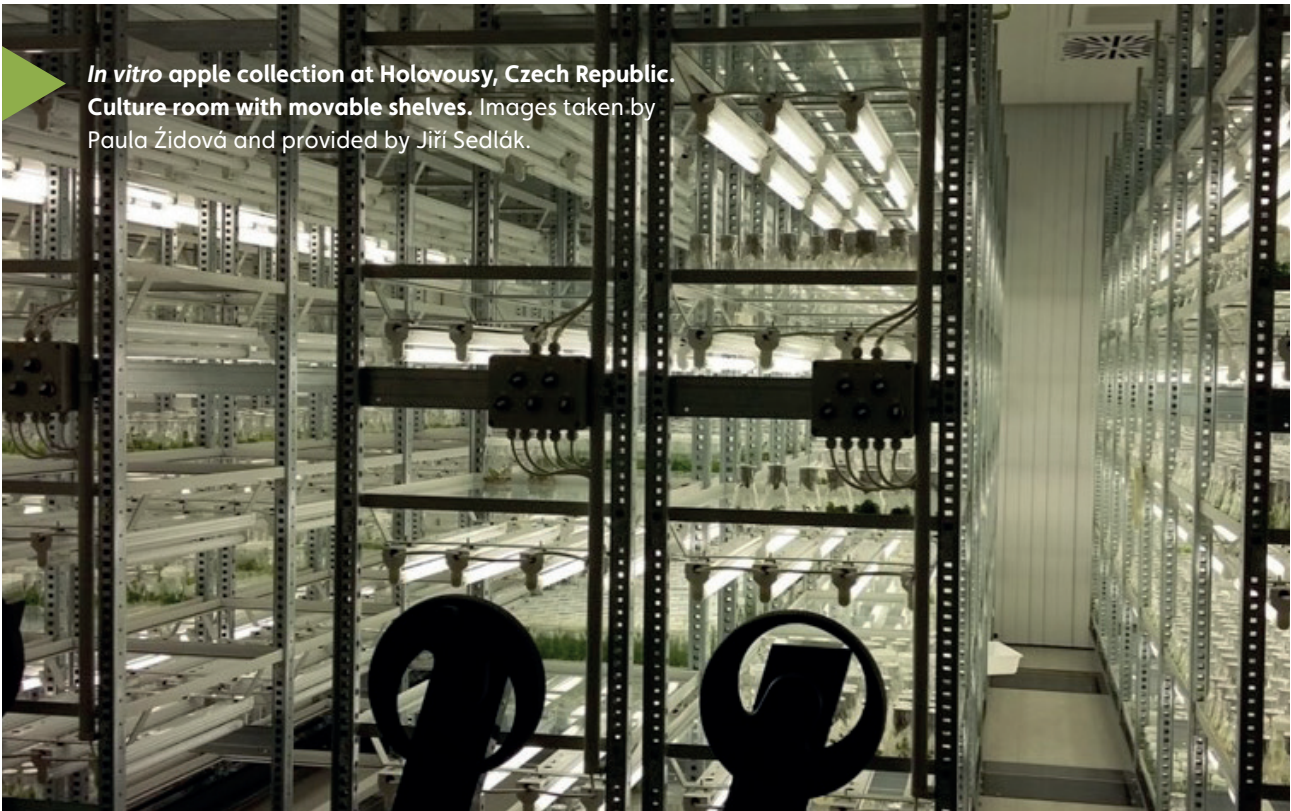
Apple cultivars are generally grafted onto improved or seedling rootstocks. Traditionally, rootstocks were grown from seeds collected from open-pollinated apple trees. In the 1800s, European apple producers recognized the value in breeding for apple rootstocks that provided orchards with uniform habit, disease resistance, preferred tree architecture, dwarf habits, and precocity. Specific

collections of accessions of apple rootstocks are found in genebanks such as the United States Department of Agriculture's Agricultural Research Service (USDA-ARS) collection in Geneva, New York. Breeding apple rootstocks is a lengthy and resource-intensive process, but modern rootstock cultivars have significant economic value as nursery stock (Fazio et al., 2015; Volk et al., 2015b).

Conservation of apple cultivars and wild species in field collections can be space- and labor-intensive to manage, and they are also susceptible to pathogens, pests, and environmental threats. Trees within orchards must be pruned and thinned to avoid cropping alternance. Depending on the rootstock and orchard conditions, trees may need to be repropagated periodically after 25–50 years. Portions of field collections may also be maintained in other forms, such as bonsai trees in pots in greenhouses.

Apples are generally amenable to *in vitro* culture; introduction, multiplication, and rooting procedures are available and are often applicable for many diverse *M. × domestica* cultivars and other *Malus* species (Kovalchuk et al., 2009; Sedlak et al., 2006, 2004, 2001). Biotechnological approaches using *in vitro* methods have facilitated the development of apple genetic transformation technologies (Malnoy et al., 2010). Some apple genetic resources (particularly rootstocks and new cultivars) have been mass-produced using *in vitro* bioreactor systems (Brischia et al., 2002; Chakrabarty et al., 2007, 2003).

**In vitro** apple collection at Holovousy, Czech Republic. Culture room with movable shelves. Images taken by Paula Židová and provided by Jiří Sedlák.



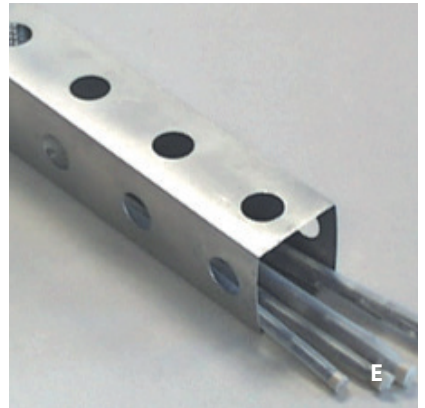
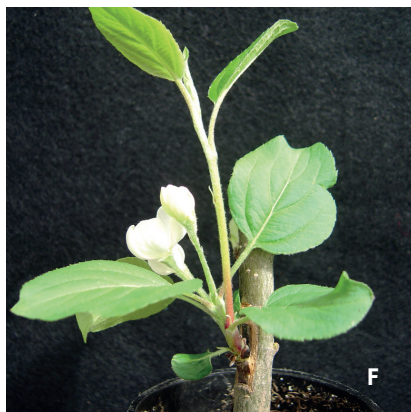
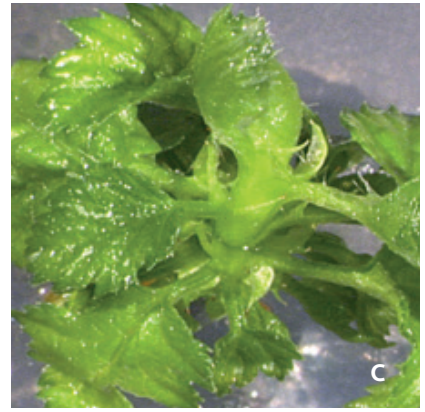
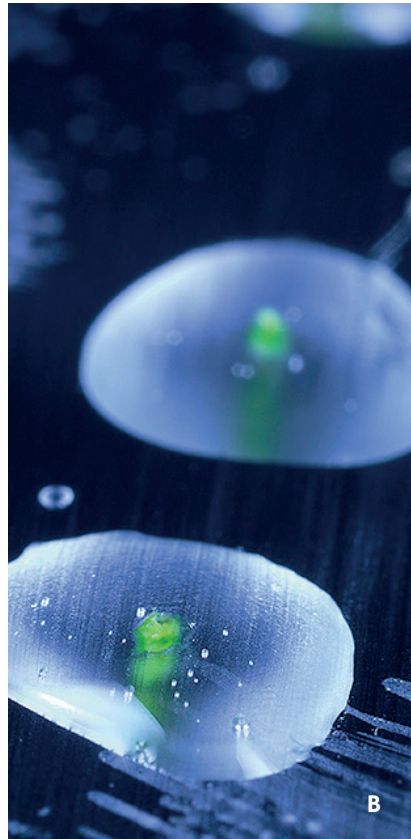
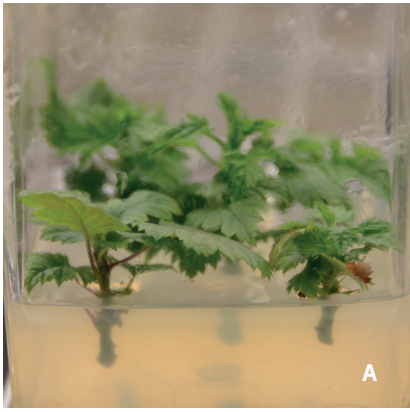
*Malus landrace* cultivar **Fragrance**.



*Malus landrace* cultivar **Mazánkův záznak**.

*In vitro* propagation is labor-intensive because culture transfers must be performed on a regular basis. Somaclonal variation is a concern, particularly when cultures are maintained *in vitro* for long periods of time (Oh et al., 2007). *In vitro* collections must be maintained in facilities that are equipped with alarm systems and have procedures in place to prevent culture loss due to system malfunctions (such as overheating or extreme cooling). Reduced-temperature slow growth storage is an option for *Malus in vitro* cultures, which reduces the time intervals between transfers (Kovalchuk et al., 2009). To our knowledge, only the genebanks in Holovousy, Czech Republic and in Egypt actively maintain *in vitro* apple collections.

Apple genetic resources can be successfully cryopreserved as dormant buds and shoot tips (Wang et al., 2018). Apple cryopreservation technologies were first implemented in genebanks with dormant buds as the explant (Forsline et al., 1998; Towill et al., 2004). The general procedure involves the collection of dormant bud scions mid-winter, desiccation to about 30% moisture content, and then slowly cooling to  $-30^{\circ}\text{C}$  to  $-35^{\circ}\text{C}$  prior to placement in liquid nitrogen vapor for long-term storage (Forsline et al., 1998; Towill et al., 2004; Höfer, 2015). Grafting warmed, rehydrated buds onto rootstocks assesses viability most effectively. Viable dormant buds have been successfully retrieved from the cryopreserved state after 10 years of storage (Volk et al., 2008a). *Malus* species



Apple conservation methods.

- (A) *In vitro* culture.
- (B) Droplets of vitrification solution with cryopreserved shoot tips.
- (C) *Malus florentina* plant recovering after cryopreservation.
- (D) Dormant apple bud.
- (E) Dormant apple buds processed for cryopreservation.
- (F) Grafted dormant bud after cryopreservation.
- (G) Liquid nitrogen tanks at the USDA-ARS National Laboratory for Genetic Resources Preservation.

vary in their response to cryopreservation, but many apple cultivars and wild relatives can be cryopreserved with high levels of recovery (Towill et al., 2004; Volk et al., 2015b).

Apple shoot tips can also be cryopreserved (Wang et al., 2018). Shoot tips (~1 mm) are excised from *in vitro* plants, pretreated as necessary, immersed in cryoprotectants (or encapsulated and dehydrated) and then plunged into liquid nitrogen. Several shoot tip cryopreservation procedures have been published for *Malus* (Li et al., 2015; Feng et al., 2013; Niino et al., 1992; Paul et al., 2000; Zamecnik et al., 2007).

Cryopreserved collections of apple genetic resources have been used to securely back up parts of collections. Through the use of cryopreservation technologies, apple genetic resources can be stored as specific genotypes at secondary sites that may not experience the same environmental threats as the field collection. Cryopreserved collections require the use of specialized equipment, reliable sources of liquid nitrogen, and specialized monitoring systems. Although the dormant bud cryopreservation technique has been implemented in several genebanks, its widespread applicability in *Malus* may be limited if field collections are not located in places which allow the trees to acquire sufficient dormancy (Bilavčik et al., 2015; Guyader et al., 2012; Höfer et al., 2006; Jenderek et al., 2011; Volk et al., 2017; Vogiatzi et al., 2012, 2011).

Pollen captures the alleles present within an accession and makes those alleles available to breeding programs (Zhang et al., 2009). Pollen is a particularly useful conservation target for breeders who wish to make crosses between trees that are not present at the same location or that have flowering times that are not synchronized (Sharafi, 2011). Rosaceae pollen is classified as desiccation tolerant and can be stored in liquid nitrogen for long-term conservation (Zhang et al., 2009). Methods are available for pollen (or anther) collection, desiccation, and storage (Ganeshan et al., 2008; Xu et al., 2014).

Some genebanks, such as the USDA-ARS National Plant Germplasm System, maintain accessions in several complementary forms, including grafted trees, seedlings, evaluation orchards, seeds (wild species), and cryogenic explants (Volk et al., 2015b). Grafted trees in the field are available for phenotypic and genetic evaluations, breeding, and budwood distribution. Seeds in long-term storage conserve the diversity of populations of wild species in an efficient manner, and cryogenic storage serves as a secure backup if trees in the field are lost (Forsline et al., 1998; Kushnarenko et al., 2010;

Towill et al., 2004; Volk et al., 2016). Currently, there are no published plans for seed conservation of apple cultivars. Apples are self-incompatible, so for seed samples, only the maternal parent is known. Thus, only half of the diversity being conserved is from the original accession.

For the 91 institutions from 24 countries that have shared collection information in the Genesys database, 92% of the accessions are stored as field collections and 70 of the 91 institutions only maintain apple accessions in the field. About 5% of the accessions are also stored as seed. Most of these seed collections are held by the Vavilov Institute in Russia (Genesys, 2017). They indicate that they have 3,114 accession of improved cultivars, breeding/research material, and traditional cultivars that are conserved as seed collections.

Old apple varieties may be found in genebanks, local gardens, meadow orchards, or in abandoned orchards. In some cases, they have been routinely grown in communities for centuries; in other cases, orchards of historic cultivars were simply abandoned as more modern cultivars became available. Currently, in the Czech Republic, old apple varieties are being threatened by habitat destruction, changes in land ownership, and new agricultural practices and priorities. Collection efforts have located, registered, conserved, and promoted old cultivars for production. The foundation ProSpecieRara in Switzerland is also focused on the conservation of old apple varieties in fruit gardens and reintroducing these into local markets to ensure their long-term conservation (ProSpecieRara, 2019). Vidal (2017) describes the effort being made in the United Kingdom to find and revive old varieties of apples and to make them available for sale in the thriving heritage fruit market.

Veteto and Carlson (2014) assessed the distribution of local diversity of heirloom apples in South/Central Appalachia in the USA. They found that only a few orchards conserved most of the diversity in the region, and the greatest risk to loss of this local diversity was from the lack of consistent intergenerational commitment to the key orchards. To ensure conservation of heirloom varieties by orchardists, Veteto and Carlson recommended a combination of governmental policies and research on sustainable agriculture with grassroots citizen movements to conserve heritage cultivars. The US Congress Office of Technology Assessment (1986) recognized and cataloged the efforts of individuals and citizen-based groups in the USA to conserve biological diversity, with a focus on local and regional crop varieties. The study concluded that individuals and grassroots organizations preserved significant



Apple accession being conserved as bonsai plants in the greenhouse in the Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium. Image courtesy of Sheri Mossbeck.





Trees conserved in *in situ* conservation sites for *Malus sieversii* in Kazakhstan. Images provided by Gayle Volk.

amounts of traditional American fruit varieties that were not found in government or public institutional collections, but they were not a secure long-term conservation approach given their limited and unstable funding and dependence on the enthusiasm and commitment of a single or small number of individuals. Knowledge and expertise within local communities could be used to identify key, financially stable collections that offer diversity to national and international conservation efforts. Collaborations with local organizations and individuals may reveal key cultivars that should be included in national public genebanks to increase their long-term security. Multiple assessments have determined that some historic cultivars are no longer available (RAFT, 2010); thus it is important to conserve key historic cultivars before they are lost (Volk and Henk, 2016).

A case study associating *ex situ* and on-farm conservation of fruit tree genetic resources in Belgium is based on the organization of an orchard network in the Walloon Region (Lateur, 2003; Lateur et al., 2010). The objectives include the following: (1) duplicating most unique material from *ex situ* collections in order to ensure their long-term safeguarding; (2) returning genetic material to the regional landscape to rebuild the local genetic diversity; (3) evaluating cultivars in their original soil and climatic conditions; (4) responding to a demand coming from farmers and the people; (5) developing economic activity with high-quality fruit processing products; and (6) coordinating various

genetic resources actions to avoid redundancy and duplication of efforts. Contracts are established that include public and private participants that define the responsibilities, objectives, place and owner of the land, cultivars planted, growing advice, and duration of the contract. As of 2017, 60 standard tree orchards on a total area of about 67 hectares have duplicated about 30% of the *ex situ* collection.

### *In situ* conservation

There have been efforts to protect wild apple species in their native habitats. The domesticated apple originates from ancient *M. sieversii* forests located in the mountains of eastern Kazakhstan and western China and nearby parts of Kyrgyzstan, Uzbekistan, and Tajikistan. The remnants of these forests still exist today, notably in the Zailiiski and Djungarskii Mountains in eastern Kazakhstan (Dzhangaliev, 2003). Efforts have been made to protect ancient apple forests in nature preserves in Kazakhstan and Kyrgyzstan (Dzhangaliev, 2003). Hokanson et al. (1997) concluded that the apple forest in Kazakhstan was suffering from human encroachment and that “about 90% of the wild apples that existed near Almaty in 1935 are now gone.” While long-term conservation of diversity is possible in field collections, they did not view this as practical given the costs and the limited sample of diversity that could be stored, except as seed. They concluded that maintaining populations in nature reserves would be more cost-effective and allow for continued evolution and adaptation.



Historic apple cultivars conserved at Yosemite National Park, USA. Image provided by Dan Schaible.

Conservation of the closely related *Malus* progenitor species in protected areas can face significant threats, not only from human encroachment and climate change, but also from hybridization with cultivated apples. Cornille et al. (2013b), Feurtey et al. (2017), and Omasheva et al. (2017) found increasing threats to the genetic integrity of *M. sylvestris* and *M. sieversii* in protected forests due to gene flow with *M. × domestica* grown in adjacent areas. Omasheva et al. (2017) concluded that the present situation with the prevalence of admixture in the wild populations in Kazakhstan is the result of current and past incidences of crop-to-wild gene flow. Areas in the low mountain zones, primarily in the piedmont-mountain border zone, have had the most impact on gene flow. This is due to the cultivation of apple cultivars in gardens and the past use of wild rootstocks for grafting *M. × domestica*. This has increased the risk of gene flow and increased the level of admixture in the adjacent wild populations.

If effectively managed in perpetuity, large and carefully selected *in situ* reserves could conserve the genetic diversity in wild populations of *Malus* species, as suggested in Omasheva et al. (2017). Although routine access to such reserves may be limited, protected sites could serve as sources of diversity for genebanks. Ideally, both genetic and phenotypic data collected on these wild populations would inform site selection and geography for protection (Hokanson et al., 1997).

Feurtey et al. (2017) concluded that wild populations need to be protected from hybridization by managing the risk for gene flow in the natural habitats and in seed nurseries set up for reforestation. Technical guidelines for the conservation of *M. sylvestris* in Europe as a key species for conservation have been established under the European Forest Genetic Resources Programme (EUFORGEN, 2017). The guidelines address the need for replanting seedling trees from genetically representative nurseries of wild species, since the isolated trees in the natural reserves restrict natural regeneration and can lead to genetic drift.

In the USA, there are also many historic orchards that are part of national, state, and regional parks (Dolan, 2009). Park managers recognize the value of these historic trees as part of the cultural landscape and *in situ* conservation of these materials may play a role in a wider conservation effort (Routson et al., 2009). Furthermore, some historic sites have records of apple cultivars that were once grown at their location. Access to these historic cultivars is valuable when site managers wish to replant the historic cultivars at their locations. With respect to local, landrace, and historic cultivars, it is critical to assure that key significant cultivars are conserved and secured, likely through a partnership amongst *ex situ* genebanks, local organizations, and the international community.

## Information availability and sharing

There are a number of sources of accession-level information on conserved *Malus* genetic resources, both institutional as well as consolidated at national, regional, or international levels. For example, passport, phenotypic, and molecular data for accessions conserved by the USDA-ARS National Plant Germplasm System are publicly available on the GRIN-Global database (USDA, 2016). The European Search Catalogue for Plant Genetic Resources (EURISCO) is a European database for accession-level information sharing (ECPGR, 2019). Genesys is a global platform for sharing accession-level information on crop genetic resources that currently makes available information on 36,654 accessions of *Malus* from 91 collections (Genesys, 2017). For each accession, Genesys maintains basic passport information, including cultivar name, species name, holding institute, biological status, country and geographic coordinates of origin, type of germplasm storage, and availability for distribution, as provided by the collection holder or interoperable databases.

The Global Biodiversity Information Facility (GBIF, 2019) maintains a global database of information on material that has been collected and maintained as live plant material or in herbarium collections. The database includes basic information such as genus, species, geo-reference of collecting site, and data source. The UN Food and Agriculture Organization's World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture (FAO WIEWS) serves as a repository of metadata (i.e. not accession-level) on the holdings of over 600 genebanks in 82 countries (FAO, 2017). This is currently archived but it is still the only comprehensive global overview of genebank holdings. The number of accessions of different *Malus* species held globally according to these sources are given in Annex I.

The Genome Database for Rosaceae is a curated web-based database that provides, genomic, genetic, and breeding data for rosaceous crops (Jung et al., 2014). In the database for *Malus*, there are data for single nucleotide polymorphisms (SNP), simple sequence repeats (SSR), genetic maps, quantitative trait loci (QTLs), Mendelian trait loci (MTLs), genome assemblies, and phenotypic measurements (GDR, 2018).

There have been some efforts to coordinate apple genetic resource conservation and use at the regional and international levels. For example, key findings and results relating to conservation and breeding are shared at conferences such as the

Rosaceae Genomics Conference and the Eucarpia Plant Breeding Symposium, in addition to national and international horticultural and genomics conferences and topic-specific symposia. In 2016, the first International Apple Symposium was held in Yangling, China. The Rosaceae International Genomics Initiative (RosIGI) committee organizes the biennial Rosaceae Genomics Conference. The US Rosaceae Genomics, Genetics and Breeding Executive Committee (RosExec) meets quarterly and is comprised of scientists that represent academia, government, and industry.

The ECPGR *Malus/Pyrus* Working Group (ECPGR, 2017) is a group of European researchers with a common interest in apple genetic resources conservation, characterization, and evaluation and promotes their utilization in different ways and particularly in breeding programs (Lateur et al., 2013). The group has sought, among other things, to define standardized phenotypic and genotypic descriptors to characterize European *Malus* and *Pyrus* collections (Watkins, 1985; Watkins and Smith, 1982; Fernández-Fernández, 2013); to incorporate phenotypic data into a European database (van Hintum and Knüpffer, 2010); and to develop and implement a comprehensive European *Malus* and *Pyrus* conservation strategy.

A number of projects have resulted in regional and international collaborative efforts with respect to the use of molecular data in breeding programs. FruitBreedomics (2017) was an international project involving partners in Europe, New Zealand, China, and South Africa between 2011 and 2015. Its goal was to bridge the gap between genetic research and breeding in apple and peach (Baumgartner et al., 2016; Bianco et al., 2016, 2014; Chagne, 2015). The European Fruit Research Institutes Network (EUFRIN, 2011) was established in 1993 as an informal, voluntary organization of university departments and research institutes in the European Union, Switzerland, and Eastern Europe. Its purpose was to prepare and submit grant proposals, enhance and facilitate research, development, and technology transfer focused on sustainable fruit production. The network is involved in an ongoing project called EUFRUIT with a consortium of 21 institutes in 12 European Countries (EIP-AGRI, 2019). The objective is to develop new varieties of fruits to meet quality standards and sustainable production systems. The US-based RosBREED project was a 10-year effort focused on developing and applying genomic and breeding methods to deliver new cultivars of apple and other Rosaceae crops. The project resulted in products with better taste, nutrition, keeping ability, and appearance (Iezzoni et al., 2016). While some of the partners in the past and current collaborations have had some international collaborators, there is as yet no truly global effort.

There are also efforts to share information on local cultivars as well as to link consumers and suppliers of planting material and fruit. The People's Trust for Endangered Species (2019) has established FruitFinder as an online catalog of local historical cultivar names, origins, uses, locality, and supplier of grafted trees. Orange Pippin (2019) is a register of fruit trees that have been contributed by individuals or orchardists globally. The database is searchable and allows users to contact owners of trees for advice or planting material. The information required for each tree includes location, description, photograph, and availability of planting material. There are many ongoing efforts such as these to share information and apple planting material through online searchable databases.

## Utilization of apple genetic resources

Assessments of diversity within genebanks can be used to reveal genetic relationships (parentage and kinship, sports, hybrids; synonyms, homonyms, mislabeling) among cultivars, facilitating both management and use (Gross et al., 2012, 2011; van Treuren et al., 2010; Patzak et al., 2012b; Urrestarazu et al., 2016; Liang et al., 2015; Lassois et al., 2016; Ordidge et al., 2018). Portions of the collections in the USA (Gross et al., 2013, 2012; Hokanson et al., 2001; Volk et al., 2009, 2008b), France (Lassois et al., 2016), Czech Republic (Patzak et al., 2012a, 2012b), Spain (Pereira-Lorenzo et al., 2007; Pina et al., 2014; Urrestarazu et al., 2012), China (Gao et al., 2015), Lithuania (Sikorskaite et al., 2012), Italy (Guarino et al., 2005; Liang et al., 2015), Latvia (Lacis et al., 2011; Lacis, 2013), Turkey (Gulsen et al., 2010; Uzun et al., 2015), the Netherlands (Van Treuren et al., 2010), Denmark (Larsen et al., 2017), Sweden and Finland (Garkava-Gustavsson et al., 2013, 2008), and Bosnia and Herzegovina (Gaši et al., 2013a, 2013b) have been genotyped using SSR or SNP marker systems. Increasingly, genomic sequence data has also been used for these assessments (Larsen et al., 2017; Duan et al., 2017; Ordidge et al., 2018).

Coordinated national and regional efforts among genebanks to compare collections at the phenotypic and genetic levels, as well as to confirm cultivar identities, will enable greater use of genebank collections in the future (Kellerhals et al., 2012; Urrestarazu et al., 2016; Liang et al., 2015; Lassois et al., 2016; Howard et al., 2017; Ordidge et al., 2018). Lassois et al. (2016) reported on one of the largest assessments of genetic diversity of national apple germplasm. Material from 13 collection holders in France was fingerprinted with 24 SSR markers from 17 linkage groups. With an overlapping 15 SSR markers, the researchers

were able to detect 34% redundancy in these collections, clarify parentage, and identify three major genetic subgroups among French cultivars. Recent results have determined that the cultivars are mislabeled about 5–20% of the time in both genebanks and local collections (Howard, personal communications), making it increasingly important to use phenotypic and genotypic assessments to determine the true identity of materials in apple collections. Ordidge et al. (2018) were able to describe the pedigrees of cultivars that dated back to the 18th century.

A large collaborative project revealed both within-collection and among-collection genetic variation in France, the Czech Republic, Spain, Sweden, Ireland, Norway, Denmark, Finland, the Netherlands, Kyrgyzstan, Switzerland, the United Kingdom, Italy, Belgium, and Russia (Urrestarazu et al., 2016). Due to the high level of allelic diversity among apple cultivars, relatively few markers are sufficient to differentiate apple cultivars (aside from sports) (Froni et al., 2012; Moriya et al., 2011).

A global collaborative research project to identify close and distant pedigree relationships has recently been initiated using thousands of accessions from germplasm collections from around the world (Howard et al. 2018). SNP array data are put through a rigorous data curation pipeline (Vanderzande et al. 2019) and evaluation procedures allow for better resolution of the results than was previously available through SSR studies. One outcome from this project has been that participating germplasm collections that contribute samples or SNP array data are able to learn of previously unknown duplicates, redundancies, mislabeled samples, and previously unknown pedigree links across collections. This project will also reveal of the extent of introgression between cultivated apples and wild species.

Efforts to screen large germplasm collections, particularly for phenotypic traits, are very costly. Thus, core collections have been established for some apple collections. These core collections are small subsets that nevertheless capture much of the diversity represented in the broader collection (Gross et al., 2013; Hokanson et al., 1998; Urrestarazu et al., 2016; Liang et al., 2015; Lassois et al., 2016). In the case of apple, the USDA-ARS National Plant Germplasm System has also identified core collections for *ex situ* populations of *M. sieversii* and *M. orientalis* (Richards et al., 2009; Volk et al., 2009, 2005). A national assessment of 2,163 accessions from French territories, including the INRA-Angers collection and 12 amateur associations and governmental, regional, and

local authorities was performed. These national assessments have identified several core collections for specific purposes, such as for cider and dessert apples (Lassois et al., 2016; LeForestier et al., 2015).

Wild *Malus* species offer diversity necessary for future breeding programs that are not available within the crop (Moriya et al., 2010; Brozynska et al., 2015). A prominent example is the Purdue University, Rutgers University, and University of Illinois apple breeding programs that made crosses between *M. × domestica* and *M. floribunda* 821 in 1945 to introgress apple scab (*Venturia inaequalis* (Cke.) Wint.) resistance into apple (Belfanti et al., 2004; Crosby et al., 1992; Gessler and Pertot, 2012; Hough and Shay, 1949). The *Vf* gene that was introgressed into *M. × domestica* is a primary source of apple scab resistance in many modern apple cultivars (Erdin et al., 2006), yet erosion of this resistance (Caffier et al., 2014) means that efforts to pyramid resistance genes are needed.

Wild species may provide novel alleles not just for disease resistance, but also desirable plant architecture, fruit quality, and increased yield (Peace and Norelli, 2009, Brown, 2012; Fazio et al., 2014, 2009). Although most *Malus* species are interfertile, undesirable fruit characteristics, non-uniform ripening times, and other traits that are not amenable to commercial apple production have deterred the use of wild species in breeding programs. With technological advances, however, it is becoming possible to transfer desirable alleles from wild *Malus* species into the cultivated apple faster and without deleterious effects on fruit quality, quantity, and production traits (Kumar et al., 2010; Yamagishi et al., 2014). New technologies, such as marker-assisted selection, genomic selection, genetic engineering, genome-wide association mapping, high-throughput genotyping, and rapid cycling have made it realistic to use a wider range of *Malus* wild species in breeding programs (Bianco et al., 2016, 2014; Brogginini et al., 2014; Chagné et al., 2012; Evans, 2013; Flachowsky et al., 2011; Kumar et al., 2013, 2012a, 2012b; Ru et al., 2015; Troggo et al., 2012; Velasco et al., 2010).

## Exchange of apple genetic resources

Genetic resources are subject to a number of international legal regimes that regulate access and benefit sharing. Germplasm exchange is also affected by a variety of regulatory frameworks – intellectual property rights, other proprietary regimes, biosecurity – and institutional arrangements. The International Treaty on Plant Genetic Resources for Food and

Agriculture (ITPGRFA), which came into force in 2006, is recognized by the Convention on Biological Diversity as a sector-specific access and benefit sharing regime (FAO, 2009). Apples are listed in Annex I of the ITPGRFA as one of the 64 crops of global significance that are included in the Multilateral System. For material held by the parties to the ITPGRFA in the Multilateral System, germplasm transfer is under the terms and conditions of the Standard Material Transfer Agreement (SMTA). Both EURISCO and Genesys flag material that has been declared to be in the Multilateral System. For germplasm held by non-ITPGRFA parties or not held in the Multilateral System, the exchange could be under the terms and conditions of the Nagoya Protocol or in other specific bilateral agreements.

Like other crops, apple varieties are subject to different forms of intellectual property rights; however, there are some interesting specificities. Prevar (2019) and Summerland Varieties (2019) describe the management of intellectual property rights over their cultivars by means of variety protection and trademarks to assist national growers in achieving sustainable profits from the new varieties, but also to generate funds from the licensing of new cultivars to support national breeding programs. The result is that new cultivars from breeding programs are managed under license agreements. Apple variety clubs (Charles, 2014) protect and trademark new apple varieties. Peil et al. (2011) suggest that the emergence of such clubs could increase competition amongst breeders and reduce the exchange of germplasm. Conversely, it could also result in an increase in demand for access to better-characterized and better-evaluated accessions. In some cases, as at Plant & Food Research in New Zealand, the funds generated are used in part to support the conservation of an *ex situ* apple collection.

In addition to access and benefit sharing and intellectual property considerations, movement of plant materials between countries and regions must be carefully managed so as to avoid the introduction of a new pest or disease, or a different strain of a pest or disease, to an uninfected region. In most countries, movement of *Malus* plant material is governed by national phytosanitary regulations, often requiring many years of quarantine or clean-up processes to minimize the risk of disease spread by budwood or live trees. These regulations, though necessary, inevitably serve to further restrict the movement of apple genetic resources internationally and their use for varietal development.

## Vulnerability of apple genetic resources

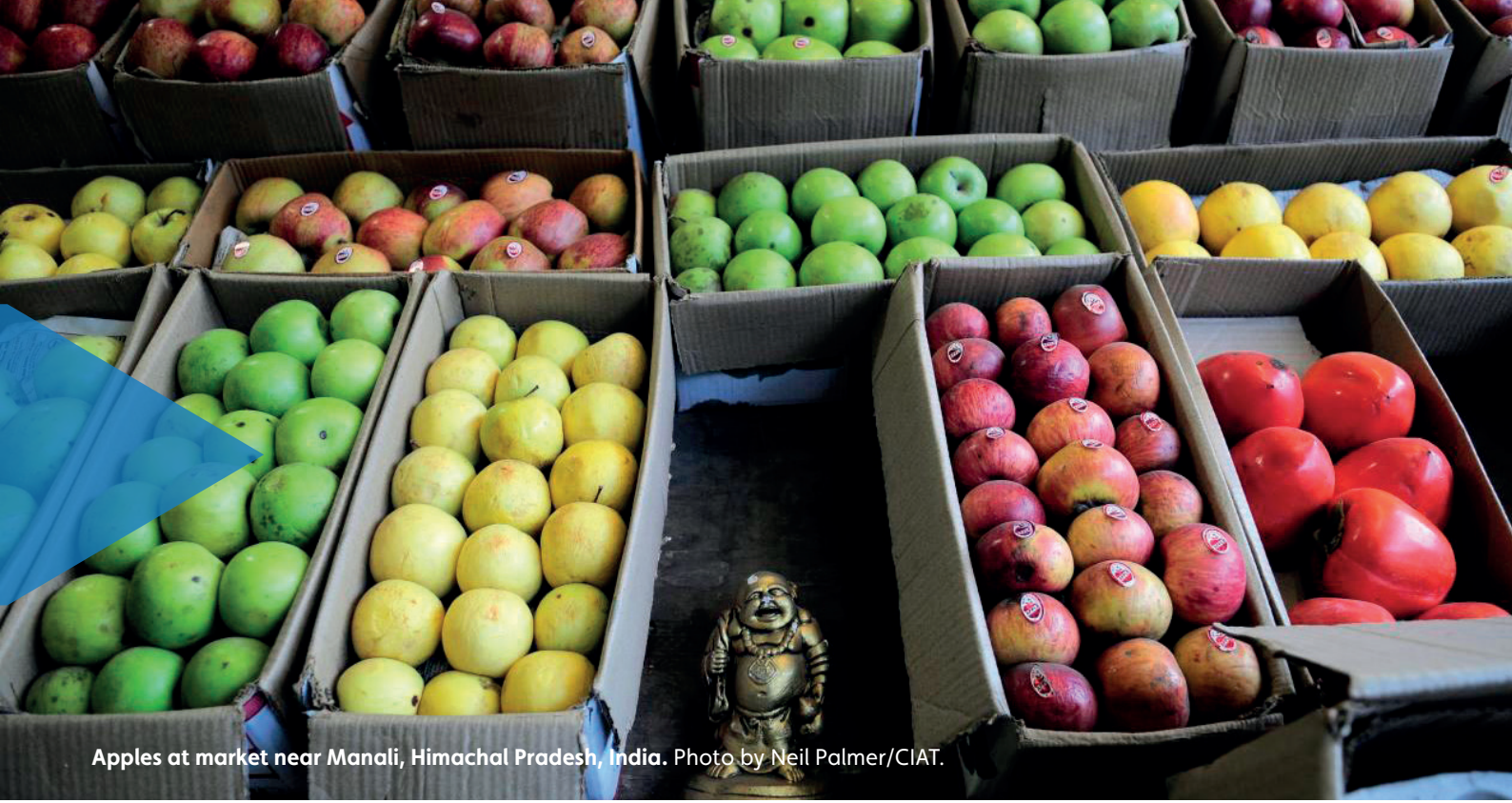
Volk et al. (2015b) assessed the vulnerability of *Malus* genetic resources in the USA. They concluded that the US apple crop is at risk because of the low number of cultivars being grown, the longevity of orchards, the cost of replanting with new cultivars, and the limited number of breeding programs. There is a need for new cultivars with improved resistance to biotic and abiotic stress, combined with a constant supply of high-quality product for the consumers. Producers are facing many challenges that are resulting in increased production costs, thus requiring cultivars that need less in the way of inputs and are easier to harvest. Volk et al. (2015b) also concluded that the apple breeding programs of the future will need to deploy advances in genomic technology as well as improved phenotyping to better use the diversity conserved in *ex situ* collections, including wild species. While their study focused on the USA, many of its findings are relevant to the vulnerability of apple in other countries and regions.

Vincent et al. (2013) reported that 55% of the *M. × domestica* wild relatives are represented by fewer than 50 *ex situ* accessions. In addition, wild species materials are often shared amongst collections, limiting the extent of diversity that has been studied. Currently, 29 *Malus* species are listed in the threatened species red list, according to the International Union for Conservation of Nature (IUCN, 2017). The majority of these (23 species) are classified as data-deficient because there is too little information on these for accurate conservation status assessments. Some of these could be listed as threatened once they are more fully evaluated. There are three species that are classified as least concern, *M. baccata*, *M. fusca*, and *M. hupehensis*, meaning they are currently not threatened in the wild. *M. sieversii* is listed as vulnerable (primarily due to habitat degradation), while *M. komarovii* and *M. niedzwetzkyana* are endangered (IUCN, 2016, 2007a, 2007b). The lack of information about *Malus* species taxonomy, genetic diversity, distribution, and conservation status has likely affected the representation of *Malus* species in the IUCN classification system.

The ancient woodlands of Central Asia and China are a center of diversity for many fruit and nut species, including *M. sieversii*. Trees within the boundaries of parks or forest preserves may have some level of protection, but natural areas with wild apple trees are at risk of being lost due to encroachment for agriculture, such as livestock production, urban development, hybridization, or other alternative uses (Forsline et al., 2003). Genebanks that maintain *ex situ* collections face a number of challenges that include increasing cost of maintenance; reduced support for routine operations; increased regulations and cost for acquisition and sharing of accessions; increased threats *in situ* to valuable local cultivars and wild relatives that are significant gaps in collections; and increased demand from users for genotyping, evaluation, and other data for accessions.



Grafted trees in the Research Institute of Horticulture and Seeds (IRHS), National Institute for Agricultural Research (INRA), Angers, France. Photo courtesy of Gayle Volk



Apples at market near Manali, Himachal Pradesh, India. Photo by Neil Palmer/CIAT.

# A GLOBAL STRATEGY FOR THE *EX SITU* CONSERVATION OF APPLES

The global nature of production and consumption means that there is global interdependence for genetic resources of foods such as apples (Khoury et al., 2015). This is a key rationale for the conservation of crop genetic resources as a global public good. This interdependence is recognized in the Second Global Plan of Action for Plant Genetic Resources for Food and Agriculture (FAO, 2011) and was the basis for the ITPGRFA.

The Crop Trust (officially known as the Global Crop Diversity Trust) is an international organization working to safeguard crop diversity, forever. The Crop Trust is recognized as an essential element of the funding strategy of the ITPGRFA. In 2006, the Crop Trust initiated an effort to work with crop conservation and use specialists to develop global *ex situ* conservation strategies for key crops listed in Annex I of the ITPGRFA, including apple. Global conservation strategies were initiated to facilitate a transition from the current complex, fragmented, and independent crop conservation system to a more integrated, collaborative, and cooperative global conservation system.

In 2012, USDA-ARS and the Crop Trust initiated the development of the global strategy for the conservation of apple genetic resources. The aim of

the strategy is to provide the evidence base to secure the long-term conservation and use of apple genetic resources as effectively and efficiently as possible. It will serve as a framework to bring together stakeholders at all levels – local, national, regional, and global – in building long-term support through greater awareness, increased capacity, greater community engagement, and sustained funding.

## Development of the Global Strategy

The *Global Strategy for the Conservation and Use of Apple Genetic Resources* is the outcome of a background study of apple genetic resources conservation and use; responses to a widely distributed survey of major apple collections; three consultations with genebank curators and key users; and site visits to key field collections. The findings from the survey provide insights into the current status of apple collections on a global scale, with some notable deficiencies. Based on the information provided in the survey, and through the consultations with genebank curators and users, a global system by which apple conservation and use efforts could become more coordinated, systematic, and unified is described. Cooperation between nationally based research institutes is desirable to make a finer delineation of the genetic

diversity and structure of apple conserved at a high-scale level.

## Status of *Malus* collections

There are many apple field genebanks located around the world, but limited information is publicly available regarding the composition and maintenance of these collections (Nybom and Garkava-Gustavsson, 2009). A survey on the status of the apple collection was initiated late in 2014. Follow-ups were made with authors of publications on apple genebank collections. In some cases, breeders and national genebanks were also contacted. There were 41 responses to the survey, representing a significant portion, but not all, of the world's national apple genebank collections. Though all regions were represented, 28 of the 41 respondent institutes were in Europe and North America. There was a gap in the Caucasus Region and Central Asia, from which only three institutes responded. Thus, the center of diversity and domestication is significantly under-represented among respondents. Responses were not received from a few countries that are believed to have apple collections with more than 300 accessions – Austria, Denmark, Finland, Romania, Slovakia, and Ukraine – as well as other countries with significant collections (Braniste, 2000; Christensen and Gaire, 2015; Gharghani et al., 2009; González-Horta et al., 2005; Kullaj et al., 2012; Marić et al., 2010). One response was received from a cryocollection in the Czech Republic, but it was not included in the collection inventory summary due its very different nature and purpose.

Of the 40 apple field collections that completed surveys, 24 (60%) were maintained by government agencies, 9 (22.5%) by universities, 3 (7.5%) by the private sector, and 4 (10%) by other entities (i.e. public research institutions, charities, state research institutes, and foundations). Botanical gardens, arboreta, breeders, and nurseries were not targeted, though some responses were

received from these sources. These non-genebank collections are also under-represented.

Amongst the respondents, 13 of the collections were established prior to 1970, while seven were established in the 2000s. The oldest collections are held by the University of Reading in the United Kingdom, the RUE Institute for Fruit Growing in Belarus, the Vavilov Institute in Russia, and the Department of Agriculture and Agri-Food in Canada.

## Collection composition

Apple collections that responded to the survey maintain a vast amount of apple diversity. The survey requested inventory information, and 38 institutions shared information on 31,349 *Malus* accessions conserved *ex situ* (Table 2). This is about 50% of the total number of accessions conserved globally according to FAO WIEWS (see Annex I). The respondents included four very large collections with more than 2,000 accessions and seven smaller collections of less than 200 accessions each. Four collections conserved only international cultivars and four institutions held only local cultivars. Of the conserved materials overall, 50% were international cultivars (defined as those that are, or used to be, produced on a large scale, and/or have been used widely in breeding and research programs); 20% were local or historical cultivars (those that have, or have had, economic, historical, cultural, and heritage value in particular localities, regions, or countries); and 24% were wild species. There were 1,074 breeding lines.

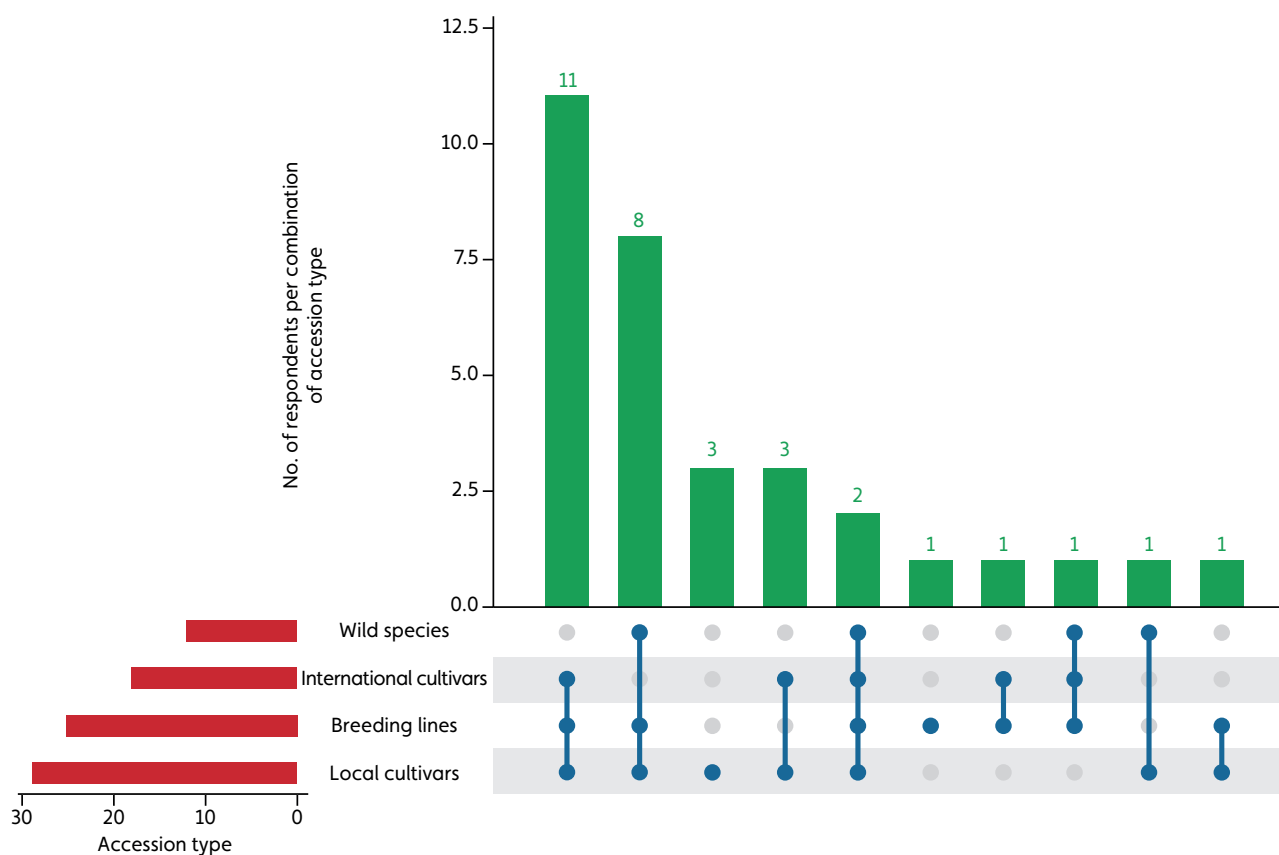
Twenty institutions held 67 wild species taxa, with between 1 and 2,300 accessions for each species. Seventeen species hybrids were also documented in the collections, with a total of 243 accessions. The survey identified *in situ* conservation projects specifically for *Malus* in Azerbaijan (*M. sieversii*), China (*M. sieversii*), Germany (*M. sylvestris*), Kazakhstan (*M. sieversii*), and the Netherlands (*M. sylvestris*).

**Table 2. Summary of accessions in collections from 38 institutions, based on the survey.**

Country	Name of collection	Total	International cultivars	Local cultivars	Breeding lines	Wild species
Albania	Institute of Plant Genetic Resources (IPGR), Agricultural University of Tirana	65	65			
Azerbaijan	Genetic Resources Institute, Azerbaijan National Academy of Sciences	437	50	125	150	112
Belarus	RUE Institute for Fruit Growing	1,545	494	44	369	638
Belgium	Walloon Agricultural Research Centre (CRA-W)	1,773	700	900		173
Bosnia and Herzegovina	Institute of Genetic Resources, University of Banja Luka	82	18	64		



Country	Name of collection	Total	International cultivars	Local cultivars	Breeding lines	Wild species
Brazil	EPAGRI / Experimental Station of Caçador	388	320	59		9
Canada	Agriculture and Agri-Food Canada	287	277	0		10
China	Institute of Pomology, Chinese Academy of Agricultural Sciences	1,011	676	58		277
Czech Republic	Research and Breeding Institute of Pomology, Holosvovsky, Ltd.	1,114	488	147	243	21
Czech Republic	Crop Research Institute Cryocollection	65		65		
Egypt	National Gene Bank	20		20		
France	Research Institute of Horticulture and Seeds (IRHS), National Institute for Agricultural Research (INRA)	2,090	1,161	885		44
Germany	Institute for Breeding Research on Fruit Crops, Julius Kühn Institute	1,343	426	451		466
Greece	Pomology Institute, Hellenic Agricultural Organization DEMETER	215	215			
Hungary	Fruitculture Research Institute, National Agricultural Research and Innovation Centre	1,210	976	234		
India	Central Institute of Temperate Horticulture	204	117	64		23
Ireland	Irish Seed Savers Association	220		200		20
Israel	Newe Ya'ar Research Center, Agricultural Research Organization (ARO)	130	57	35	35	3
Italy	University of Udine	256	220	11		25
Italy	Department of Agricultural Sciences, Bologna University	40	8	29		3
Italy	Trees and Timber Institute (Ivalsa), National Research Council (CNR)	40	11	29		
Japan	Apple Research Division, Institute of Fruit Tree Science, National Agriculture and Food Research Organization (NARO)	1,350	850	250		250
Kazakhstan	Institute of Plant Biology and Biotechnology	38	21	15		2
Latvia	Latvia State Institute of Fruit-Growing	698	572	72		54
Lithuania	Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry (IH LRCAF)	937	524	37	71	305
Netherlands	Centre for Genetic Resources (CGN)	173	19	111		43
New Zealand	New Zealand Institute for Plant & Food Research Limited	400	375	0		25
Poland	Center for Biological Diversity Conservation, Polish Academy of Sciences Botanical Garden, Powsin	429	429			
Russia	N.I. Vavilov Research Institute of Plant Industry	3,821	2,640	566	206	409
Slovenia	Biotechnical Faculty, University of Ljubljana	119	106	13		
South Africa	Agricultural Research Council (ARC) Infruitec-Nietvoorbij	520	480	40		
Spain	Agrifood Research and Technology Centre of Aragón (CITA de Aragón)	191	23	191		
Spain	Public University of Navarre (UPNA)	397	20	377		
Sweden	Swedish University of Agricultural Sciences (SLU) Balsgård	550	350	200		
Switzerland	Agroscope	1,300		1,300		
Turkey	Egirdir Fruit Research Station	330	330			
United Kingdom	University of Reading	2,247	2,174			73
USA	USDA-ARS Plant Genetic Resources Research	5,291	1,372			3,919
<b>Total</b>		<b>31,349</b>	<b>15,564</b>	<b>6,592</b>	<b>1,074</b>	<b>7,121</b>



**Figure 3. The number of respondents to the survey who indicated a priority for wild species, international cultivars, breeding lines, and local cultivars for conservation in their collection, solely and in combination.**

The survey respondents were asked about the priority they gave to conservation of internationally important cultivars, local cultivars, breeding lines, and wild species (Fig. 3). Thirty-four institutions considered local apple cultivar conservation to be a priority of their collection. Breeding lines and international commercial cultivars were priorities for 24 of the institutions that responded. Nineteen of the collections included wild species materials as a priority, while 7 considered crabapples and 10 considered public garden materials as priorities.

The types of accessions held in genebanks correspond to the overall goal of their conservation efforts. When the combination of the different accession types was assessed, there were three institutions that had a focus only on local accessions and an additional one whose sole priority was breeding lines (Fig. 3). The majority of the respondents had a focus that included local cultivars, international cultivars, breeding lines, and wild species. Only four of the institutions gave no priority to local varieties. Some collections identified specific collection gaps, including wild species (China, Russia, the USA), local cultivars (Azerbaijan, China, Israel, the Netherlands, the USA), breeding lines (Canada, South Africa), and international cultivars (Sweden).

### Security of collections

Apple collections are primarily maintained in the field and are thereby threatened by environmental conditions, diseases and pathogens (Table 3). The extent of disease monitoring, spraying, and eradication varies among the surveyed collections, with most of the monitoring performed as visual inspections. In some cases, trees are removed and replaced if they are infected. For some critical accessions, targeted pruning or thermotherapy methods may be used to eradicate diseases that are identified. None of the field collections were maintained in a completely pathogen-free state; however, they were maintained free of quarantine diseases specific to each country.

Respondents gave details related to security of the collection in terms of primary threats, major disease and insect pests, and status of safety backup. Based on the survey responses, collections vary widely in the threats they face. Some collections, such as those of Greece, New Zealand, and Turkey, did not indicate any serious threats. Many collections stated that their most significant threat was a lack of stable funding, including those in Azerbaijan, Belgium, Canada, the Czech Republic, Israel, Italy, Lithuania, Spain, Sweden, and the United Kingdom.

**Table 3. Primary biotic threats for *Malus* production and conservation**

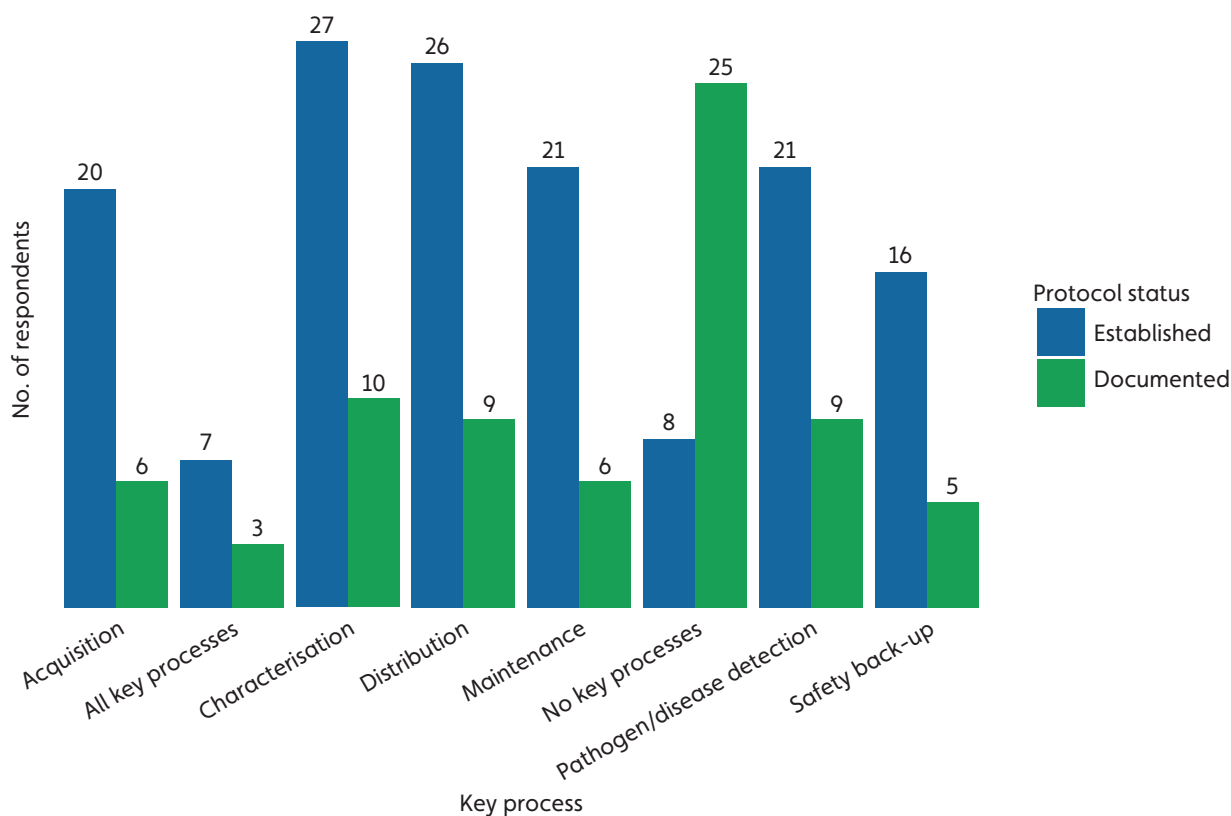
Disease or pathogen	Common name	Type
<i>Erwinia amylovora</i> (Burrill) Winslow et al.	Fire blight	Bacteria
<i>Pseudomonas syringae</i> pv. <i>syringae</i> van Hall	<i>Pseudomonas</i>	Bacteria
<i>Alternaria alternata</i> (Fr.) Keissl	Alternaria rot	Fungus
<i>Alternaria mali</i> Roberts	Alternaria blotch	Fungus
<i>Botryosphaeria dothidea</i> (Moug.) Ces. & De Not.	White rot	Fungus
<i>Botryosphaeria obtusa</i> (Schwein.) Shoemaker	Black rot (fruit)/frog eye leaf spot	Fungus
<i>Monilia fructigena</i> / <i>M. laxa</i>	Brown rot	Fungus
<i>Neonectria ditissima</i>	Apple canker	Fungus
<i>Penicillium expansum</i> Link <i>Penicillium</i> spp.	Blue mold	Fungus
<i>Pezicula malicorticis</i> / <i>Neofabraea malicorticis</i>	Apple anthracnose	Fungus
<i>Phyllosticta solitaria</i> Ellis & Everh.	Fruit blotch	Fungus
<i>Podosphaera leucotricha</i> (Ellis & Everh.) E.S. Salmon	Powdery mildew	Fungus
<i>Valsa mali</i>	Valsa canker	Fungus
<i>Venturia inaequalis</i> (Cooke) G. Wint.	Apple scab	Fungus
<i>Apria crataeli</i> L.	White butterflies	Insect
<i>Cydia pomonella</i> L.	Codling moth	Insect
<i>Dysaphis plantaginea</i> Passerini	Rosy apple aphid	Insect
<i>Eriosoma lanigerum</i> Hausmann	Woolly apple aphid	Insect
<i>Zeuzera pyrina</i> L.	Leopard moth	Insect
<i>Microtus</i>	Voles	Mammal
<i>Candidatus phytoplasma mali</i>	Apple proliferation	Phytoplasma
Apple chlorotic leafspot virus	ACLSV	Virus
Apple stem grooving virus	ASGV	Virus
Apple stem pitting virus	ASPV	Virus

Diseases and insect pests (Brazil, Canada, China, the Czech Republic, Germany, Israel, Italy, Latvia, the Netherlands, Spain, Switzerland, and the USA) as well as climate change (Azerbaijan, Belarus, Hungary, Lithuania, and the Netherlands) are also significant concerns.

According to the survey, apple collections define “backing up” in different ways. In some cases, an accession is considered backed up because multiple trees are available within a single orchard or planting. Duplicate plantings at a secondary site (or availability of the same materials through an alternative source) increases collection security. Some collections have at least a portion of the collection backed up either *in vitro* or through the use of cryopreservation. Only a few of the surveyed collections were completely backed up at a secondary site. Overall, most collections do not have the resources to devote to securely backing up their collections in multiple sites or in complementary methods. Of the 38 collections that responded, 26 (61%) had at least a portion of the collection duplicated at a second site, *in vitro*, or in

cryo. Of the 22 collections with a partial backup, 17 had a portion of the collection conserved at a secondary site, two were partially conserved *in vitro* (the Czech Republic and Egypt), and four were partially conserved in cryo (Germany, India, the United Kingdom, and the USA).

Ensuring long-term conservation and availability of a collection is the product of mitigating risks to the collections with consistent, high standards for routine operations. In order to manage quality of operations, there is a need to have clear, well-documented protocols for key processes that are up to date, transparent, and continuously monitored. Thus, the survey asked about the status for specific key processes in terms of protocol development and documentation. More than 20 of the collections that responded to the survey have established protocols for acquisition, characterization, maintenance, pathogen/disease detection, and distribution (Fig. 4). Eight respondents had not developed formal protocols for any of the key processes, while 25 respondents had no formal written documentation for any protocols. Only the Genetic



**Figure 4. The number of respondents who indicated that a key process had a protocol formally established and/or documented.**

Resources Institute in Azerbaijan, the Institute for Breeding Research on Fruit Crops in Germany, and the Vavilov Institute in Russia have developed and documented all key processes.

Another risk for genebanks that needs to be managed is the inadequate and often fluctuating annual budgets that affect genebank operations, including staffing. Survey participants were asked about the adequacy of staffing. Only 13 of the collections indicated they were able to adequately retain qualified and skilled staff at their genebanks. The main concerns given for staffing were: 1) inadequate level of staffing; 2) negative impact of upcoming retirements; 3) poor retention of trained staff due to inadequate wages; 4) competition from other projects of institutes; and 5) the short-term nature of the contracts, given the total reliance on project funds.

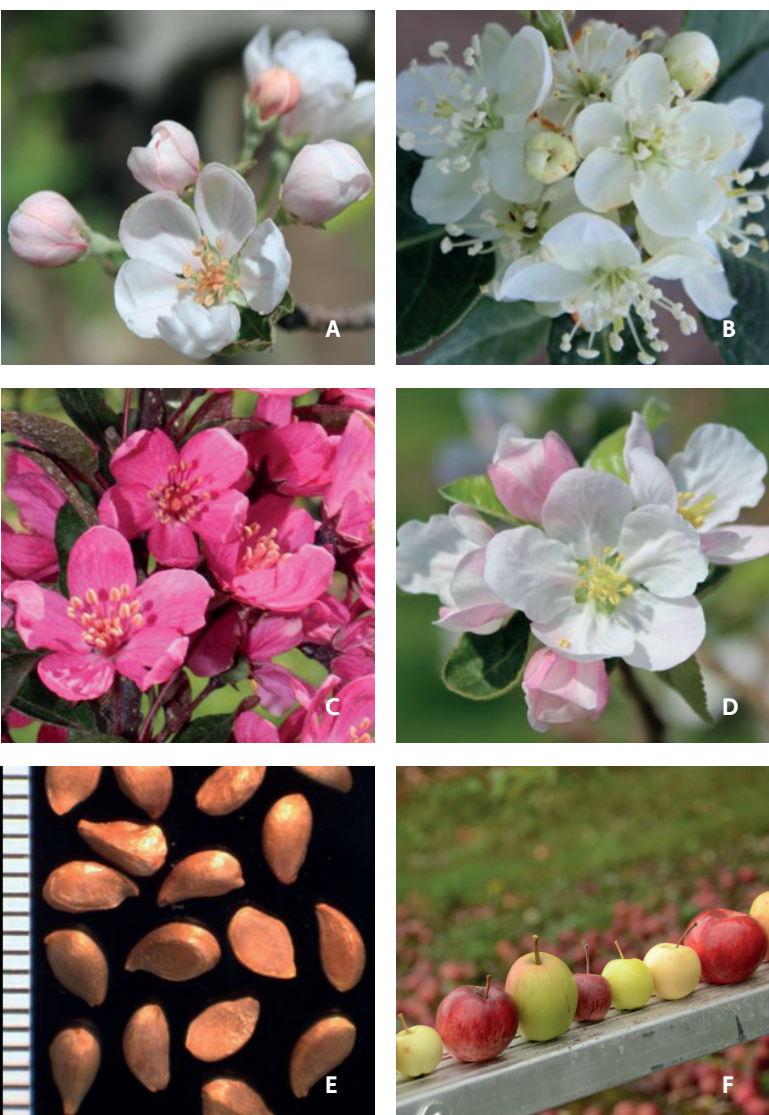
The survey respondents were asked about the adequacy of the annual funds for the conservation of the collection. Overall, 1 institute had an increasing budget, 17 had a stable but not necessarily adequate budget, 15 had a decreasing budget, while 6 institutions had no budget allocated for conservation at all. The institutes listed a number of ways in which they were working to ensure a stable, adequate budget: 1) including collection costs in other projects; 2) rationalizing the collection

to reduce costs; 3) increasing sales of cuttings and trees to producers; 4) increasing research on the collection; and 5) increasing public awareness of the need for conservation of apple genetic resources.

### Accession-level documentation

The survey respondents were asked about the accession-level information they manage, who could access it, and how it could be accessed. Only three institutions had no accession-level data. Fourteen had only internal databases but some of these indicated data were available upon request from the curator or that they had plans to make it available on their website. Over half of the institutes had accession-level information available online through their own database, through Genesys, or both. Many of the websites were in the local language, but ten institutes had data available in English also. It is clear that data availability may limit future collection comparison efforts if global sharing is not encouraged, especially through global platforms such as EURISCO or Genesys.

More than half of the apple collections that responded to the survey had accession-level data for passport (26) and phenotype (23) from characterization and/or evaluation work. Eighteen institutes had both passport and phenotype data. Nineteen institutes had taxonomy and genotype



**Phenotypic characterization for (A-D) flower color, (E) seed size, and (F) apple size.**

Images courtesy of Gayle Volk.

data but only 16 had images. Nine institutes had all the data types documented and three had all but taxonomy or images. Some of these institutes indicated that a portion of their data was internal but could be shared upon request.

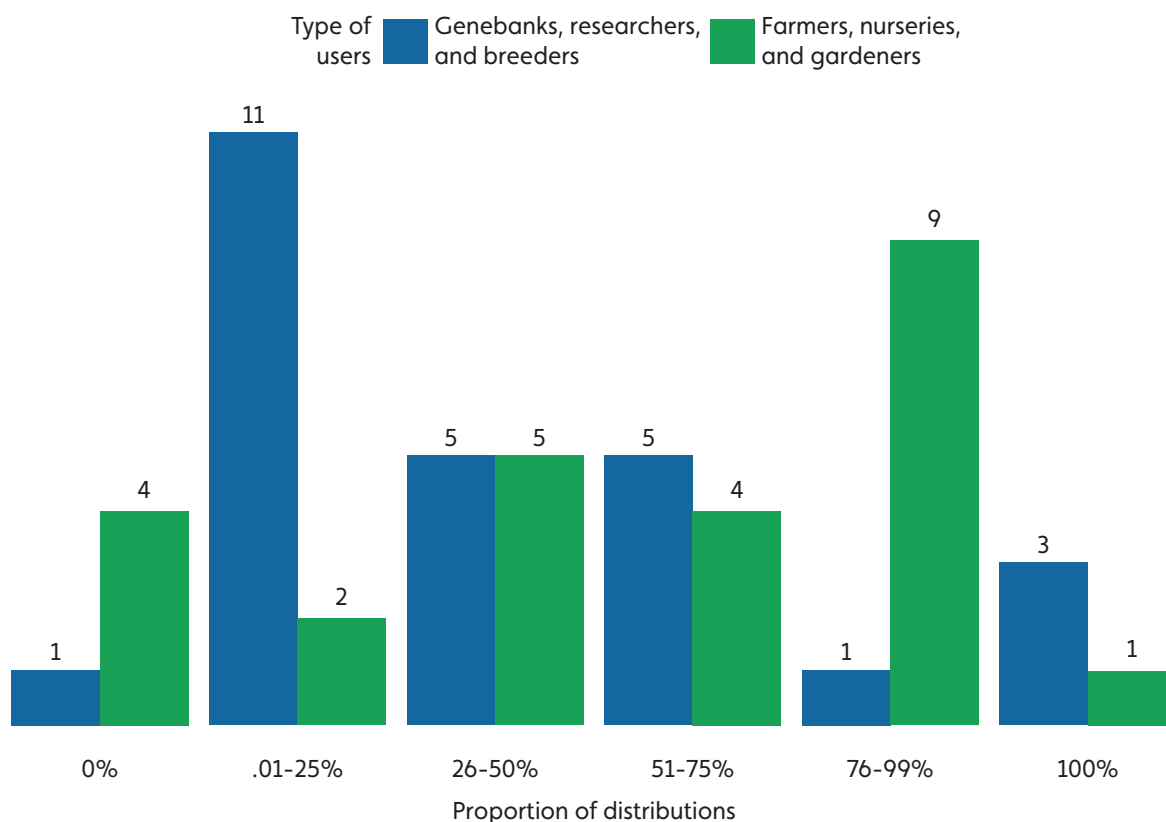
Phenotypic characterization and evaluation of collections for traits needed for improved collection maintenance (such as taxonomic classification) and for the user community (such as fruit traits, tree architecture, or pathology) varies considerably across collections. The standards used for data collection also vary, and include such methods as those described by GRIN-Global (USDA, 2016), the former International Plant Genetic Resources Institute (FAO/IPGRI, 2001), FruitBreedomics (Attanasio et al., 2015) and individual countries such as Russia (Vavilov Institute, 1989) and China (Wang et al., 2005; Lateur and Populer, 1994).

Twenty-four of the respondents indicated that all or part of their accessions have been genotyped, mainly using SSR markers, such as the ECPGR SSR set used by four institutes or the array used in the FruitBreedomics project, as well as with specific linked markers. While significant efforts have been made, or are planned, better standardization of future phenotyping, genotyping, and imaging efforts across all apple collections would facilitate collection management, comparison, and use.

## Distribution

Distribution of accessions to users is an important function of genebanks, but distribution faces many constraints. These constraints include institutional and national access policies, germplasm health regulations, and the cost of sending plant material. Thirty-four of the respondents currently distribute accessions in some form to others. Six collections did not distribute materials; one respondent could not distribute because of fire blight; and three indicated they could distribute accessions but were not doing so currently. The collections that distributed the largest numbers of material annually were USDA-ARS (6,000), Irish Seed Savers (4,000), China (600), and Belgium (6,000, primarily budwood to nurseries). Twelve of the collections only met within-country requests while 21 distributed materials in response to both domestic and international requests. Of the collections that have both national and international distributions, the proportion of the total number of samples sent annually to international users varied from 5% to 30%, with an average of 7%. In summary, amongst the survey respondents, there is a low level of international exchange of apple germplasm.

According to the survey, six of the respondents used only the SMTA and 24 used only a bilateral material transfer agreement, while five institutes used both. Some collections have limitations on access (particularly for cultivars that are under plant variety protection), but most accessions are available for research purposes. Distributions are affected by the quality of budwood available, such as the age of tree, pruning status, and disease status. They may also be affected by the ability of a recipient to accept materials. In some cases, genebanks cannot guarantee that propagules are free of quarantine pathogens, as may be necessary to obtain phytosanitary certificates and import permits to ship outside of their country or region. Furthermore, national and regional quarantine and plant protection organizations may limit the quantity and type of imported plant materials. If most genebanks are primarily serving residents of their own countries, this is likely due to strict



**Figure 5. Number of institutes making the given proportion of their distributions to research users (genebanks, researchers, and breeders) and producer users (farmers, nurseries, and gardeners).**

quarantine requirements that limit the international movement of apple germplasm.

Distributions are costly. The costs include maintaining and extracting disease- or insect-free planting material; obtaining the required phytosanitary permits; and the cost of the actual shipping. Seventeen of the respondents indicated that their institute charged recipients for distributions. The costs to recipients vary. Some collections charge to reimburse the cost of budwood collection, permits, and shipping, while others only charge for shipment.

The respondents of the survey distributed samples to a range of different users that fall into two main categories (Fig. 5). The first category is “other genebanks, researchers, and breeders” (researchers). The second category is “farmers, nurseries, and gardeners” (producers). Overall, slightly more than half of the samples distributed from respondent institutes are to producers for direct use. However, institutes vary widely. Eleven institutes distribute a very low proportion (0-25%) of their material to research users, but only six institutes had a very low proportion of their distributions going to producers. Fifteen institutes distributed more than 50% of their samples to producers, while only nine institutes distributed the vast majority to researchers.

The importance of both research use and direct use of accessions for production has resulted in a diversity of types of plant material distributed by institutions. Four main types of distributions were made: 1) vegetative replica of the trees in the field genebank, specifically dormant buds, softwood cuttings, and rooted trees; 2) recombinants, either open- or control-pollinated, as seeds; 3) pollen for use as male parent in breeding; and 4) tissues or DNA for research purposes. Dormant buds were most frequently distributed. Overall, 31 of the 33 collections that responded to the question distributed dormant buds, and half of these (16) distributed only dormant buds. Softwood cuttings and pollen were distributed by about 50% of the institutes, while all the other forms were distributed by less than one-third of the institutes. All institutes distributed at least one form of vegetative propagule, and fewer than 50% distributed pollen or seeds.

### Summary of the size and composition of apple collections

Forty institutions responded to the survey, and overall, they conserve about 31,000 accessions, with nearly 70% being international or local cultivars. Twenty-three of the genebanks conserved at least one accession of a wild *Malus* species. Five of the respondents indicate that they are involved in *in situ* conservation efforts in their country.

The respondents gave the highest priority to the *ex situ* conservation of unique local cultivars, but priority was also given by some to international cultivars, breeding lines, and wild species.

Overall, the respondents indicated that collections are not securely conserved for the long term, with field collections not adequately safely duplicated. The respondents identified a number of key biotic and abiotic threats to their collections. These were managed to some degree but with a focus on pests and diseases. There was a risk to conservation due to poorly established and documented protocols for key processes, insufficient staffing, and inadequate annual budgets. In fact, 21 of the respondents indicated that they are facing a reduction in funding or they had no funds allocated for conservation.

Most of the institutions indicated that while they had accession-level documentation, there were inadequacies in the types of data available, the form of the storage of that data, and the public sharing of their data. There is germplasm exchange with researchers, breeders, and producers, but there are constraints to global sharing of accessions and the associated information due to policies, phytosanitary issues, and budget shortfalls.

In conclusion, the current "system" for *ex situ* conservation of apple genetic resources includes a large number of collections that are held by a wide range of governmental organizations, universities,

private companies, botanical gardens, foundations, public parks, and individuals. They share a commitment to the conservation and use of apple genetic resources but face many constraints. Most collections are fairly isolated from other collections, although some are linked together in national or regional programs, especially in Europe.

## Consultations with genebank curators and users

The Global Strategy for the Conservation and Use of Apple Genetic Resources is the outcome of a number of consultations with genebank curators and key users. Three meetings were held with experts from the apple community. An initial discussion was held as an Expert Consultation on the use of apple wild relatives for pre-breeding in Trento, Italy on 1 October 2012. The participants had some key concerns in relation to apple genetic resource conservation and use. These included:

- ▶ Lack of an international network to facilitate sharing and exchange.
- ▶ Little global-level information available on accessions conserved in collections and their availability for distribution.
- ▶ No globally-integrated database for accession-level information.
- ▶ The lack of high-quality passport data that could be shared on crop wild relatives.



Participants in an expert group meeting in Bologna in 2014. Image by Paula Bramel.

- ▶ No standardized characterization and phenotypic evaluation data on accessions.
- ▶ No international core collection identified.
- ▶ Restrictions on access for certain countries in collection of wild relatives.

A meeting was then held in Seattle, Washington, USA on 23 June 2014, prior to the 7th International Rosaceae Genomics Conference. The objective was to discuss the global strategy development process and to obtain input on a proposed survey tool. The global strategy was introduced; there were presentations from some key apple genebanks about their collections; and then a discussion was held to determine needs for the development of a strategy. Input was received on the survey tool and this was incorporated into its development.

Finally, the initial survey results were shared with an expert group at a workshop held in Bologna, Italy on 14 June 2015 (Volk and Bramel, 2017). There were 11 participants in the workshop, representing both genebanks and users. The workshop focused on the implications of the survey for the identification of the main elements of a global conservation system for apple.

## Summary of apple genebank visits

Site visits were made to Plant & Food Research, Havelock North, New Zealand; USDA-ARS Plant Genetic Resources Research, Geneva, New York, USA; Agroscope, Wädenswil, Switzerland; the Agrifood Research and Technology Centre of Aragón (CITA de Aragón), Zaragoza, Spain; the INRA Research Institute of Horticulture and Seeds, Beaucouze, France; the Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium; the Research and Breeding Institute of Pomology, Holovousy, Ltd., Horice, Czech Republic; the Julius Kühn Institute, Institute for Breeding Research on Fruit Crops, Dresden/Pillnitz, Germany; the National Apple Repository, Xincheng, China; and an *in situ* site for *M. sieversii* in Almaty, Kazakhstan. It was clear from the survey and these visits that there is limited or no ongoing international collaboration on conservation, conservation research, documentation, or phenotyping. However, there are some collaborations with respect to genotyping, cryopreservation (at least in Europe, North America, New Zealand, and China), and plant explorations. There is a limited extent of collaboration on enhanced use, except through genomics.

There are collection holders at national, provincial, and local levels that have limited opportunities for cooperation or interaction. Collection holder linkages vary from a centralized network within countries to decentralized networks of regionally



Marc Lateur and Paula Bramel at the Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium.

or locally based conservers who may have some interactions with national or NGO institutions. In many cases, the national genebank serves as a primary collection that helps link others together within a country.

There are policy constraints that limit or restrict the use of the SMTA in some locations. Some of this is due to the private nature of some collections, which are not considered to be part of the Multilateral System of the ITPGRFA. Some key countries currently have a policy of no external distributions. Phytosanitary regulations also form a significant barrier for germplasm exchange. These barriers include strict export requirements as well as onerous, costly processes for import and clearance of quarantine in country. In some cases, seeds or virus-free stocks may ease import and export constraints. Another phytosanitary challenge for field collections is the limited timescale and options posed when there is a need for an urgent national-level response to new threats, such as any regulation that requires immediate removal of diseased trees or fields that threaten a whole



field site, such as that seen in France and Germany for fire blight. Different approaches for safety duplication or management of the orchard site are being utilized to overcome this risk, but this can significantly increase the cost of maintaining collections. For example, the increased effort required in China for routine management of Valsa canker in the field collections has significant long-term costs for securing conservation.

Only a few institutes that maintain collections have dedicated funds that cover the full or partial cost of routine operations. In many cases, there were some institutional funds from research or commercial activities, but often the conservation costs are covered by research or breeding programs with short-term projects. These efforts are often dependent on the commitment of individual scientists managing the collections. Even if institutions do have annual funds allocated for maintenance of the collection, they often do not meet the increased needs of salaries, maintenance, and supplies. Overall, there is a need to better understand and detail the annual costs required for secure, efficient long-term conservation. This better understanding can be used to advocate for greater annual support for routine operations.

The genebank visits support the description of the current global system as not rational, cost-effective, sustainable, or secure, although there are some interesting ongoing efforts to address these inadequacies for the long term at the national level. One of the biggest challenges for the collections that were visited is the need for conservation of a large number of local or national cultivars. Global sharing of the experiences of some of the key collection holders to address these challenges at the national level may reveal some new partnership

opportunities to include smaller, local collections that focus on these heritage materials. In some national collections, particularly in China where there is an abundance of crop wild relatives, there is a need to fill gaps in the collection of wild species and to encourage protection for the key collection sites. Local and international expertise is needed to ensure these protected *in situ* conservation sites are properly designed and managed.

The genebank visits revealed some key components of a global conservation system. There is a need for greater opportunities for nationally based collection holders to develop international-level links and collaborations on conservation, rationalization, cultivar identity, accession-level documentation, enhanced use, conservation research, policy, and advocacy to increase critical mass, raise awareness, and increase resources allocated for conservation. National-level collaborations would benefit with more cooperation for the conservation of local or national cultivars, including policy, rationalization, formal agreements, resource allocations, agreed-upon standards, and monitoring systems. There is also a need for greater engagement with local conservers and users that could secure accession on farm, in orchards, or in public places. The conservation of wild species needs greater collaborations between national, provincial, and local *ex situ* and *in situ* conservation efforts.

## Securing long-term conservation and use of apple genetic resources

The current *ex situ* conservation system for apple includes public entities (national, provincial, state, and local), universities, botanical gardens, nurseries,

**Table 4. A summary of the conservers and types of accessions found in the current global conservation and use system for *Malus* genetic resources.**

Conservers	Types of accessions		
	International commercial cultivars	Local cultivars/ landraces	Wild species
National collections held by public genebanks	Yes	Some	Yes
Public- and private-sector apple breeding programs holding own collections	Yes	Some	Few
University and other research programs holding own collections	Yes	Some	Some
Botanical gardens	Some	Some	Some
NGOs with conservation or marketing objectives	Some	Yes	
Individual farmers, land holders, orchardists, and gardeners	Yes	Yes	
Forests and other protected areas		Some	Yes

private breeding companies, and private collections in many countries (Table 4). There are essentially three different types of apple genetic resources that need to be conserved for the long term:

- ▶ International cultivars (including rootstocks)
- ▶ Local cultivars/landraces
- ▶ Wild species

International cultivars have been previously defined as those that are (or used to be) produced on a large scale, that have been used in breeding programs as key or historical parents, or that have been used in genomics, physiology, and pathology research programs. Although multiple accessions of such cultivars are often found in collections throughout the world, they have not been systematically compared at a genetic level to ensure that identical genotypes are in fact being conserved with the same cultivar name. Duplicates could be identified based on cultivar names but should be confirmed with genetic profiling. With the challenges of moving apple genetic resources between countries and regions, due to quarantine considerations, it is reasonable that many genebanks would want to keep copies of important cultivars. Such redundancy in the global system could also be seen as an advantage for safety duplication to secure their conservation for the long term. A diverse set of international cultivars present in many collections could be used as part of core collections and as reference standards. They could also make high-quality, true-to-type cultivars available to local nurseries and producers.

Local cultivars and landraces are genotypes that have (or have had) economic, historical, cultural, and heritage value in particular localities, regions, or countries; show interesting or unique local traits; are adapted for specific high-quality local food products; and represent considerable diversity. Individually, these have a lower global profile and impact than international cultivars, but they could be useful in breeding programs as sources of both new traits and local adaptation. Some local cultivars have not been selected for commercial cultivation and have usually not been evaluated for wide-scale production, while others – especially those planted as standard trees in orchard meadows – have been selected for local commercial cultivation. They have been produced and heavily consumed locally but may have attributes that limit their wide-scale commercial value. In some cases, the cultivars were produced in important apple-growing areas in the past but there had been a considerable decline in production in the region that has left significant diversity abandoned in old orchards or in gardens.

Due to the longevity of apple trees, trees in many old (often abandoned) orchards remain available for decades or even centuries.

Many of these local collections are not securely conserved, since they are maintained by interested individuals with a strong personal interest or by groups who do not have a stable long-term funding base. Establishing networks between national genebanks and local collections may help document and secure these collections. Including local cultivars in a national *ex situ* field genebank is only one option of many that would contribute to securing long-term conservation. Networks of local collectors can be part of the national effort to conserve, characterize, evaluate, develop local economical uses for, and share responsibility for the safety duplication of local cultivars. This is being done in Belgium, France, Germany, and Switzerland as well as many other countries. There are a number of models being used for these networks but there is a need for the establishment of a positive public-private partnership, clear coordination, and standard protocols and agreements to clarify the rules of collaboration and to enhance the security and efficacy of the national system.

In apple, every genotype is unique and could be named as a cultivar. That does not mean, however, that every cultivar that has been named must be preserved for the long term in the national genebank. Identifying the highest priority materials on the basis of both genetic and phenotypic novelty would be a useful first step. Due to the prevalence of synonyms, it is particularly important to assess genetic relationships and trueness to type amongst local cultivars. Genetic diversity assessments utilizing a standard reference set of markers and a low-cost provider of the genotyping services would allow for genetic assessments to compare sets of local cultivars with those in national genebanks (Urrestarazu et al., 2016). Projects are currently ongoing to track putative accession denomination errors, synonyms, homonyms, and to compare apple genebank collections using both SSR and SNP marker systems (ECPGR, 2017; Howard et al., 2018). Phenotypic assessments help confirm synonyms and unique traits of local cultivars (Bühlmann et al., 2015).

Many wild species (or key populations of wild species) are under-represented in current collections. These gaps should be clearly identified and filled. Wild *Malus* species could be represented as exemplar accessions in grafted field plantings, as orchards of seedlings, as seeds, as pollen, or any combination of these. Field collections of wild species should also be maintained (to some degree) for immediate

use in breeding programs or for evaluation for desirable novel alleles and traits. Genetic diversity and phenotypic studies of trees *in situ* could be used to choose a restricted number of trees to represent each wild population. In some cases, *Malus* species diversity could be captured as seeds by making specific crosses (Volk et al., 2016) or by collecting seeds from wild populations. In addition, pollen could be collected and conserved for distribution and use. Apomixis is known in at least 21 *Malus* species. This offers an additional mechanism to conserve wild species without the risk of contamination during regeneration (Chao and Zhong, 2017). Seeds could then be stored in optimized cold storage conditions and a fraction of these made available. Seeds have the advantage of being more easily distributed across international borders.

### How should apple germplasm be conserved in the global system?

Cultivars are clonally propagated and not faithfully produced by seeds, so the main focus of apple conservation is clonal integrity. Field collections are recognized as the primary conservation approach and the most secure form of conservation. The maintenance of apple trees in the field does not, however, lend itself to a single-genebank, centralized approach for long-term conservation. Globally, the development of standard operating procedures based upon collectively agreed best practices for processes such as acquisition, characterization, maintenance, germplasm health, distribution, and safety backup would be a key activity for global collaboration. The implementation of these procedures across genebanks will guide collection managers in their management decisions, enhance the security of conservation of collections, and facilitate capacity development (Benson et al., 2011; FAO, 2014). Standardized genotyping among collections will help identify current duplications within and among collections. Herbarium vouchers and images of accessions also need to be much more systematically considered as part of collection management (*ex situ* as well as *in situ* conservation).

Is seed or pollen conservation an option? Yes, apple pollen and seeds can be conserved, often less expensively than trees, but the key unit for conservation of apple cultivars will continue to be the specific clonal genotype. Thus, it is not advisable to *only* store seeds or pollen from cultivated apples. For wild species, it is possible to store only seeds. Field collections of wild species representatives should also be maintained, to some degree, so that these can be readily used in breeding programs or for evaluation for desirable novel alleles and traits.

*In vitro* cultures could be used for international distribution, since they are easier to clean of bacteria and virus infections than apple scion wood. They are also easier to transport. However, due to cost and concerns about somaclonal variation, *in vitro* cultures in slow growth has not been systematically applied as a method to conserve apple genetic resources for the long term or to back up field collections.

Cryopreservation of dormant vegetative buds is a useful approach for safety duplication of individual collections but is not currently being applied on a systematic basis to securely conserve apple collections at a global level. Dormant bud cryopreservation will be particularly amenable to collections maintained in climatic conditions that allow the buds to achieve adequate levels of cold hardening. Shoot tip cryopreservation, on the other hand, could be performed using *in vitro* plants that originate from any climate. It could be very valuable to apply such techniques for virus-free nuclear stocks in order to maintain them free of new infections. Overall, cryopreservation is a space-efficient, low-maintenance, relatively inexpensive option that would, however, require some expertise, equipment, and start-up capital. It is being used to back up some field collections, such as by the USDA-ARS. The conserved germplasm is not easily accessible in the short term, given the process required for post-thaw recovery and grafting of dormant buds to produce flowering trees to use in a breeding program.

### What are the issues for access and distribution?

A significant impact of the global system would be to facilitate increased access to and use of apple genetic resources for the long term. International cultivars are important for breeding and commercial production, and increased access to these genetic resources could benefit local production economies, research, and breeding programs. Access to local cultivars and landraces may be of particular interest to better understand adaptive responses to the environment or pest/pathogen pressures, and for use for specific traits in breeding programs, such as robustness, hardiness, tolerance to biotic stresses, tolerance to high temperatures and humidity, and cider quality. Wild species provide genetic resources that are largely untapped.

There are many policy issues around the use of the SMTA for apple germplasm exchange that could be explored in a global context. Halewood et al. (2013) reviewed some of the fundamental issues around the implementation of the ITPGRFA. A few of these have relevance to germplasm exchange of *Malus* and have contributed to complexity in

access and benefit sharing for conserved genetic resources. For example, there is a need to increase the availability of non-publicly held genetic resources through greater voluntary inclusion of material into the multilateral system. There is also a need to distinguish between access for use in research, breeding, and commercialization to better facilitate germplasm exchange.

International distribution of *Malus* genetic resources is challenging, particularly for clonally propagated cultivars. Documentation of existing *in vitro* cultivar collections could be particularly valuable for distribution purposes. At some point, it may become possible to develop a pathogen-free set of apple genetic resources to facilitate global distribution.

### Are information systems adequate?

Efforts to compare national and regional collections of *Malus* genetic resources will be facilitated by the availability of accession-level information that includes the use of the appropriate set of descriptors as well as phenotypic and genetic data in standardized formats (Kellerhals et al., 2012; Schmitz et al., 2013; Wegrzyn et al., 2012; Rubenstein et al., 2006; Endresen and Knüpfper, 2012). Comparisons among existing descriptor lists would be the first step toward developing a single standardized global descriptor set (Evans et al., 2012; FAO/IPGRI, 2001; Iezzoni et al., 2010; Kellerhals et al., 2012; Lateur and Populer, 1994; Mratinić and Akšić, 2011). The comparison of descriptors would identify existing available data and provide methods for conversions amongst datasets.

There is a need to agree upon a set of standard passport data as well as descriptors for baseline phenotyping. The provenance data may vary depending upon the original source and form of the materials. Wild materials could be documented based on their taxonomy, original collection site (geographic coordinates), population, and inventory information, as well as any available genotypic data. Cultivars could be documented by name, pedigree, genotype, source, phenotype, etc. There is also a need to have taxonomic identities and cultivar identifications that have been verified (Evans et al., 2011).

Current efforts to coordinate data collection and use in the European community could serve as a nucleus for a global information system that might include sharing through a database. The adequacy and possible expansion of existing databases should be explored. Genesys (2017) already provides accession-level passport information about apple genetic resources. However, an apple information system will need to manage data at the tree level. An example of an effort to facilitate

sharing of information on trees and their location for apples can be seen in the tree registry of the website Orange Pippin (2019), which has been established to share information on apple varieties. FruitFinder (People's Trust for Endangered Species, 2019) and other efforts being made to develop online databases to share information on local rare or heritage varieties in orchards could be expanded as a source of information for the global system.

The development of a standard reference set of molecular markers (SSRs, SNPs, or another system agreed upon by the community) will allow for low-cost, consistent genotyping that is needed in wider application for the assessment and conservation of apple diversity. Whole-genome resequencing may also play a role (Duan et al., 2017). A number of studies have demonstrated the use of genotypic data and recommended identifying a standard format for collecting and sharing genotypic data to facilitate apple collection comparisons on a global scale (Evans et al., 2011; Lassois et al., 2016; Sehic et al., 2013; Nybom and Weising, 2010; Urrestarazu et al., 2016; Howard et al., 2018). Ideally, the data would also facilitate use of apple collections by the local user community. Curators could use the comparisons to identify and manage internal redundancy, mislabeling, or to initiate efforts for safety duplication. The genomic/genotypic data could be generated and made available by a single or an identified set of laboratories that could offer reasonably priced analysis.

Providing curators and researchers access to training opportunities with respect to regulations, pathology, standardized phenotyping, use and application of molecular data, cryopreservation, field management, databases, and other topics would build collaborations among participants, develop networks, and educate participants about new and standard technologies.

### Priority actions for the global *ex situ* conservation system for *Malus*

The current system is neither efficient, cost-effective, rational, nor secure. There are a number of key actions that are needed to facilitate the development of a global system that will ensure conservation and use for a very long time. For example, the long-term conservation of the international cultivars can be better secured by taking advantage of redundancies across genebanks. The long-term conservation of the wild species will be enhanced with greater links of *ex situ* seed conservation and *in situ* seed gardens for restoration. Local cultivars may be viewed as a national or local responsibility; however, the global system could facilitate the monitoring,

security backup, establishment of protocols for characterization and genotyping (for establishment of trueness to type), and information sharing to ensure their long-term conservation and use.

The most important first step is to facilitate linkages between the diversity of conservers to address the urgent challenges facing *Malus* genetic resources conservation. This would also allow for greater links with the wide diversity of users, who are increasingly involved in conservation efforts. Greater collaboration within the community will enable global actions to better secure the diversity, through comprehensive accession-level information systems, and greater cooperative actions. Thus, the highest-priority action for the global system is to establish a collaborative platform for securing apple diversity, a mechanism by which *ex situ* apple collections, *in situ* conservation efforts, and the user communities cooperate and collaborate to secure apple genetic resources, ensure long-term sustainability for conservation, and enhance use. The first step in implementing the global strategy would be to hold an international meeting on *Malus* genetic resources with all the key genebanks, international and national governmental bodies, some NGOs, and key engaged individuals and researchers. This meeting would be used to initiate a Global Apple Diversity Platform, with knowledge sharing and action planning for the full implementation of the global conservation strategy.

Participation in the Global Apple Diversity Platform would be through registration, either as a collection holder or as an individual with expertise or a desire to contribute to the conservation and use of apple diversity. The registration process for a collection would include providing basic accession-level information about the collection. Care would be taken to ensure that participation does not become onerous, but instead yields benefits that far exceed the time and energy necessary to be an active participant. Efforts will be made to operate in a range of languages to facilitate participation.

The management of the Global Apple Diversity Platform would include a secretariat with a coordinator, a scientist with expertise in apple/temperate fruit tree genetic resources, and a database manager, who would be overseen by a steering group. The secretariat could be housed at an international organization such as the Crop Trust, a national genebank, or an existing regional initiative such as ECPGR. A few key national genebanks should take a leadership role. The exact nature of the governance and management of the Global Apple Diversity Platform would be defined through stakeholder consultations.

The minimum resources required for the platform for the long term would need to include the cost for the secretariat, meetings of the steering group, and one global platform meeting at least every 2-3 years. Additional resources would be needed for database development and management, priority platform collective activities, addressing urgent threats to collections, and short-term projects related to priority actions. These have been identified from the background study, the survey of major collections, the strategy development workshop, and the key genebank visits. Some of the initial activities of the Global Apple Diversity Platform could be as follows:

- ▶ Build a global apple conservation and use community that is composed of a wide range of public, private, non-governmental, and individual actors involved in the apple value chain.
- ▶ Ensure access to an information platform with an international registry and database for *ex situ* and *in situ* apple collections, including tree- and accession-level phenotype, passport, and genotype information.
- ▶ Facilitate working groups that would be established to focus on key issues such as best practices for securing local cultivar conservation; best practices or common standard operating procedures, especially for acquisition, curation and distribution; or the development of complementary coordinated strategies for safety duplication, including the use of seed for long-term conservation.
- ▶ Monitor the status and vulnerability of apple genetic resources *ex situ* and *in situ*, especially urgent threats.
- ▶ Coordinate a global genotyping effort to initially assess and map diversity within and among accessions in collections, verify accession identity, confirm redundancies, identify mislabeled accessions, and identify unique accessions. The diversity assessment could also be used to identify key gaps in global collections that need to be secured or protected.
- ▶ Collate information about key local cultivars and verify identity or uniqueness.
- ▶ Develop regional or global core collections, or trait-specific subsets.
- ▶ Put in place a global communication and advocacy strategy for conservation and use.
- ▶ Share news and information within the community.
- ▶ Facilitate training opportunities to improve collection management.

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# ANNEX I: MALUS SPECIES AND HYBRIDS LISTED IN ONLINE CATALOGS AND COLLECTION INVENTORIES

Malus species or hybrid	Synonym, common name, or hybrid parentage (GBIF, 2019)	Number of accessions or occurrences			
		FAO WIEWS (FAO, 2017)	Genesys (2019)	BGCI PlantSearch (BGCI, 2016)	GBIF (2019)
<i>M. angustifolia</i> (Aiton) Michx.	Southern crab	65	60	26	1,512
<i>M. baccata</i> (L.) Borkh.	Siberian crab	214	101	278	2,902
<i>M. bhutanica</i> (W.W.Sm.) J.B. Phipps			52	3	186
<i>M. brevipes</i> Rehder		5	2	20	10
<i>M. caspiensis</i> Langenf.			2		
<i>M. cerasifera</i> Spach			9	5	10
<i>M. chitralensis</i> Vassilcz.					3
<i>M. coronaria</i> (L.) Mill.	Sweet crab		105	77	2,334
<i>M. crescimannoi</i> Raimondo			2	1	
<i>M. dasyphylla</i> Borkh.					57
<i>M. denticulata</i> Lavallo		3		3	5
<i>M. doumeri</i> (Bois.) A. Chev.			12	8	354
<i>M. eleyi</i> Hesse		1	1		
<i>M. florentina</i> (Zuccagni) C. K. Schneid.	Hawthorn-leaf crab	10	6	33	169
<i>M. floribunda</i> Siebold ex van Houtte	Japanese crab	36	23	139	375
<i>M. fusca</i> (Raf.) C.K. Schneid.	Oregon crab		235	54	2,148
<i>M. glaucescens</i> Rehd.	<i>M. coronaria</i>	2		4	19
<i>M. halliana</i> Koehne	Hall crab	31	18	58	254
<i>M. hislop</i>			2		
<i>M. hi-hopa</i>			2		
<i>M. hillieri</i>			2		
<i>M. honanensis</i> Rehder		8	6	7	75
<i>M. hupehensis</i> (Pamp.) Rehder	Chinese crab	230	210	110	1,473
<i>M. ibrido</i>		5	4		
<i>M. ioensis</i> Britton	Iowa crab	94	79	74	1,550
<i>M. kansuensis</i> (Batalin) C.K. Schneid.		70	64	37	413
<i>M. kirghisorum</i> Al.Fed. & Fed.	<i>M. sieversii</i> var <i>kirghisorum</i>	18		15	5
<i>M. komarovii</i> Rehder			1	2	109
<i>M. lancifolia</i> Rehder	<i>M. coronaria</i> var <i>lancifolia</i>	1		5	14
<i>M. leiocalyca</i> S.Z. Huang				2	21
<i>M. maerkangensis</i> M.H. Cheng & al.	<i>M. × xiaojinensis</i> M.H. Cheng & N.G.Jiang				5
<i>M. mandshurica</i> (Maxim.) Komarov	Manchurian crab	8	6	28	602
<i>M. muliensis</i> T.C. Ku					



<i>Malus</i> species or hybrid	Synonym, common name, or hybrid parentage (GBIF, 2019)	Number of accessions or occurrences			
		FAO WIEWS (FAO, 2017)	Genesys (2019)	BGCI PlantSearch (BGCI, 2016)	GBIF (2019)
<i>M. niedzwetskyana</i> Dieck	<i>M. pumila</i> var. <i>niedzwetzkyana</i> (Dieck) C.K. Schneid	1	21	24	30
<i>M. ombrophila</i> Hand.-Mazz.		7	6	1	241
<i>M. orientalis</i> A.N. Uglitzk.	<i>M. sylvestris</i> subsp. <i>orientalis</i> (A. Uglitzk.) Browicz	956	976	63	2,985
<i>M. orthocarpa</i> Lavallee		1	1	9	8
<i>M. pallasiana</i> Juz.	<i>M. baccata</i> (L.) Borkh.		1	13	41
<i>M. paradisiaca</i> (L.) Medik.	<i>M. pumila</i> Mill.	1	1		
<i>M. platycarpa</i> Rehd.	Bigfruit crab	14	10	17	49
<i>M. praecox</i> (Pall.) Borkh.	<i>M. sylvestris</i> subsp. <i>praecox</i> (Pall.) Soo	1		11	129
<i>M. prattii</i> (Hemsl.) C.K. Schneid.	Pratt apple		89	26	525
<i>M. prunifolia</i> (Willd.) Borkh.	Chinese crab	170	80	89	905
<i>M. pumila</i> Mill.	<i>M. domestica</i> Borkh.	4,986	238	194	115,956
<i>M. purpurea</i> (Barbier & Cie) Rehder	<i>M. × purpurea</i> (Barbier & Cie) Rehder	7	13	124	258
<i>M. robusta</i> Rehder	<i>M. × robusta</i> Rehder	33	21	79	91
<i>M. rockii</i> Rehder		3	1	26	312
<i>M. sachaliensis</i> Juzep	<i>M. mandshurica</i> (Maxim.) Komarov		4	7	12
<i>M. sargentii</i> (Rehder) Bean	Sargent's crab	40	28	161	167
<i>M. scheideckeri</i> (L.H. Bailey) Spath ex Zabel	<i>M. × scheideckeri</i> (L.H. Bailey) Spath ex Zabel	2	3	47	19
<i>M. sieboldii</i> Rehder	Toringa crab apple	287	3	103	4,543
<i>M. sieversii</i> (Ledeb.) M. Roem.		86	2,991	54	8,962
<i>M. sikkimensis</i> (Wenz.) Koehne		27	19	55	194
<i>M. soulardii</i> Britt.	Soulard crab or <i>M. × soulardii</i>	2	4	10	53
<i>M. spectabilis</i> Borkh.	Asiatic apple	19	17	67	316
<i>M. spontanea</i> Makino			9	4	27
<i>M. striata</i> hort. ex Juz.	unresolved				
<i>M. sylvestris</i> Mill.	European crab	3,934	285	110	71,462
<i>M. theifera</i> Rehd.	<i>M. hupehensis</i> (Pamp.) Rehder		1		21
<i>M. toringo</i> (Siebold) de Vriese & Tuinb.-Fl.	<i>M. sieboldii</i> subsp. <i>sieboldii</i> Rehder	352	147	68	2,230
<i>M. toringoides</i> Hughes	Cut-leaf crab	8	116	69	337
<i>M. transitoria</i> C.K. Schneid.		109	99	56	450
<i>M. trilobata</i> (Labill. Ex Poir.) C.K. Schneid	<i>Eriolobus tribatus</i> M. Roem.	8	3	28	64
<i>M. tschonoskii</i> (Maxim.) C.K. Schneid	<i>Macromeles tschonoskii</i> Koidz or Pillar apple	13	6	56	597
<i>M. turkmenorum</i> Juz. & Popov	<i>M. sieversii</i> var. <i>turkmenorum</i> (Juz. & Popov) Ponomar	2	2	2	3
<i>M. yunnanensis</i> (Franch.) C.K. Schneid.	<i>Macromeles yunnanensis</i> Koidz.	32	24	60	604
<i>M. zhaojiaoensis</i> N.G. Jiang		59	59	6	206
<i>M. zumi</i> (Matsum.) Rehder	<i>M. sieboldii</i> subsp. <i>sieboldii</i> Rehder	7	13	104	111
<i>M. × adstringens</i> Zabel	<i>M. baccata</i> × <i>M. pumila</i>	10	4	23	44

Malus species or hybrid	Synonym, common name, or hybrid parentage (GBIF, 2019)	Number of accessions or occurrences			
		FAO WIEWS (FAO, 2017)	Genesys (2019)	BGCI PlantSearch (BGCI, 2016)	GBIF (2019)
<i>M. × arnoldiana</i> (Rehder) Sarg. ex Rehder	<i>M. arnoldiana</i> Rehder		2	28	28
<i>M. × asiatica</i> Nakai	<i>M. asiatica</i> Nakai	22	22	16	305
<i>M. × astracanica</i> hort. ex Dum. Cours.		1	1	4	3
<i>M. × atrosanguinea</i> (Spath) C.K. Schneid.		3	2	25	27
<i>M. × dawsoniana</i> Rehder	<i>M. dawsoniana</i> Rehder	2	2	10	37
<i>M. × denticulata</i> Lavalley	Unresolved			3	5
<i>M. (×) domestica</i> Borkh.	<i>M. domestica</i> Borkh. or <i>M. pumila</i> Mill. or Apple	32,283	34,149	3,260	100,476
<i>M. × gloriosa</i> Lemoine	<i>M. × scheideckeri</i> × <i>M. pumila</i> or <i>Niedzwetzkyana</i>		3	8	7
<i>M. × hartwigii</i> Koehne	<i>M. baccata</i> × <i>M. halliana</i>		6	18	21
<i>M. × heterophylla</i> Sumner	<i>M. heterophylla</i> Sumn. or <i>M. sieversii</i> (Lebed.) Roem.			3	2
<i>M. × kaido</i> (Siebold) Dippel	<i>M. × micromalus</i> Makino				
<i>M. × lemoinei</i>					
<i>M. × magdeburgensis</i> Hartwig	<i>M. pumila</i> × <i>M. spectabilis</i>		3	8	8
<i>M. × micromalus</i> Makino	<i>M. micromalus</i> Makino	10	28	37	171
<i>M. × moerlandsii</i> Door.	<i>M. × purpurea</i> × <i>M. toringo</i>		3	26	44
<i>M. (×) platycarpa</i> Rehder	<i>M. platycarpa</i> Rehder or <i>M. domestica</i> × <i>M. coronaria</i>	14	2	18	49
<i>M. (×) purpurea</i> (Barbier) Rehder	Purple crab		2	124	258
<i>M. ringo</i> Siebold ex Carriere	<i>M. asiatica</i> Nakai		2		1
<i>M. (×) robusta</i> Rehder	Siberian crab ( <i>M. baccata</i> × <i>M. prunifolia</i> )		2	81	91
<i>M. × scheideckeri</i> (L.H. Bailey) Spath ex Zabel	<i>M. floribunda</i> × <i>M. prunifolia</i>	2			19
<i>M. (×) soulardii</i> (L.H. Bailey) Britton	Soulard crab ( <i>M. ioensis</i> × <i>M. pumila</i> )	2			53
<i>M. (×) sublobata</i> (Dippel) Rehder	<i>M. prunifolia</i> × <i>M. toringo</i>		5	11	25
<i>Malus</i> hybrids		503	375		
<i>Malus</i> species		6,307	1,269		
<b>Total accessions</b>		<b>51,129</b>	<b>42,200</b>	<b>6,435</b>	





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