

Ensuring the genetic diversity of apples

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1 Introduction: the importance of apples

Apples are a healthy and nutritious part of the human diet. They provide antioxidants, polyphenolics, vitamins and fibre. Consumption of apple products has been shown to be associated with a reduced risk of major diseases, including cancer, cardiovascular disease and asthma (Boyer, 2004; Hyson, 2011). Apple consumption may also improve human responses to Alzheimer's disease, cognitive decline due to ageing, diabetes, weight management and bone health (Hyson, 2011).

Apple cultivars have been propagated by grafting since the time of early Greek civilizations (Mudge, 2009). In fact, 'Herefordshire Pearmain' and 'White Winter Pearmain' are apple cultivars that are available today and that have been clonally propagated ever since they were described in the 1200s (Smith, 1971). Many of the early European apple cultivars were valued as fruit used in hard cider production (Leforestier, 2015; Luby, 2003). By the 1700s, apple cultivars that were sought for fresh consumption were widely grown (Luby, 2003). Some of the fresh consumption apple cultivars that are grown today have been around for over a century, including Cox's Orange Pippin (1825), McIntosh (1811), Red Delicious (1880) and Rome Beauty (1848) (Smith, 1971). Many of these older varieties are vulnerable to abiotic and biotic threats.

This chapter reviews the threats to apple production and the resulting need for new varieties of apple. It considers the advantages of using progenitor species of *Malus* × *domestica* in traditional breeding programmes, and then presents and addresses three challenges to ensuring the genetic diversity of apples: understanding *Malus* diversity,

capturing and maintaining this diversity, and ensuring that researchers and breeders have access to living apple accessions.

2 Threats to production and the need for new varieties

Apple orchards are particularly susceptible to threats as most apple-growing regions are dominated by the production of only a few cultivars (Hampson, 2003). These cultivars are propagated clonally by grafting, resulting in genetically identical orchard trees. This lack of genetic diversity in orchards makes entire orchards susceptible if new diseases or pathogens become threats to non-tolerant cultivars. The concern regarding orchards of identical cultivars is further magnified by the fact that many of the popular cultivars share a common ancestry. A study of worldwide genetic diversity among cultivars in apple breeding programmes revealed that 'Cox's Orange Pippin', 'Golden Delicious', 'Red Delicious', 'Jonathan' and 'McIntosh' are the most common parents of new cultivars (Noiton, 1996). The status effective numbers (the equivalent number of panmictic-mated, unrelated, non-inbred genotypes) were 8–10 for the mainstream cultivars and those with resistance to apple scab (*Venturia inaequalis*) (Noiton, 1996). Consequently, different cultivars may not exhibit a wide range of tolerance to threats, resulting in similar levels of susceptibility across orchards that are thought to be diverse. Orchards are long-term investments, with a relatively low turnover in cultivars or rootstocks over the decades (Volk, 2015a). The high cost of planting and relatively slow return on investment results in low orchard turnover and therefore a slow rate of adoption of new varieties that may provide resistance to biotic threats (Volk, 2015a).

Pathogens continue to threaten orchards. Some apple cultivars that carried a single gene resistance to a disease, such as apple scab, are now showing signs of susceptibility (Parisi, 2004). Through mutation and selection, the apple scab pathogen is overcoming the resistance in apple cultivars that carry the most common resistance gene, *Rvi6* (*Vf*), which was introgressed from *Malus floribunda* 821 (Crosby, 1992; Soriano, 2009). Durable cultivars with new forms of resistance, ideally in pyramided form, are needed so that orchards remain resistant to pathogens over time (Gessler, 1994; Patocchi, 2009; Soriano, 2009).

Apple orchards are also threatened by the effects of changing climate. Warm winters may not result in adequate chilling hours for budbreak in the spring (Petri, 2004). Alternatively, warm spring weather may cause trees to flower early, and spring frosts could easily damage the crop if they occur during bloom or when fruit has set on the trees (Eccel, 2009).

There is a need for new apple varieties that can resist pathogens and climatic threat. These new cultivars would ideally have pyramided forms of disease resistance, desirable fruit quality and storage characteristics, and minimal need for pesticide applications. In addition, these new cultivars would flower at times to avoid spring frost damage, and have foliar and fruit architectures that are amenable to high-density production and mechanical harvesting (Volk, 2015a).

3 Sources of genetic diversity

3.1 Wild apple species

Wild apple species offer genetic variation for desirable traits for improvement: disease and insect resistance, fruit quality, growth attributes and physiological characters (Table 1;

Table 1 Crop wild relatives (excluding progenitor species) of apple (*Malus*) that have been identified to potentially have pathogen resistance useful to breeding programs

Trait	Pathogen	<i>Malus</i> species source	References
Crown gall resistance	<i>Rhizobium radiobacter</i>	<i>Malus sieboldii</i>	Moriya, 2010
Fire blight resistance	<i>Erwinia amylovora</i> (Burill)	<i>M. x robusta</i> 5	Broggini, 2014; Giovanni, 2014; Peil, 2007
Powdery mildew resistance	<i>P. leucotricha</i> (Ell. Et Everh.) Salm.	<i>M. coronaria</i>	Sestras, 2011
Powdery mildew resistance	<i>P. leucotricha</i> (Ell. Et Everh.) Salm.	<i>M. niedzwetzkyana</i>	Sestras, 2011
Powdery mildew resistance	<i>P. leucotricha</i> (Ell. Et Everh.) Salm.	<i>M. robusta</i>	Dunemann, 2007; Markussen, 1995
Powdery mildew resistance	<i>P. leucotricha</i> (Ell. Et Everh.) Salm.	<i>M. zumi</i>	Sestras, 2011
Scab resistance	<i>Venturia inaequalis</i> (Cke.) Wint.	<i>M. atrosanguinea</i> 804	Crosby, 1992
Scab resistance	<i>V. inaequalis</i> (Cke.) Wint.	<i>M. baccata</i>	Erdin, 2006; Gygax, 2004; Kumar, 2012
Scab resistance	<i>V. inaequalis</i> (Cke.) Wint.	<i>M. coronaria</i>	Sestras, 2011
Scab resistance	<i>V. inaequalis</i> (Cke.) Wint.	<i>M. floribunda</i> 821	Belfanti, 2004; Benaouf, 2000; Crosby, 1992; Gessler, 2012
Scab resistance	<i>V. inaequalis</i> (Cke.) Wint.	<i>M. micromalus</i>	Crosby, 1992; Patocchi, 2005
Scab resistance	<i>V. inaequalis</i> (Cke.) Wint.	<i>M. niedzwetzkyana</i>	Sestras, 2011
Scab resistance	<i>V. inaequalis</i> (Cke.) Wint.	<i>M. pumila</i>	Crosby, 1992
Scab resistance	<i>V. inaequalis</i> (Cke.) Wint.	<i>M. zumi</i>	Sestras, 2011

Brozynska, 2015; Hokanson, 1997; Luby, 2001). Wild *Malus* species are native throughout the northern hemisphere in North America, Europe and Asia (Fig. 1). The species centre of diversity is believed to be China, where at least 31 species of *Malus* have been identified in the wild (Zhi-Qin, 1999). Most *Malus* species are considered to be temperate, with winter chilling requirements that must be satisfied for fruit bud formation. However, there are some wild *Malus* species (and cultivars) that are not winter hardy, including *M. doumeri* (Robinson, 2001).

Taxonomic sections of the genus *Malus* have been previously described based on phenotypic traits (Table 2; Phipps, 1990; Robinson, 2001; Yu, 1979). More recently, chloroplast-based sequence analyses have been performed to identify genetic relationships among *Malus* species. The phylogenies based on chloroplast sequence



Figure 1 Distribution of wild *Malus* species throughout the world.

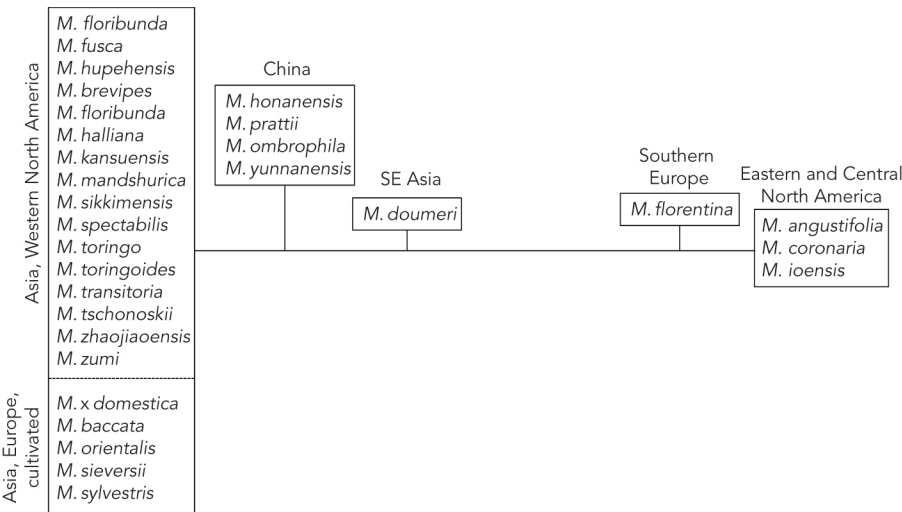


Figure 2 Diagram depicting relationship among clades of apple species, summarized from Volk (2015b).

did not align with the previously described *Malus* sections. The 47 chloroplast genome sequences compared by Nikiforova et al. (2013) identified five differentiated clades, with only the Chloromeles section aligning with the previous definition. Volk et al. (2015b) sequenced four regions of the chloroplast from 412 individuals representing 30 *Malus* species, and found that only three clades of species could be differentiated (Fig. 2; Table 2). Further assessments of the taxonomic relationships among *Malus* species will require the use of both chloroplast and nuclear data for multiple individual representatives per species (Volk, 2015b).

Table 2 Taxonomic sections or clades of *Malus* based on Robinson (2001), Nikiforova (2013) and Volk (2015)

Robinson, 2001	Nikiforova, 2013	Volk, 2015
Eumalus	A	A
<i>M. asiatica</i>	<i>M. asiatica</i>	<i>M. brevipes</i>
<i>M. baccata</i>	<i>M. baccata</i>	<i>M. baccata</i>
<i>M. floribunda</i>	<i>M. fusca</i>	<i>M. fusca</i>
<i>M. halliana</i>	<i>M. halliana</i>	<i>M. halliana</i>
<i>M. hupehensis</i>	<i>M. hupehensis</i>	<i>M. hupehensis</i>
<i>M. mandshurica</i>	<i>M. mandshurica</i>	<i>M. mandshurica</i>
<i>M. orientalis</i>	<i>M. orientalis</i>	<i>M. orientalis</i>
<i>M. sieversii</i>	<i>M. sieversii</i>	<i>M. sieversii</i>
<i>M. spectabilis</i>	<i>M. pumila</i>	<i>M. spectabilis</i>
<i>M. zhaojiaoensis</i>	<i>M. kirghisorum</i>	<i>M. zhaojiaoensis</i>
<i>M. prunifolia</i>		<i>M. prunifolia</i>
<i>M. micromalus</i>	B	<i>M. sikkimensis</i>
<i>M. sylvestris</i>	<i>M. sylvestris</i>	<i>M. sylvestris</i>
	<i>M. domestica</i>	<i>M. domestica</i>
		<i>M. toringo</i>
	C	<i>M. toringoides</i>
	<i>M. sargentii</i>	<i>M. tschonoskii</i>
	<i>M. prunifolia</i>	<i>M. zumi</i>
Sorbomalus	<i>M. floribunda</i>	<i>M. floribunda</i>
<i>M. transitoria</i>	<i>M. transitoria</i>	<i>M. transitoria</i>
<i>M. florentina</i>	<i>M. zhaojiaoensis</i>	
<i>M. fusca</i>	<i>M. sieboldii</i>	
<i>M. ombrophila</i>	<i>M. spectabilis</i>	
<i>M. sargentii</i>	<i>M. rockii</i>	
<i>M. sieboldii</i>	<i>M. sikkimensis</i>	
<i>M. toringo</i>		
<i>M. toringoides</i>	D	B
<i>M. kansuensis</i>	<i>M. kansuensis</i>	<i>M. kansuensis</i>
<i>M. honanensis</i>	<i>M. honanensis</i>	<i>M. honanensis</i>
<i>M. prattii</i>	<i>M. prattii</i>	<i>M. prattii</i>
<i>M. yunnanensis</i>	<i>M. yunnanensis</i>	<i>M. yunnanensis</i>
		<i>M. omprophila</i>
Chloromeles	E	C
<i>M. angustifolia</i>	<i>M. angustifolia</i>	<i>M. angustifolia</i>
<i>M. ioensis</i>	<i>M. ioensis</i>	<i>M. ioensis</i>
<i>M. coronaria</i>		<i>M. coronaria</i>
Eriolobus		
<i>M. trilobata</i>		
Docyniopsis		
<i>M. doumeri</i>		
<i>M. tschonoskii</i>		

Wild species may offer sources of resistance or desirable traits to overcome abiotic and biotic threats (Table 1; Hokanson, 1997; Luby, 2001). The challenge to using wild species in breeding programmes is that the wild species usually have undesirable fruit, non-uniform ripening times and other traits that are not amenable to commercial apple production. Desirable traits are difficult to introgress from wild species using traditional breeding methods because of the long juvenile phases and possible linkages between desirable and undesirable traits.

3.2 Progenitor species of *Malus × domestica*

Traditional breeding programmes may be able to make use of the progenitor species of *M. × domestica* more easily than more distant wild species of *Malus*. Given the closer relationship between the progenitor species and the cultivated apple, these species may be more easily introgressed into cultivars, thus possibly reducing the time to new cultivar development (Cornille, 2012). Progenitor species could be desirable in breeding programmes because they are usually diploid and often have larger and more desirable fruit characteristics than the more distantly related species. The primary progenitor species of *M. × domestica* are likely *M. sieversii*, *M. sylvestris*, *M. orientalis*, *M. baccata* and *M. prunifolia* (Fig. 3; Cornille, 2014; Volk, 2015a).

Malus × domestica was domesticated thousands of years ago. Progenitor species *M. sieversii* served as the source species. Other western species, including *M. orientalis* and *M. sylvestris*, were hybridized with *M. sieversii* as seedlings and were transported from the region of the Tien Shan Mountains in Central Asia through the Middle East and into Europe. Alternative domestication processes may have occurred as *M. sieversii* was transported across China, where it may have hybridized with *M. baccata* and *M. prunifolia* (Luby, 2003). 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, 2012, 2014).

Some accessions representing *M. × domestica* progenitor species exhibit desirable traits. For example, *M. baccata* species representatives have resistance to apple scab, *Botryosphaeria* canker (*Botryosphaeria* (Sawada) Shang), fire blight (*Erwinia amylovora* (Burrill) Winslow et al.) and powdery mildew (*Podosphaera leucotricha* (Ellis and Everh.) E.S. Salmon), in addition to cold hardiness and resistance to waterlogging (Dunemann, 2009; Hokanson, 2001; Le Roux, 2012; Luby, 2002; Wan, 2011; Yang, 2011; Zhi-Qin, 1999). Similarly, *M. sieversii* accessions have resistance to apple scab, *Botryosphaeria* canker,

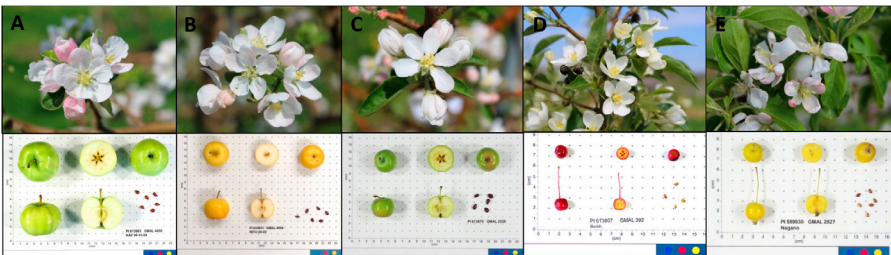


Figure 3 Images of fruits and flowers of *Malus* progenitor species: (A) *M. sieversii*, (B) *M. orientalis*, (C) *M. sylvestris*, (D) *M. baccata* and (E) *M. prunifolia*. Flower images provided by C.T. Chao.

fire blight, powdery mildew, woolly apple aphid (*Eriosoma lanigerum* Hausmann), light brown apple moth (*Epiphyas postvittana* Walker), apple leaf curling midge (*Dasyneura mali* Kieff.), Phytophthora, western white root rot (*Rosellinia nacatrix* Prill.), violet root rot (*Helicobasidium mompa* Tanaka), apple maggot (*Rhagoletis pomonella* Walsh), blue mould (*Penicillium expansum* link) and apple replant disease (Bus, 2005; Forsline, 2004; Hokanson, 2001; Isutsa, 2000; Kumar, 2010; Luby, 2001, 2002; Norelli, 2014; Zhi-Qin, 1999). *M. sieversii* also exhibits cold hardiness, late flowering, variation in water use efficiency, short juvenility, resistance to sunburn, some variants of fruit quality and drought tolerance (Bassett, 2011; Khan, 2014; Luby, 2001; Maguylo, 2014; Yan, 2008). Progenitor species *M. sylvestris* has resistance to blue mould (Jurick, 2011). Accessions of *M. orientalis* have been documented to have resistance to apple scab, cedar apple rust (*Gymnosporangium juniperi-virginianae* Schwein.), fire blight, sooty blotch (*Peltaster fruticola* Johnson, Sutton and Hodges, *Geastrum polystigmatum* Batista and M. A. Farr, *Leptodontium elatus* (F. Mangelot) de Hoog, *Gloeodes pomigena* (Schwein) Colby), and flyspeck (*Schizothyrium pomi* (Mont. and Fr.) Arx; Höfer, 2013; Volk, 2008). *M. orientalis* is also later blooming, adapted to a wide variety of habitats, and can be stored for extended lengths of time (Höfer, 2013). *M. prunifolia* has exhibited resistance to apple scab, Botryosphaeria canker and fire blight (Fischer, 1999; Hokanson, 2001; Le Roux, 2012; Wan, 2011; Zhi-Qin, 1999).

4 Understanding *Malus* diversity

Taxonomic relationships among *Malus* species have been difficult to discern. Molecular marker and sequence data have been used for species differentiation and to determine genomic relationships among *Malus* species (Forte, 2002; Nikiforova, 2013; Robinson, 2001; Velasco, 2010; Volk 2015b). Much less is known about the diversity of *Malus* species in the wild. Overall, species have a weak spatial genetic structure, likely due to the long-distance dispersal of pollen and seeds (Cornille, 2013b). Localized, regional structures among some subsets of populations have been identified (Richards, 2009b; Volk, 2008).

Assessments using microsatellite markers have shown that populations sampled over one thousand kilometres in Kazakhstan were significantly differentiated. Two primary clusters of individuals were identified based on the microsatellite markers. The southwestern *M. sieversii* collection sites were more admixed and diverse than the northern sites. There were higher levels of differentiation among half-sib seedlings than among sites, likely due to the self-incompatible, outcrossing nature of the species (Richards, 2009b). *Malus sieversii* from the Xinjiang Province of China exhibited significant differences among populations, with low levels of gene exchange (Zhang, 2007).

Malus orientalis populations in Turkey, Georgia, Armenia, and Southern Russia were genetically distinguishable and exhibited high levels of diversity (Volk, 2008). *M. orientalis* from Russia was more homogeneous, whereas the populations sampled from Turkey were highly diverse and exhibited disease resistance. Some pockets of novelty were identified, which could possibly be attributed to local hybridization with domesticated cultivars (Volk, 2008).

Wild *Malus sylvestris* is distributed throughout Europe in a manner that has been partially dictated by high-disturbance events, including winds, fire, and floods. Analyses of the population structure of Rhine Valley revealed, similar to other wild apple species, a weak

population structure, despite the clumped settings (Schnitzler, 2014). Further analyses of the population structure of *M. sylvestris* in Europe revealed that there were three differentiated populations in Europe: (1) a western population from France to Norway, (2) a northeastern population near the Carpathian Mountains, and (3) a southeastern population along the Balkan Peninsula (Cornille, 2013a). Genetic assessments of these populations suggest that *M. sylvestris* populations retreated to glacial refugia in the Iberian Peninsula and the Balkan region during the times of the ice ages (Cornille, 2013a).

Malus diversity can also be assessed at the cultivar level. Apple cultivars have been vegetatively propagated for centuries, and many of these ancient cultivars remain available as either local cultivars or within genebanks (Guarino, 2006; Pereira-Lorenzo, 2007; Urrestarazu, 2012). Cultivar diversity assessments within genebanks reveal genetic relationships (parentage, sports, synonyms, mislabelling) among cultivars (Gross, 2012; van Treuren, 2010). Due to the high level of allelic diversity in *Malus × domestica*, relatively few markers can be used to differentiate distinct apple cultivars (aside from sports), which makes for straightforward cultivar identification of the diploid cultivars (Foroni, 2012; Moriya, 2011). Collections with genetic fingerprints available also serve as standards for the identification of unknown cultivars (Routson, 2009).

Dozens of genebanks worldwide maintain *Malus* collections including both local cultivars and those with international economic significance. Regional genebank collaborations as well as those between individual genebanks have performed collection comparisons that have revealed novel local varieties (Gao, 2015; Patzak, 2012; Zhang, 2012). These local varieties may have novel alleles for key traits that are not commonly available in the cultivated genepools (Leforestier, 2015; Tartarini, 2004). A large-scale comparison among dessert apples in European genebanks identified strong historical processes of cultivar migration and selection as well as adaptive responses to diverse environments. These findings demonstrate the limited population structure, yet extensive variation among apple cultivars (Urrestarazu, 2016).

The past decade has brought about many advances in understanding the genetic diversity in *Malus*; however, there is much to be done. Diversity assessments have not been performed for many *Malus* species. In addition, correlations among unique habitat or environmental niches (or selection pressures) and genetic adaptations may reveal desirable new genes that could be used in breeding programmes. Access to phenotypic, genotypic and sequence data for individuals representing wild populations of *Malus* species will facilitate progress in this area. The use of genetic marker and sequence data to compare individuals within and between collections will greatly aid in the understanding of collection composition, overlaps and vulnerabilities. The use of novel local cultivars with desirable phenotypes in breeding programmes may generate new cultivars that make apple production more sustainable in the future.

5 Capturing, maintaining and accessing genetic diversity

5.1 Capturing and maintaining the diversity

Natural settings with wild apple trees are at risk of being lost due to the encroachment of humans and the presence of livestock (Forsline, 2003; Fowler, 2004; Williams, 2005). Forest

settings may be destroyed as habitations expand and lands are cleared for alternative uses (Forsline, 2003). Trees within boundaries of parks or forest preserves may have some level of protection. Obtaining access to protected areas may be a challenge.

The role of genebanks for species conservation has grown as the natural habitats of species continue to be threatened (Esquinas-Alcázar, 2005; Forsline, 2003). Representatives of most *Malus* wild species are available in national genebanks; however, in many cases, the diversity of wild species is poorly represented in *ex situ* genebanks (Vincent, 2013). Many national genebanks and arboreta include *Malus* species, crab apples or domesticated apples in their collections. These genebanks may be limited in size or scope because of the expense of maintaining collections of living trees. In some cases, collections may focus on ornamental crab apple collections, whereas others may focus on historic cultivars, crop wild relatives or species diversity. Vincent (2013) reported that 55% of the *M. × domestica* crop wild relatives are represented by fewer than 50 *ex situ* accessions.

The USDA-ARS National Plant Germplasm System (NPGS) maintains a large apple collection as part of the Plant Genetic Resources Unit in Geneva, New York. This collection currently has 3070 unique grafted trees in the field representing *M. × domestica* cultivars



Figure 4 USDA-ARS National Plant Germplasm System apple collection in Geneva, New York. (A) Grafted apple cultivar orchard and (B) own-rooted seedling orchard of *M. sieversii* trees.

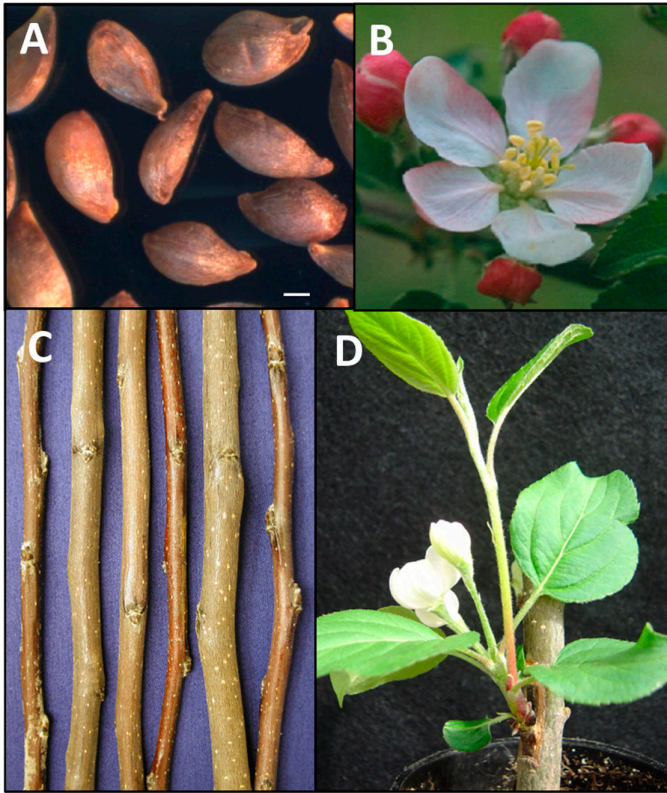


Figure 5 Images of *Malus × domestica*: (A) seeds, bar: 1 mm; (B) flower; (C) dormant budwood for use in cryopreservation and (D) grafted apple bud after cryopreservation.

and 33 *Malus* species, and 15 hybrid species. The collection also has an orchard that has been planted with own-root seedling trees grown from seeds of *Malus* species collected in the wild (Fig. 4). In addition, there is a planting of trees that are the progeny of crosses between ‘Gala’ and seven *Malus sieversii* accessions (GMAL 4335, GMAL 4448, GMAL 4331, GMAL 4333, GMAL 4327, GMAL 4455 and GMAL 4334; Volk, 2015a, Thomas Chao, pers. comm.). These populations segregate for key traits such as resistance to blue mould, fire blight, apple scab, oblique banded leaf roller (*Choristoneura rosaceana* (Harris)) and codling moth resistance as well as water use efficiency (Wisniewski, 2016). Quantitative trait loci and linked markers have been identified for blue mould resistance traits (Norelli, 2014).

The NPGS apple collection was established as part of a national network of clonal repositories in the 1980s (Postman, 2006). Budwood for grafting trees representing cultivars was originally donated from breeders, arboreta and other genebank collections. In addition, a series of collection trips were performed between 1989 and 2015 to acquire seeds from wild *Malus* species (Forsline, 2003; Volk, 2015a; Thomas Chao, pers. comm.). The NPGS apple collection is actively maintained in the field and propagules are distributed for research purposes. A seed collection, composed primarily of wild-collected seeds from plant explorations, is maintained at -18°C . In addition, 2052 of the trees in the

field collection are backed up as dormant buds at cryogenic temperatures as a long-term secure base collection at the National Laboratory for Genetic Resources Preservation in Fort Collins, Colorado (Fig. 5; Volk, 2016).

The apple collection is maintained using a complementary conservation approach that includes grafted trees for the permanent collection, seedling trees in evaluation orchards, seeds for conserving the diversity of wild species and a cryogenic back-up of the vegetatively propagated accessions (Volk, 2010b; Walters, 2008). 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 wild species in an efficient manner, and cryogenic storage of vegetatively propagated collections serves as a secure back-up if trees in the field are lost (Forsline, 1998; Towill, 2004; Volk, 2010b).

In the 1990s, a core collection of the field apple collection was proposed as a smaller subset of NPGS apple accessions that was believed to capture much of the diversity represented in the collection (Gross, 2013; Hokanson, 1998). Microsatellite markers have since been used to assess how well the original core collection captured the genetic diversity, and additional accessions were suggested for inclusion in the core collection based on these results (Gross, 2013). In addition, core collections have been proposed for *M. sieversii* and *M. orientalis* species collections within the NPGS (Richards, 2009a; Volk, 2005, 2009).

5.2 Access to the diversity

Genebank collections provide access to living apple accessions (in either tree or seed form) for use in taxonomy, physiology, molecular and evolutionary biology, horticulture and breeding programmes. Available accessions in the NPGS apple collection are distributed both within the United States and internationally using the appropriate permits (Volk, 2015a). Genebank accessions are known to be most valuable when standardized characterization and evaluation data are available (Rubenstein, 2006; Volk, 2010a). Genebank customers are most readily served by the use of descriptors that are relevant to their communities. They also benefit from collections that have materials whose taxonomic identities are correct and cultivar identifications have been verified (Evans, 2010). Descriptor sets that are selected to correctly identify wild species may be different than those that are used to evaluate materials for potential use in breeding programmes (Phipps, 1990; Schmitz, 2013; Yu, 1979).

Passport, phenotypic and molecular data for NPGS accessions are publicly available on the GRIN-Global database (<http://www.ars-grin.gov/npgs/gringlobal/webpages/publicwebsite.html>). Future database interoperability should provide seamless data access on *Malus* collections from genomic database portals (such as the Genome Database for Rosaceae, GDR). Efforts to compare national and regional collections of *Malus* genetic resources will be facilitated by the collection of phenotypic and genetic data in standardized formats (Kellerhals, 2012; Schmitz, 2013; Wegrzyn, 2012).

6 Future trends: new technologies to facilitate enhanced and sustainable crop production

Traditional apple breeding is a complex process that involves many years of crossing, field evaluations, multi-site testing and consumer trials to ensure that new varieties meet the expectations of both producers and consumers (Evans, 2013). The multiple generations of

modified backcrosses that were previously necessary to introgress genes of interest from a wild species into a desirable background made it an arduous process to use wild species in breeding programmes.

New technologies that make use of marker-assisted selection, genomic selection, genetic engineering, genome-wide association mapping, high-throughput genotyping and/or rapid-cycling plants are paving the way towards the increased use of wild *Malus* species in apple breeding (Bianco, 2014; Broggini, 2014; Evans, 2013; Flachowsky, 2011; Kumar, 2012a,b, 2013; Troggio, 2012), thus facilitating the movement of desirable alleles from *Malus* species into the cultivated apple and reducing deleterious effects on fruit quality, quantity, and production traits (Kumar, 2010). These techniques become feasible as markers linked to key traits, trait-associated breeding values and quantitative traits become better understood (Gardner, 2014; Kouassi, 2009; Longhi, 2013; Troggio, 2012). The process of identifying allelic variation in *Malus* wild species will be aided by an improved understanding of the diversity available within wild species and the characterization of the key genes that influence desirable traits.

Technological advances in the area of whole-genome sequencing will soon enable the genomic sequencing of entire apple collections (Velasco, 2010). This will allow users to determine the genetic relationships among accessions within apple genebanks. The use of fine resolution genomic data in breeding programmes focused on wild species will be dependent upon access to high-quality phenotypic evaluation data that allows users to link desirable traits to their genetic control (Volk, 2015a). New genomic assessment tools that easily accommodate polyploid accessions will also facilitate the analyses of polyploid cultivars and species (Bianco, 2014).

Access to wild *Malus* species will be critical for advancements in future plant breeding programmes. Wild *Malus* species are most readily available to the breeding community from genebanks. Many species are, however, poorly represented in genebank collections, and phytosanitary restrictions and treaty agreements may limit access to wild materials, particularly at the international level (Bretting, 2007; Varaprasad, 2010). Ongoing coordinated efforts among genebanks to compare collections at the phenotypic and genetic levels as well as confirm cultivar identities will facilitate the use of genebank collections in the future (Kellerhals, 2012; Volk, 2011). These efforts will enable future researchers specializing in apple improvement to provide consumers with higher quality fruit that can be produced more sustainably.

7 Where to look for further information

There are many challenges in conserving and utilizing apple genetic resources. With limited funding and high costs associated with genebanks and breeding programmes, researchers must work together to capitalize on their strengths. Key findings and results relating to conservation and breeding are shared at many conferences including the Rosaceae Genomics Conference and the Eucarpia Plant Breeding Symposium, in addition to national and international horticultural and genomics conferences and topic-specific symposia.

The Rosaceae Genomics Conference is organized by a committee that meets in the form of the Rosaceae International Genomics Initiative (RosIGI; <https://www.rosaceae.org/community/rosgi>). In the United States, the U.S. Rosaceae Genomics, Genetics and Breeding

Executive Committee (RosExec; https://www.rosaceae.org/community/us_rosexec), is comprised of scientists specializing in Rosaceous crops who represent academia, government and industry programmes. This group meets quarterly to serve as a communication and coordination focal point in for Rosaceae genomics, genetics and breeding in the United States. In Europe, the *Malus/Pyrus* European Cooperative Programme for Plant Genetic Resources (<http://www.ecpgr.cgiar.org/working-groups/maluspyrus/>) has working groups with meetings to ensure long-term conservation and facilitate the utilization of plant genetic resources in Europe. In the United States, the Apple Crop Germplasm Committee (CGC; <http://www.ars-grin.gov/npgs/cgclist.html#Apple>) is comprised of NPGS users who provide collection management recommendations to the NPGS apple collection curator. Apple CGC members published an apple crop vulnerability report in 2015 that summarizes the status of apple production and conservation in the United States (Volk, 2015a). Rosaceae passport, taxonomic, genomic, genetic and phenotypic data are available in English in multiple databases, including, but not limited to, the GDR (Jung, 2014), GRIN-Global, NCBI (<http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi>), GBIF (<http://www.gbif.org/>), Brogdale National Fruit Collection Database (<http://www.nationalfruitcollection.org.uk/index.php>) and Genesys (<https://www.genesys-pgr.org/welcome>).

8 References

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