Chapter 10
Genebank Conservation of Germplasm Collected from Wild Species

Christina Walters, Christopher M. Richards, and Gayle M. Volk

Abstract Crop genebanks are tasked with maintaining genetic resources that support agriculture. They must keep a diverse array of samples alive for decades to centuries. Controlled conditions within the genebank are necessary to maintain quality and ensure consistency of the sample through time. Challenges for providing quality and consistency increase with samples that are mostly unstudied and highly heterogeneous and respond in unpredicted ways, as is the case for samples collected from the wild. The task of genebanking will be facilitated by better definitions of the "conservation target," meaning the level of diversity that the sample is intended to represent. With that definition, collectors will have better knowledge of what and where to collect – and when to stop – and "fit-for-purpose" samples will be preserved. Major uncertainties persist about the life expectancy of the sample and whether genebanking imposes genetic shifts. Standards have been recommended by the international community to ensure lasting quality of samples despite a large number of unknowns. We believe some of these standards will be counter-productive or unobtainable for wild-collected samples, and we have offered alternatives that stress documentation so future genebank users can predict whether a sample will suit their needs.

Keywords Conservation target · Cryopreservation · Germplasm · Longevity · Preservation · Propagule · Sampling · Storage · Ex situ conservation · Genebank

10.1 The Challenge of Ex Situ Collections: Maintaining Wildness in Captivity

North America's rich flora has the potential to contribute genes to make our crops more resilient to disease, pests, and weather extremes. The unique features of many North American taxa also offer the opportunity to find sources for new products and

---

C. Walters (✉) · C. M. Richards · G. M. Volk
USDA, Agricultural Research Service, Center for Agricultural Resources Research, National Laboratory for Genetic Resource Preservation, Fort Collins, CO, USA
e-mail: christina.walters@ars.usda.gov

© This is a U.S. government work and not under copyright protection in the U.S.; foreign copyright protection may apply 2018

S. L. Greene et al. (eds.), *North American Crop Wild Relatives, Volume 1*,
https://doi.org/10.1007/978-3-319-95101-0_10

better nutrition or healthier, more sustainable landscapes. Many of the plants described in this book are congeneric with plants commonly used in agriculture. For these, breeders will select the genes of interest from the wild relative and assimilate them into modern cultivars, leaving behind undesirable genes that contribute to a weedy phenotype. The breeder's job is facilitated by low barriers to interspecific hybridization incumbent with wild species that are closely related to domesticated species (i.e., crop wild relatives, CWR). Similar concepts apply to other wild species that may offer new products or have greater resilience in a changing landscape (Urban, 2015). In these cases, modern-day domestication efforts may be invoked and provide an important reminder of the reservoir of services that wild plants provide, bringing new opportunities for economic growth, ecological sustainability, or aesthetic sensibility.

It makes sense to collect North American genetic resources in genebanks and make them available to agriculture, conservation, engineering, and scientific disciplines that explore biological diversity and the environment. We envision collections of genetic resources as an inventory of "nature's solutions" to tough environmental problems. Genebank users will sift through these collections for patterns of diversity or traits of interest. Therefore, genebanks must be careful not to "edit" nature's work in case it masks or removes the very trait that was sought. This creates a real challenge because the highly controlled conditions that genebanks must use can create strong pressure for plastic living systems to adapt and become domesticated. The signature of domestication is particularly recognizable in seed traits (Fig. 10.1) (Schoen and Brown 2001; Meyer et al. 2012). In essence, the crop genebank mission is to maintain "wildness" of the stored germplasm so that genebank users have full responsibility for domestication efforts.

Fig. 10.1 Seed of wild progenitors (left) and crops (right) showing domestication traits of lighter color and larger seeds. Top to bottom are *Pistacia*, *Coffea*, *Glycine*, *Hordeum*, *Zizania*, and *Sorghum*. Photo taken by LM Hill

Table 10.1 Requirements to ensure scientific collections are fit-for-purpose in studies of biological diversity

1	Maintain samples that are structurally intact (i.e., lack signs of physical damage) and are genetically representative of the source material (i.e., the conservation target)
2	Ensure samples are accompanied by data that describe the sample and the population from which it came
3	Authenticate data using accepted calibrations and standardized measurements
4	Allow access to samples and data
5[a]	Keep samples healthy and able to be regenerated

[a]A special requirement for germplasm banks

The purpose of this chapter is to describe some of the challenges one might experience when capturing and maintaining diversity inherent in collections of CWR – whether originating in North America or elsewhere. While we appreciate that plant (and animal) genebanks are often rationalized in an ethical context of conserving natural diversity (e.g., Soulé 1991; Guerrant et al. 2004, 2014), our premise in this chapter is that all the goals for genebanks – economic sustainability, environmental services, ethical considerations, opportunity for new applications, and aesthetic potential – are realized through scientific approaches. Hence, we view plant genebanks as scientific collections and that the scientists who use these collections need access to well-characterized, high-quality materials that are quality-assured so that the genebanking experience doesn't affect experimental outcomes (ISBER 2012; Walters et al. 2008; Guerrant et al. 2004, 2014). Overall requirements to gather and document materials that are fit-for-purpose for studies of biological diversity are summarized in Table 10.1.

Elements in Table 10.1 interact to contribute to the success (or failure) of genebanks in delivering samples of interest to users. Our focus in this chapter is element one and its interaction with element five. Providing viable samples (i.e., germplasm) differentiates a genebank from other types of plant collections such as DNA banks or herbaria. Arguably, ensuring viability while preventing genetic change is one of the greatest challenges facing genebanks today. Tools to validate how well a sample represents the source population and how it is maintained through time in the genebank are rapidly developing (Kilian and Graner 2012).

Plant genebanks can serve as an important tool for conservation. Ex situ conservation, made possible by genebanks, can complement in situ strategies that operate at habitat and landscape levels (Soulé 1991). Safely preserved at locations protected from social pressures or environmental disasters, genebanks can amass significant genetic diversity from a great range of taxa in a relatively small volume. Numerous land managers and conservation groups within the USA incorporate seed banking to forestall attrition of plant genetic diversity and ensure excellent sources of germplasm for land restoration (Guerrant et al. 2004; Hay and Probert 2013; Haidet and Olwell 2015; PCA 2015). Collections of plants of conservation concern (sensu USFS https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprd3848211.pdf (visited October 3, 2017)) exist already. These can be especially valuable as a source of agronomic traits (Khoury et al. 2013). For example, the highly endangered plant

Zizania texana Hitchc., which grows in a 7 km stretch of the San Marcos River near San Antonio, TX, has a desired perennial growth habit and produces seeds with exceptionally high lysine (Kahler et al. 2014).

Germplasm may be maintained in genebanks as samples actively growing, under field greenhouse or tissue culture conditions, or as alive-but-not-growing samples maintained by highly controlled conditions. Collections of the former are often referred to as living, and the latter can be referred to as stored, suspended (for suspended animation), or preserved. Often stored germplasm and seed banks are synonymous, although technologies have rapidly advanced to preserve many other germplasm forms in addition to seeds (see next section). Living collections allow curators to observe traits and regenerate samples, but they also increase the risk of losing samples to inclement weather, pests, pathogens, social unrest, and old age. Genetic erosion through drift, inadvertent selection, or introgression with neighboring related plants can also occur while growing or regenerating a sample. And, regeneration is especially expensive in terms of land and labor for large plants that may take years to sexually mature. Risks to field collections can be partially mitigated by maintaining plants in vitro, but labor and suitable space to maintain these collections can be cost-prohibitive (Pence 2011). Preserved collections are less expensive and more space efficient, carry lower risk from natural or anthropogenic disasters, and make genetic resources readily available regardless of season, year, or location (Li and Pritchard 2009; Volk et al. 2009; Pence 2011). Moreover, risks of genetic erosion during regeneration are mitigated when storage conditions are exceptional and maintain high viability with no mortality over extended periods (Richards et al. 2010; Walters et al. 2015a).

Plant genebanks are proliferating worldwide, and currently, about 1750 exist to serve agriculture, conservation, and studies of ecology, evolution, and diversity (Hay and Probert 2013; FAO 2014). These germplasm collections focus on a wide array of plant genetic resources and usually invoke a combination of living and stored approaches to maintain and evaluate samples. Major questions challenging genebank operations include forms of germplasm that can be preserved, propagated, and utilized, indicators for when a collection is "complete," life expectancy during storage, and assessment of genetic quality and potential uses of genebanked samples.

10.2 The "Conservation Target," Germplasm that Is "Fit-For-Purpose," and Genebank Management Plan

Germplasm samples must align with genebank mission, which is defined by the genebank's specific objectives or rationale. For example, the purpose of the USDA National Plant Germplasm System (NPGS) is to provide diversity that benefits research and education about agriculture. For this reason, NPGS collections focus on the subset of the world's approximately 300,000 plant species that have economic potential. Currently the NPGS collection contains only about 16,000 species, but

this is represented by nearly 600,000 accessions (an accession is a sample with unique identifying information, such as taxon, location, and harvest details (this information is also called passport data)) – one of the world's largest plant germplasm collections globally (https://npgsweb.ars-grin.gov/gringlobal/query/summary.aspx visited October 3, 2017). NPGS accessions are roughly divided into named cultivars (50%), genetic stocks (20%), and wild relatives or landraces of crops (30%). About 250,000 accessions are distributed each year to users. This strongly suggests that interest in genebank collections among the scientific community resides in questions at the sub-taxonomic level.

Twenty five years ago, conservation targets for genebanks hovered at the taxonomic level (Soulé 1991). For example, botanical gardens used the living genebank strategy and broadly collected species but just had a few exemplars for each. This strategy provides support for phylogenetic distinctions but is unlikely to reveal variation within a species (Marshall and Brown 1975; Hokanson et al. 1998; Lawrence et al. 1995). Proliferation of genebanks that take advantage of advancing storage technologies and data management offer the opportunity for collections to explore a finer scale of genetic variation (Charlesworth et al. 2001; Lockwood et al. 2007a, b; Franks et al. 2008; Walters et al. 2008; Engelmann 2011). Conservation targets at these finer scales include populations, ecotypes, families (e.g., maternal lines), and individuals with exceptional characteristics, traits, or even particular gene expression patterns (Khoury et al. (2015). At the writing of this chapter, we feel that the conservation target(s) for CWR is/are mostly undefined for most crop collections. Conservation targets might range from samples that provide phylogenetic representation to samples that confer particular traits or ecotypes (e.g., drought tolerance). In many instances, collections of CWR are sought to provide a general representation of population diversity of the species, as a contingency against outbreak of disease or pests. When the conservation target is defined below the taxonomic level, stringency for maintaining genetic identity of the sample tightens (Table 10.1, element 1) and requires metrics to demonstrate the proficiency by which a genebank delivers samples that reflect the finer-scaled conservation target (Van de Wouw et al. 2010).

10.2.1 Germplasm

What part of the plant should be sampled for genebanking purposes? For DNA analyses, a fresh leaf or other non-senescent tissue is usually sufficient (Walters and Hanner 2006). However, for genebanks with the additional responsibility of providing live material (Table 10.1, element 5), the choice of propagule is a critical decision (Volk and Walters 2004). Within the genebank, ability to provide samples of high structural integrity that faithfully represent the conservation target usually rests on whether the material can be stored and easily distributed. Costs of processing and storage should figure significantly into the genebank's business model to determine the volume of material that can be managed effectively. Additional

criteria for the collector might be timing of the collection trip in relation to plant phenology, remoteness of populations, permitting allowances, amenability to harvest, impact to the population and site, and potential for opportunistic collection of other species. Users may have additional preferences for the ease and required time for growing or propagating the material as well as the immediate availability of germplasm. Luckily, many plants are fairly plastic in their reproductive behavior and offer numerous propagule types to meet a range of requirements, preferences, and constraints (Table 10.2). Genebanks frequently distinguish between propagules that are sexually-derived (i.e., seeds and pollen) and those that arise from vegetative cuttings (i.e., clonally propagated). In agriculture, this distinction usually occurs because the conservation target is a specific genotype and the plant is highly heterozygous and outcrossing, for example, fruit crops (Volk and Walters 2004). Clonal propagation may be necessary for plants of conservation concern if there is reproductive failure in the wild (e.g., inbreeding, no pollinators) or if population sizes are inviable (Pence 2013). Since the conservation target of CWR is usually at either the population or gene level, stringent control of the genotype may be unnecessary and may actually impede broader representation of diversity within the population or incorporation of useful genes into a cultivar (Volk and Walters 2004).

Seeds are the most commonly used propagule for plant genebanks. Usually compact, plentiful, storable, growable, and representative of maternal and pollen-donor lines, seeds might just be the ideal medium for plant genebanking. Indeed, over 95% of the USDA National Plant Germplasm System collection uses seeds as the propagule form of choice. Seed-related traits such as fertility, fecundity, uniformity, germination speed, harvesting ability, and longevity – traits that facilitate genebanking – reflect traits selected during domestication (Meyer et al. 2012) (Fig. 10.1). We should expect disparities in these seed traits between untamed wild progenitors and their derivative modern cultivars. Consequently, we should also expect contrasting response to genebanking from domesticated and wild-collected germplasm. Moreover, we can expect wild-collected germplasm to be more prone to genetic erosion arising from the highly artificial conditions implicit within a genebank. Anecdotal accounts of greater difficulties genebanking seeds from the wild are increasing (Hay and Probert 2013; Walters 2015a). Despite these challenges, conservation groups and land managers have demonstrated the feasibility of genebanking wild seeds and the utility of this germplasm in restoration work (Maschinski and Haskins 2012; Guerrant et al. 2004; Haidet and Olwell 2015). As collaborators in these efforts, we have gained experience working with the seeds of truly wild species and can describe some of the pitfalls we've encountered that reduced the efficiency and accountability of genebanking efforts. These are not insurmountable problems; they simply indicate the need for adjustments in procedures, expectations, and anticipated costs for properly genebanking populations from natural populations compared to established methods using crop seeds.

Depending on several factors, seeds may be a less preferred germplasm form for sampling CWR in natural populations. Some plant species produce seeds that are less suited for genebanking because viability is lost quickly when standard genebanking conditions (sensu FAO 2014) are used. Seeds exhibiting low survival

Table 10.2 Some common propagules used in plant genebanks

Propagule	Advantages	Disadvantages	Exceptions
Seeds: Conservation target at population and/gene level	Compact High fecundity of some plants make it possible to collect many individuals Highly developed, low-cost, storage technology for orthodox seeds Efficient for propagation and regeneration and distribution Represents progeny of extant population (can capture many genotypes and many genes) May present barrier to some diseases Demonstrated ability to efficiently capture diversity	Heterogeneous traits in wild populations multiple harvest times needed, and timing can be unpredictable Asynchronous germination can lead to poor stand establishment and drift Long time to sexual maturity in perennials Potentially unknown pollen source Mating systems may preclude maintaining desired maternal traits	Non-orthodox seeds require cryogenic storage Possible low seed production in wild due to reproductive failure (endangered species), drought, late frost, non-mast year, herbivory
Pollen: Conservation target at gene level	Very compact Available for immediate use in breeding programs Available during flowering Amenable to storage Captures diverse genes Maybe the fastest, least labor-intensive way to achieve some form of back-up	A gamete, not an individual Ephemeral Difficult to harvest Must make crosses to regenerate populations Must be genebanked immediately after collection (short processing timeline)	
Shoot tips: Conservation target at individual level	Compact Captures specific genotype, OK as an exemplar of species Amenable to in vitro culture Preservation technologies rapidly developing Clonal propagation reduces concern about genetic drift	Requires large amounts of quality source materials at correct phenological stage Unexplained variation in response to growth medium among genotypes Processing and growth are labor intensive Many individuals needed to capture diversity of a heterogeneous population	

(continued)

Table 10.2 (continued)

Propagule	Advantages	Disadvantages	Exceptions
Dormant buds or overwintering vegetative structures: Conservation target at individual level	Compact Captures specific genotype, OK as an exemplar of species Does not require in vitro culture (less labor than shoot tips) Preservation technologies are advancing Clonal propagation reduces concern about genetic drift	Plants must be winter-adapted and in acclimated state Recovered by grafting Many individuals needed to capture diversity of a heterogeneous population	Variable responses within and among species result from complex bud structures
Somatic embryos and cell cultures: Conservation target at individual level	Compact Captures specific genotype; May be more amenable to preservation than non-orthodox seed Can generate huge numbers of individuals	Successful propagation is highly genotype-specific, tends to narrow captured diversity High risk of somaclonal variation Labor intensive for establishing and processing	

See also Havens et al. (2004) for complementary information

under standard genebanking conditions are nominally classified as “recalcitrant” or “intermediate” (and collectively as “non-orthodox”) compared to counterparts that are considered “orthodox” (Walters 2015b; RBG 2017). In interspecies hybridization zones, cuttings may provide more certain taxonomic identification than progeny from uncertain pollen sources. At sites where there are few individuals or low fecundity, cuttings may provide a means to collect germplasm with lower potential impact to the natural population; these can then be grown-out in field collections to facilitate characterization and regeneration through seeds. Pollen is under-appreciated as a germplasm form in plants, which contrasts with animal genebanks in which semen, the counterpart to pollen, is the most commonly used germplasm form (Mazur et al. 2008). Pollen might be an effective alternative germplasm form that can capture genes of interest and deliver them to a breeding population when seeds are unavailable or have poor storage characteristics or when maintaining cuttings is cost-prohibitive. For example, pollen from oak trees is desiccation tolerant, while oak seeds tend to be recalcitrant (Franchi et al. 2011). Pollen is storable (Hoekstra 1995; Volk 2011), but it lacks the longevity traits exhibited in seeds of the most common agronomic species (Dafni and Firmage 2000). The requirement for rapid processing of pollen samples makes it a less-ideal germplasm form when collecting from remote natural populations.

Quality of seed set and phenology are also important factors; a plant collector should sample seeds in the fruiting period, vegetative tissues when plants show active growth flushes or cold-adapted twigs are available, and pollen if plants are flowering. Alternative germplasm forms, such as pollen or cuttings, may augment genetic diversity lost by high mortality during banking of non-orthodox seeds.

10.2.2 Sampling Strategies and Management Plans.

In addition to the type of germplasm collected, the conservation target also defines the sampling strategy (Guerrant et al. 2014; Hoban et al. Chap. 8, this volume) as well as the genebank management plan. For germplasm banks, management plans must be suitable to deliver viable germplasm (Table 10.1, element 5); however management plans can vary depending on the conservation target. When the conservation target is an exemplar of phylogenetic representation, sampling probably occurred at one or a few convenient locations, and there probably wasn't great effort expended to get an accurate genetic representation of the species or particular populations. In this case, management at the genebank should complement the sampling effort to ensure sufficient viability for representatives of the taxon. Conservation targets for agricultural-based genebanks are usually at the sub-taxonomic level, for example, diversity is sought for a specific trait (e.g., aluminum tolerance, salt tolerance, and disease resistance) or for broad population representation needed for contingencies in the future. For conservation targets at trait or population levels, sampling usually occurred across diverse locations, and the sites and number of sites were selected carefully to maximize the sought diversity with fewest possible samples (so as to not overwhelm genebanking operations). Stringent genebank management plans are needed to ensure that the sample remains genetically representative of the source population. These are discussed further in Sect. 10.4 (Standards and Best Practices).

An important question arises about collection completeness, "completeness" being defined as how well the samples in the collection represent the diversity within the conservation target. There are few specific metrics to determine completeness (though see Hoban et al. Chap. 8, this volume for fuller discussion). It is important to note that metrics will differ among collections that are focused on different conservation targets such as a species representative, a specific trait or a contingency collection. International policy and legislation (e.g., International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA-FAO 2001), the Convention on Biological Diversity Strategic Plan (SCBD 2010), and the Global Strategy for Plant Conservation (SCBD 2014)) encourage strategies for ex situ conservation of CWR but provide few recommendations for the conservation target or metrics for effectiveness of sampling or management plans. Using species distribution models along with validating genetic data (Hoban et al. Chap. 8, this volume) may provide collectors more sophisticated tools to locate and monitor genetic variation and estimate uncertainty about collection "completeness."

10.3 Genebanking Wild-Collected Germplasm

Maintaining germplasm collections within allotted resources is a top priority for most genebanks. As mentioned earlier, living genebanks are limited in the amount of genetic diversity they can represent, and living germplasm is more vulnerable to stressful conditions. Preserved germplasm is maintained under highly controlled conditions. There is an expectation that it will remain viable into the future, but that duration is usually poorly defined. Often genebanking duration is defined as short-, medium-, or long-term, which most commonly define the storage conditions (ambient, refrigerated, and freezer, respectively) (FAO 2014), rather than the needed longevity, such as over a breeder's career, until an imminent restoration project is implemented, or forever. At NLGRP, we target a 100-year lifespan for most of our seed accessions (Walters et al. 2004).

The apparent stasis imposed by preserving germplasm often lures the naïve into a perception of simplicity; however the complexity of the effort is revealed by considering the timescale in which genebanks operate – usually decades. It is not generally appreciated that the impacts of seemingly minor deviations today won't be evident until sometime in the future; hence, an unsuspecting genebank manager may inherit a "ticking time-bomb." Genebank failures can go unexplained without standardized methods or stringent documentation. Herein lies a paradox: how do genebanks use standardized treatments for diverse materials and not encounter highly variable responses? And, do the variable responses impact how well the sample can represent the conservation target? A better understanding of time-scales is required to address these questions.

10.3.1 Stopping the Clock: A Primer in Preservation Technologies.

Understanding how to stabilize biological materials and predict the effects of time is a highly practical science needed for everyday problems. The food industry needs to provide expiration dates for product quality. Effectiveness of drugs and dosage response must consider the variable conditions that occur in household medicine cabinets. Plastic products, coverings, and packaging lose form and function over time. Everyone experiences the yellowing of old paper, the brittleness of aged rubber bands, and the failure of worn tape to hold documents together. Like all materials, the fundamental process for structural stability (i.e., preservation) is solidification (Menard 2008; Walters et al. 2010) – in the case of germplasm, this involves solidifying, or vitrifying, cytoplasm (i.e., forming a "glass") without too much disruption to the cell structure.

Most cytoplasm vitrifies at room temperature when samples dry to between 30% and 50% RH. If this level of drying can be accomplished without too much cell shrinkage, the cell survives; loss of more than 50% cell volume is considered lethal (Walters 2015b). During embryogenesis, food reserves (starch, protein, or lipid) are

deposited into cells, displacing water which consumed as much as 80–90% of the cell volume in an immature embryo and 60–80% of cell volume in a mature recalcitrant (i.e., desiccation-sensitive) seed. The cell volume of a mature orthodox seed changes very little during desiccation (less than 30%), and we believe this explains their extreme tolerance to desiccation as well as subsequent longevity (Walters 2015b). The process of drying without dying makes desiccation tolerant organisms, including orthodox seeds, the original material engineers. This process is highly regulated during embryogenesis (Righetti et al. 2015), and incompletion or disruption of the established program, like any material, can have dire consequences to the functionality and stability of the end product – in this case, seed survival through time (Walters et al. 2010; Walters 2015b).

Once cells are in the glassy state (at temperatures below the glass transition temperature or Tg), they are relatively stable, meaning that change occurs, but over a much longer time scale than reactions occurring in fluid systems. In other words, preservation doesn't stop the clock, it just slows it down. In solids, such as vitrified cytoplasm, structure and mobility become two sides of the same coin. The "structure" is defined by how the compressed molecules impede movement of neighboring molecules. Pores formed during glass formation and molecules in the glass now shift to fill those pores and pack more efficiently. This rearrangement defines the "mobility" as well as the rate of change within the glass (Menard 2008). The movement brings molecules slowly into closer proximity, where they interact and oxidize; the material becomes brittle. The time scale is often experimentally intractable, which is one of the reasons why seed longevity is difficult to predict or measure. Water is a "plasticizer" of biological glasses, meaning it promotes larger pore space, hence greater mobility and faster aging. If seeds are not sufficiently dried, molecules in the cytoplasm move faster, causing more rapid deterioration. Anti-plasticizers (e.g., cryoprotectants) stabilize structure by a number of mechanisms. There is some speculation that anti-plasticizers of unknown identity accumulate in long-lived seeds (Walters 2015b).

A glass is stabilized by lowering the temperature. For orthodox seeds, which formed glasses during drying at ambient temperatures, molecular rearrangements to form lethal ice crystals at sub-zero (°C) temperatures are improbable, and so longevity increases progressively with lower temperature (to a point) (Walters, 2004). Most genebanks use freezers at −18 °C for conventional storage, because it is highly accessible technology, being easily achieved using a single-stage compressor. Freezer storage of plant germplasm began in the 1970s, and there was strong debate about its benefits until the early 2000s (Zheng et al. 1998; Walters 1998).

Cryogenic storage for plant germplasm became accepted in the mid-1980s and routine in the mid-1990s. Cryogenic storage may occur through a number of platforms, and the appropriate treatment and storage temperature for plant germplasm relies on the vitrification temperature, Tg. Technically, all storage below 0 °C is cryogenic; however, here, we consider it as storage below temperatures achieved by conventional freezers. Mechanical freezers with a dual-stage compressor cool to −80 °C and may be effective for germplasm with a relatively low Tg or unstable glass or when lipid transitions are important to survival. Most frequently, cryogenic

storage is associated with the use of Dewar flasks or cryovats that are cooled by liquid nitrogen. Germplasm is either immersed into the fluid and stored at −196 °C or stored in the vapor above liquid nitrogen (between −150 and −190 °C, depending on distance from fluid surface and convection within the tank). A few status reports on longevity of cryopreserved germplasm are available (Towill et al. 2004; Walters et al. 2004; Volk et al. 2008; Ballesteros and Pence 2017; Pence et al. 2017).

Many plant propagules do not survive the desiccation stress required to form glasses at ambient temperatures (e.g., vegetative propagules and some non-orthodox seeds) (Table 10.2). Lowering the temperature of these non-vitrified systems poses high risk of lethal ice formation, which can only be avoided by forming a glass during the cooling process and maintaining it below Tg so that glasses don't melt and ice doesn't form during storage. Inhibiting ice formation at sub-zero (°C) temperatures, while maintaining cell viability, requires optimization of interacting treatments for moisture adjustments, additions of cryoprotectants and rapid cooling (Walters et al. 2013; Wesley-Smith et al. 2014).

10.3.2 Conservation Targets, Sample Quality, and Preservation Success

We preface this section by the infamous story of the Pará rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg., which produces a non-orthodox seed. Mostly told for political intrigue, the story describes how less than 1% of over 50,000 seeds survived to domesticate the species and initiate rubber industries in Malaysia and Singapore (Brockway 1979). Here, the conservation target for *H. brasiliensis* was primarily its rubber-producing trait, a species characteristic that can be captured by exemplars. Thus, loss of 99% of the collected seeds, as a result of poor shelf-life, was considered acceptable. We would likely find those losses unacceptable by today's standards because our conservation targets tend to be at finer scales: genetic diversity representative for the whole species or for key populations or individuals. The salient point, for the context of this chapter, is that preservation success is defined by whether loss of genetic diversity during genebanking is acceptable, which is largely dependent on the stated conservation target.

The *Hevea brasiliensis* story also illustrates that it is possible to genebank even when seeds are not orthodox. Usually a species producing non-orthodox seeds will be harvested as a cutting or fresh seed and immediately grown out to form a living collection. As described in the previous section, it is now possible to preserve non-orthodox seeds and other germplasm forms that do not survive cytoplasmic solidification at ambient temperatures. However, this effort requires exacting coordination between collector and curator.

An initiative to bank wild species requires background information on how propagules respond in storage. To that end, we cross-referenced prioritized CWR from the USA (Khoury et al. 2013) with information available from Royal Botanic Kew's

Seed Information Database (SID) (RBG 2017) (Table 10.3). A limited number of US species were included in the SID, emphasizing the lack of information available for storage behavior of germplasm from CWR native to the USA. Extrapolating from the genus level, we believe that 75% of the 179 genera from the Inventory will produce orthodox seeds and that at least 8% will not produce orthodox seeds. High variability in seed storage response is exhibited in about 8% of congeners, and no records are given for 8% of the genera.

Even orthodox seeds present challenges for storage, especially with conservation targets at the population level, which is where most agronomic- and conservation-based collections are poised. The increasing number of anecdotal accounts that seeds collected from the wild are harder to store are not surprising (Hay and Probert 2013; Walters 2015a; Balleseros and Pence 2017). We know that embryo development is critical to longevity, and metabolic pathways expressed during embryogenesis are keys (Righetti et al. 2015; Walters 2015b). Seed quality is dependent on processes that are uncontrolled in the wild during the growing season, such as moisture availability, nutrition, competition, and pathogens, and it will decline if developmental programs are not completed (Probert et al. 2007) or extended toward germination (Tarquis and Bradford 1992). Seed quality is also under genetic control (Clerkx et al. 2004; Schwember and Bradford 2010; Nagel et al. 2011; Righetti et al. 2015), with ecotypes within a species having contrasting storage behavior (Tweddle et al. 2003; Clerkx et al. 2004; Daws et al. 2004; Walters et al. 2005; Probert et al. 2009; Kochanek et al. 2009; Mondoni et al. 2014). Phenology, fecundity, carbon partitioning, composition, seed coverings, resistance to pests, and drought tolerance are all inherited traits that affect seed longevity. These traits are more uniform in domesticated plants but vary considerably in seeds from natural populations; hence, an accession of seeds collected from the wild will be heterogeneous, and this will result in differences on how individual seeds within the sample respond to genebanking conditions.

Genebanking wild-collected seeds carries inherent risks for genetic erosion: when the shorter-lived seeds in an accession die, seed traits will tend toward greater uniformity, and when seeds are regenerated, germination, flowering, and maturity are likely to become more synchronized. Unless extraordinary measures prevent these tendencies, genebanking seeds collected from the wild can be an exercise in domestication as a result of inadvertent selection of traits controlling preservability, growth habit, morphology/physiology, and reproductive capacity (e.g., Burton and Burton 2002; Gilligan and Frankham 2003; Harding 2004; Aubry et al. 2005; Falk et al. 2006). Even though sample quality may remain high during genebanking, there may be an attrition of sought-after genetic diversity and an increasing tendency for genetic representation of the sample to veer away from the source population (Table 10.1, element 1).

Table 10.3 Probable seed storage behavior for taxa native to the USA that are congeneric to domesticated species

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Abutilon Mill.	0	8	O	0
Acer L.	0	6	O to R	1
Actaea L.	47	1	O	0
Aegilops L.	0	5	O	0
Agave L.	20	4	O	0
Agropyron Gaertn.	0	2	No data	
Agrostis L.	0	15	O	0
Allium L.	0	47	O	1
Alopecurus L.	0	4	O	0
Amaranthus L.	3353	40	O	0
Andropogon L.	0	13	O	0
Annona L.	48	1	O to U	
Apios Fabr.	0	1	No data	
Apium L.	0	1	O	0
Arbutus L.	0	3	O	0
Armoracia G. Gaertn.	0	1	O	0
Aronia Medik.	0	3	No data	
Arrhenatherum P. Beauv.	0	2	O	0
Artemisia L.	0	50	O	0
Artocarpus J.R. Forst. and G. Forst.	0	1	R	
Asimina Adans.	1024	9	U	
Asparagus L.	0	3	O	0
Atriplex L.	0	37	O	0
Avena L.	0	3	O	0
Bassia All.	0	1	O	0
Beta L.	0	4	O	1
Boehmeria Jacq.	0	1	O	0
Brassica L.	0	5	O	2
Bromus L.	0	35	O	0
Camelina Crantz.	0	1	O	0
Canavalia Adans.	0	6	O	0
Capparis L.	0	2	O to R	
Capsicum L.	5084	2	O	0
Carica L.	53	1	I	
Carthamus L.	0	1	O	0
Carya Nutt.	4078	13	U	
Castanea Mill.	15	5	R	

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Chenopodium L.	386	51	O	0
Chrysanthemum L.	0	1	O	0
Chrysophyllum L.	0	2	R	
Cinnamomum Schaeff.	0	1	R	
Cochlearia L.	0	1	O	0
Cocos L.	0	1	R	
Coix L.	0	1	O	0
Colocasia Schott.	0	1	U	
Corchorus L.	0	2	O	0
Coreopsis L.	0	8	O	0
Corylus L.	803	3	I	
Crataegus L.	0	70	O	0
Crotalaria L.	0	6	O	0
Croton L.	0	15	O	0
Cucumis L.	0	4	O	1
Cucurbita L.	3392	8	O	1
Cuphea P. Browne	0	5	O to I	
Cynara L.	0	3	O	0
Cyperus L.	0	48	O	0
Dactylis L.	0	1	O	0
Daucus L.	1578	2	O	0
Digitaria Haller	0	20	O	0
Dioscorea L.	0	3	O	0
Diospyros L.	0	7	O to R	
Diplotaxis DC.	0	2	O	0
Echinacea Moench.	0	13	O	0
Echinochloa P. Beauv.	0	15	O	0
Elymus L.	0	43	O	0
Eragrostis Wolf	0	27	O	0
Eruca Mill.	0	2	O	0
Eugenia L.	0	3	R	
Fagus L.	0	2	O to I	
Festuca L.	0	36	O	0
Ficus L.	0	4	O	0
Foeniculum Mill.	0	1	O	0
Fragaria L.	1907	21	O	0
Gaylussacia Kunth.	0	8	O	0
Glycyrrhiza L.	0	1	O	0

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Gossypium L.	10,582	3	O	1
Hedysarum L.	0	7	O	0
Helianthus L.	5158	72	O	1
Hibiscus L.	0	18	O	0
Hordeum L.	0	18	O	1
Humulus L.	626	6	O	0
Hydrastis J. Ellis	0	1	No data	
Hypericum L.	0	1	O	0
Ilex L.	0	21	U	
Illicium L.	0	1	R	
Ipomoea L.	1251	40	O	0
Jatropha L.	0	4	O	0
Juglans L.	702	9	U	
Lactuca L.	2943	11	O	1
Lathyrus L.	0	31	O	0
Lepidium L.	0	37	O	0
Lespedeza Michx.	0	11	O	0
Leymus Hochst.	0	17	O	0
Licania Aubl.	0	1	No data	
Lilium L.	0	5	O	0
Limnanthes R. Br.	82	1	O	0
Linum L.	0	21	O	0
Lolium L.	0	3	O	0
Lotus L.	0	77	O	0
Lupinus L.	0	95	O	0
Malus Mill.	6203	4	O	1
Manihot Mill.	21	4	O	1
Manilkara Adans.	55	1	I to R	
Medicago L.	0	10	O	0
Melilotus Mill.	0	3	O	0
Mentha L.	0	4	O	0
Mespilus L.	0	1	No data	
Morus L.	0	2	O	0
Nasturtium W.T. Aiton	0	4	O	0
Nicotiana L.	2342	9	O	0
Olea L.	0	1	O	0
Oplopanax (Torr. and A.Gray) Miq.	0	1	O	0
Opuntia Mill.	286	31	O	0

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Oxalis L.	0	8	O	0
Pachyrhizus Rich. Ed DC	11	0	O	0
Panax L.	0	1	No data	
Panicum L.	1731	37	O	0
Papaver L.	0	14	O	0
Parthenium L.	151	7	O	0
Paspalum L.	0	42	O	0
Passiflora L.	0	13	O to I	
Pastinaca L.	0	1	O	0
Pennisetum Rich.	0	10	O	1
Penstemon Schmidel	0	39	O	0
Persea Mill.	173	3	R	
Phalaris L.	0	6	O	0
Phaseolus L.	17,856	17	O	1
Phleum L.	0	2	O	0
Phlox L.	479	9	O	0
Physalis L.	0	13	O	0
Physaria (Nutt. ex Torr. and A. Gray) A. Gay	237	4	O	0
Pinus L.	0	4	O	0
Piper L.	0	1	O to I	
Pistacia L.	356	1	O	0
Poa L.	0	42	O	0
Portulaca L.	0	10	O	0
Pouteria Aubl.	85	0	R	
Prosopis L.	0	9	O	0
Prunus L.	2970	30	O	0
Psathyrostachys Nevski	0	2	No data	
Pseudoroegneria (Nevski) Á. Löve	0	1	No data	
Psidium L.	67	2	O	0
Pueraria DC.	0	3	O	0
Pyrus L.	0	1	O	0
Raphanus L.	0	1	O	0
Rhododendron L.	0	30	O	0
Ribes L.	1273	65	O	0
Rorippa Scop.	0	9	O to U	

(continued)

Table 10.3 (continued)

Genus with a 1A, 1B, or 2 priority ranking[a]	# accessions in NPGS for genus[b]	# priority taxa for priority collection in the USA[a]	Predicted response to storage[c]	# of species within genus with reported constants for Seed Viability model[d]
Rosa L.	0	27	O	0
Rubus L.	2109	67	O	0
Rudbeckia L.	0	11	O	0
Ruellia L.	0	2	O	0
Rumex L.	0	19	O	0
Saccharum L.	0	9	O	0
Salsola L.	0	4	O	0
Sambucus L.	0	11	O	0
Satureja L.	0	1	O	0
Scorzonera L.	0	1	O	0
Setaria P. Beauv.	1081	27	O	0
Simmondsia Nutt.	324	1	O	0
Solanum L.	18,016	39	O	0
Sorbus L.	0	11	O	0
Sorghum Moench.	0	4	O	1
Stillingia Garden	0	2	No data	
Syzygium R.Br. ex Gaertn.	0	2	R	
Theobroma L.	271	0	R	
Thinopyrum Á. Löve	0	2	No data	
Thlaspi L.	0	1	O	0
Tragopogon L.	0	4	O	0
Triadica Lour.	0	1	O to R	
Trifolium L.	0	96	O	0
Tripsacum L.	294	4	No data	
Vaccinium L.	1786	39	O	0
Vanilla Mill.	0	2	No data	
Vernicia Lour.	0	1	No data	
Vicia L.	0	14	O	0
Vigna Savi	0	2	O	2
Vitis L.	5028	29	O	0
Zizania L.	0	6	I	

Genera are from supplemental material supplied by Khoury et al. (2013). Information about seed responses to storage are from congeners listed in Kew's SID (http://data.kew.org/sid/sidsearch.html accessed 14 Feb 2017). Information on constants for the Viability Equation model come from a different page on the SID website (http://data.kew.org/sid/viability/ accessed October 3, 2017) and represent the number of species within the listed genus with reported constants (no data were available for specific taxa listed the Inventory)

[a]Definitions of priority rankings for collection (1A, 1B, and 2) were taken from Khoury et al. 2013, and associated taxa were retrieved from the Inventory provided at http://www.ars-grin.gov/misc/tax/ (accessed 6 Feb 2017)

(continued)

Table 10.3 (continued)

[b]Presence of accessions for genera within NPGS collections were retrieved from GRIN (site) (Courtesy of C.K. Khoury)
[c]Seed storage behavior was taken from Kew's SID and reflect behaviors listed for the majority of congeners with reported data (no data were available for specific taxa listed in the Inventory) (http://data.kew.org/sid/sidsearch.html accessed October 3, 2017), O, orthodox; I, intermediate; R, recalcitrant; U, unclear
[d]Congeners with Viability Equation information come from a different page on the SID website (http://data.kew.org/sid/viability/ accessed October 3, 2017) (no data were available for specific taxa listed the Inventory). Zero indicates no information listed for that genus; blanks indicate seeds with probable non-orthodox storage behavior

10.3.3 Predicting Longevity and Detecting Aging

The intention to use genebanking to conserve genetic diversity extant in the wild underpins genebanking practices designed to prolong seed shelf-life. Storage treatments, viability monitoring frequencies, and regeneration are all based on assumptions about longevity. However, longevity varies tremendously among seed lots within a species due to uncontrolled and unknown factors of seed quality. Therefore, the actual longevity of a seed lot is only known after-the-fact. Genebanks need reliable assessments of longevity before and during storage.

Longevity might be predicted using an empirical model that is parameterized by constants for moisture and temperature effects for different species. The Seed Viability Equations (Ellis and Roberts 1980) [http://data.kew.org/sid/viability/ (visited October 3, 2017)] provide "ball-park" estimates of survival with time when conditions are not-too-cold or not-too-dry; this model becomes unreliable beyond the limits of inference of the data used to parameterize it (Walters 1998; Walters et al. 2004). Since freezer storage is a relatively new practice (it was first used routinely at NLGRP in 1978), there are few data sets that actually demonstrate longevity in the freezer, let alone predict it reliably.

Models such as the Viability Equations allow us to "standardize" different laboratory experimental conditions, so diverse species can be ranked for longevity in a similar context (Hay et al. 2003). Not surprisingly, information for CWR species from the USA (Khoury et al. 2013) are not available. The information might be gleaned from behavior of congeners, but only 15 of the 135 genera believed to be orthodox had species coefficients listed in the SID (Table 10.3). More extensive comparisons of seed longevity within the genebank, and estimates of within-species variation for some CWR, come from early genebank results of seeds stored initially at 5 °C (Walters et al. 2005; Nagel and Börner 2010). Additional insights come from seed aging experiments conducted at warmer temperatures under high humidity challenges (Probert et al. 2009) or drier conditions (e.g., Fig. 10.2). There is general agreement that seeds from Apiaceae tend to be short-lived and seeds from Chenopodiaceae tend to be long-lived. Seeds from Asteraceae and Poaceae exhibit a wide range of longevities. In the future, we hope to adjust species-level information with data about habitat, location, and weather data associated with the sample.

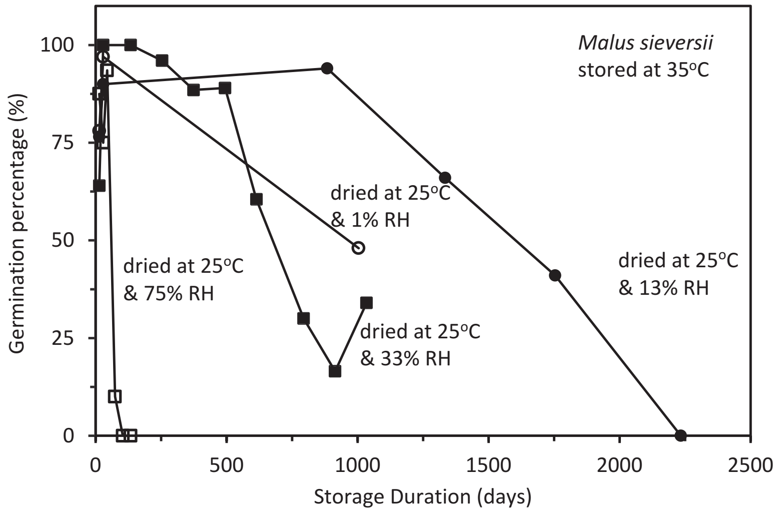

Fig. 10.2 Survival of a seed sample of *Malus sieversii*, progenitor species of domesticated apple. Seed moisture was adjusted at 25 °C at indicated relative humidity for a 3-week period; seeds were then sealed in foil laminate packages and placed at 35 °C for indicated time and then germinated. The increasing longevity with decreasing moisture treatment (75–13% RH) is indicative of orthodox behavior, and the faster aging at very low RH (1%) demonstrates a limited benefit of drying. This seed lot survived for about 3 years (+1000 days) at 35 °C. Extrapolating longevity to −15 °C using a general rule for temperature effects (doubling for every 10 °C; Q10 = 2), we can predict this seed lot would survive for about 100 years in a genebank (original data)

Wide variation of longevity within a species makes it difficult to accurately predict how a particular sample will behave in the genebank (e.g., Walters et al. 2005). These differences are accommodated in the Viability Equations using an initial quality factor, which is dependent on a highly accurate measure of initial germination as well as an assumed high correlation between initial germination and longevity. Despite model predictions, there is a poor correlation between initial germination and longevity (Walters et al. 2005; Nagel and Börner 2010; Ballesteros and Pence 2017), which we attribute to quality factors that have initial but not long-term effects (Mead and Gray 1999) and factors that have long-term but not initial effects (Hay and Probert 1995; Tarquis and Bradford 1992; Walters et al. 2004).

Monitoring viability is currently the genebank's only tool for assessing whether quality is maintained. For seeds, this involves a germination assay which, like the initial test, is a snapshot having little predictive power of future change. Statistical considerations related to sample size also influence how well change can be detected (Guerrant and Fiedler 2004; Richards et al. 2010). Accumulation of deaths in time, marked by changes in germination potential, is a poignant demonstration that responses of individuals *within* a sample vary during storage.

Genebanks must monitor seed viability, but without a priori information on longevity and germination, there is no guidance for monitoring interval or seed counts. When a priori knowledge is insufficient, frequent viability monitoring is recom-

mended (FAO 2014); this demands extensive resources and can quickly deplete the sample, rendering the entire exercise useless. Better processing and storage conditions to prolong longevity, better methods to relate aging at higher temperatures to freezer conditions, and new tools to monitor nondestructively (Colville et al. 2012; Mira et al. 2016; Fleming et al. 2017) will aid the genebanking operation.

10.4 Standards and Best Practices

Standards or best practices communicate how state-of-art science should be implemented in order to maintain quality samples for a desired timeframe (ISBER 2012). They also communicate to future users how samples were treated (ISBER 2012). Intentions of standards are quality control, predictability, and metrics for how the genebanking experience affected the sample relative to its conservation target. This is particularly important if methods to accomplish bio-banking goals (sensu Table 10.1) are not yet established. Future users deserve to know the history of the sample and how protocols might affect the sample's usefulness to them. Therefore, best practices and standard operating procedures (SOPs) must convey information to users so that they can access whether the sample is fit for *their* purpose.

Standards and best practices for genebanking must align with the sampling strategy, which ultimately must align with the stated conservation target. In our opinion, the conservation target is often not sufficiently defined for samples collected from wild populations, and this can cause a mismatch between sampling and genebanking protocols. In the example of *Hevea brasiliensis,* ability to produce latex for rubber is expressed at the species level, which meant collectors needn't search for this useful trait among certain ecotypes or individuals. Collectors harvested an excessively large number of seeds because they had prior failures due to the difficult physiology – not because they wanted to capture genetic diversity (Brockway 1979). In other words, the primary conservation target was a few specimens of the species, and extraordinary measures to keep all 50,000 of the harvested seeds alive would have been initially unappreciated. Therefore, in this context, stringent management of the collection wasn't that necessary. Eventually, though, the new rubber industries learned that greater genetic diversity from higher seed survival would have been beneficial, and collections for genetic diversity within the species would have required more stringent genebanking protocols. Linking this analogy to the case of CWR within the USA, we need to know whether the agronomic traits we seek (e.g., Khoury et al. 2013) are expressed by all individuals of a species, by specific ecotypes, or by some rare individuals. We believe the answer to this question can help to define the conservation target as well as the technical investment required to maintain US collections.

Developing a set of agreed-upon standards or "best practices" (sensu ISBER 2012) for collecting and maintaining diversity invariably develops into discussions of practicalities, impossibilities, and available resources. Experienced genebanks know that working with wild-collected materials usually involves choosing among

less-than-optimum options. Often, quality control of samples coming into the genebank is limited by the biology of the organism. Standards, such as FAO's Genebank Standards (FAO 2014), can guide genebanks established for agricultural purposes (domesticated plants and CWR), and modifications for wild plant species collected for conservation purposes have been suggested (Hay and Probert 2013). Currently genebanking standards for samples collected in the wild require initially high viability and frequent viability monitoring as well as management decisions to regenerate or recollect when viability degrades to 85% of initial viability (FAO 2014, Seed Conservation Standards for "MSB Partnership Collections" at http://www.kew.org/sites/default/files/MSBP%20Seed%20Conservation%20Standards_Final%2005-02-15.pdf (visited on October 3, 2017)). These stringent guidelines are to ensure the sample remains genetically representative of the wild population from which it is harvested (Table 10.1, element 1) (FAO 2014). If the conservation target is not specific to a population, is it necessary to follow these technically stringent standards? If the conservation target is specific to a population, does stringency of SOPs increase risks of genetic erosion by premature consumption of the sample through too much testing or too frequent or infrequent regeneration (Richards et al. 2010)?

FAO's standards for orthodox seeds (Chap. 4 in FAO 2014) serve as the foundation for many national and international seed banks around the globe, including the USA. These standards were developed about 7 years ago through consensus of a large group of experts having different opinions on the intent of standards and even the interpretation of existing storage data to guide standards. Remaining ambiguities and inaccuracies were place-marked for future research and understanding, and sufficient scientific knowledge has accumulated to call for an update, or at least modification, to meet the needs of seed banks. Improved methods are especially needed to address uncertainty associated with genebanking seeds when there is little knowledge about the species or contingencies when samples are heterogeneous and prone to genetic erosion. Current standards may also be too stringent for some conservation targets. Therefore, we take this opportunity to examine these standards for orthodox seeds and suggest areas for better alignment with conservation targets for germplasm collected from wild populations. To encourage conversation among genebanks, we have listed some standards we feel need adjustment and have provided alternative language (Table 10.4). Standards for non-orthodox seeds in Chap. 6 of the Genebanking Standards (FAO 2014) can also be modified to reflect rapidly developing technologies.

10.5 Sample Regeneration and the Nexus of Different Genebanking Strategies

Genebanking seeds collected from wild populations will likely impose genetic bottlenecks (Falk et al. 2006) that can be minimized by careful collection (Hoban and Schlarbaum 2014) and curation (Richards et al. 2010) as well as large enough sample sizes and treatments informed by the biology of the sample (Hay and Probert 2013). The extent and direction of genetic shifts can have large impact on the

Table 10.4 Best practices for acquisition and storage of orthodox seeds having agricultural utility

Standard	Explanation for difference from current FAO standard	Alternative best practice
Sampling from wild populations	The conservation target (e.g., a specimen, sample that is representative of the population, or single/multiple maternal line(s)) determines the sampling and maintenance strategies which, in turn, determines rigor of genebanking operations as well as costs	*Establish goals and comply with legal requirements* Clearly state conservation target Comply with all legal obligations for collection, transport, propagation, and distribution of samples. Obtain permission from landowners and any regulatory agencies to make seed collections and report permit or agreement numbers as well as handling and distribution restrictions in accession records. Acquire necessary permissions several months before the collecting season
	Sampling strategy should align with conservation target and population size. If Best Practice/Standard can't be met, consider redefining the conservation target to make it appropriate to sample from fewer individuals. For example, site has a single tree, the conservation target is more likely a maternal line rather than a whole population Sufficient seeds should be collected to accommodate curation, distribution, and regeneration of the accession. Sampling must not impact wild populations.	*Basic information about the population and how much to take* Distribute seed harvests among a representative proportion of maternal plants to meet conservation target goals. Standard number of plants for a population-based conservation target is about 50, and a greater number is needed if a desired trait or gene is believed to be rare in the population. Fewer maternal plants are acceptable if the conservation target is a specimen or exemplar of the species or a trait that is characteristic to the species To represent a natural population that is not of conservation concern, strive to collect at least 2500 seeds from a representative cross-section of plants, but do not collect more than 20% of available seeds. To represent a natural population that is of conservation concern, collect no more than 10% of the available seeds, and distribute sampling across at least half of the seed-bearing plants. If seed numbers are too low, consider various options: revision of conservation target, recollection at the site, or immediate regeneration of seeds to get requisite numbers for curation and distribution. Initial sample size should accommodate at least 20 average requests and 2 unusually large requests. Therefore, target ~1400 seeds (distribution) + 800 seeds (maintenance) + 250 seeds (regenerations × 3) + 50 seeds (archival) = 2500 seeds. Report the numbers of seed-bearing plants in the target population and proportion of plants sampled for seeds.

(continued)

Table 10.4 (continued)

Standard	Explanation for difference from current FAO standard	Alternative best practice
	Associated data should be in format compatible with national and international databases that compile information about biological collections. Standard should reflect information commonly sought by users.	*Associated data* Record data about the collection site using standard definitions and units. Direct measurements, rather than inferred information, especially for geospatial data, are always preferred. Using Darwin Core standards will ensure compatibility across institutions (Wieczorek et al. 2012, Endresen and Knüpffer 2012). Basic "passport" data should include categories for institution and sample identification, taxonomic classification, collector and collection date, general locality information, georeferenced latitude and longitude, and site ownership. Geospatial data should be measured directly and not inferred from locality information. Habitat fields should be consistent across institutions and include data on physical attributes of the site, such as elevation, slope, and aspect. Sampling data should include the number of individuals in the population at the site and number or proportion of individuals from which seeds were harvested (both reflect conservation target level for which a sample is appropriate/suitable).
	Seed maturity is a complex trait under genetic and environmental regulation. Sampling only mature seeds at a single date may impose a genetic bottleneck during sampling. Immature seeds age faster than fully mature seeds, and the faster aging may impose a genetic bottleneck during storage. If the conservation target is a representative sample of the population, it's important that the collection include seeds that matured at different times. If this requires inclusion of immature seeds (because only a single sampling date is possible), special treatment of the sample is needed to avoid bottlenecks during storage.	*Collect seeds that will survive banking* Collect mature seeds for greatest potential longevity. If goal is for sample to genetically represent the population, sample over the entire fruiting season, making multiple trips to the collection site. If only one harvest is possible (because the site is remote), sample the entire range of seed maturities representatively, and alert seed bank curator of need for expedited handling. A sample containing more than 10% immature should be targeted for cryogenic storage and needs to be processed quickly. For plants with very short fruiting season, obtain on-the-ground monitoring of seed maturity, and use collection procedures to capture only mature seeds. Report date(s) of seed collection; associated phenology data such as flowering time and range of maturity of seeds in the population are useful. Do not include seeds from other species in the sample. Immediately after harvest, put seeds under cool (12–15 °C for tropical seed, 5–10 °C for temperate seeds) aerobic conditions, out of direct light, and away from free water. Transmit samples to laboratory-controlled conditions within 1 week.

Processing before storage and storage conditions and safety back-up	Current standards don't provide guidance on initial processing of seeds arriving at genebank.	*Receiving, cleaning, and weighing* Assign a unique, identifying number to each incoming sample and associated collection information, and record date of receipt. Clean sample to reduce bulk and apparent diseased materials, but do not aim for perfection if it takes time away from more vital activities. Keep sample cool and dry (RH < 50%) while cleaning. If debris within sample affects consistency among germination assays, the genebank may wish to prepare a subset for subsequent viability monitor tests. Record mass of seed using IS units. Convention is to record a single mass for 100 seeds; however, replicated measurement of individual seeds is more informative. The relative humidity associated with the seed at time of measurement is useful to assess dry mass. Record number of seeds in sample and an indication of whether number was a direct count or inference based on mass.
	Standards need to be revised to emphasize the point that postponing low temperature storage will negatively impact longevity at storage temperature. Thus, guidelines should emphasize drying times and anomalies in storage temperature. A requirement for "equilibration" to a specific moisture level suggests a certain exactness that is not supported by the literature. Moreover, some combinations of drying RH and drying temperature will result in costly over-drying of seeds when stored at −18 °C. For example, drying at 15% RH will always result in less than 15% RH if the sample is stored at lower temps. Re-equilibration will also always occur once the sample is moved to the storage temperature. Hence, there are many options for drying that will achieve the recommended moisture level, and choice of conditions should be a matter of ambient conditions, costs to dry, and workflow decisions.	*Drying samples to maximize longevity at storage temperature* Dry samples quickly to the appropriate moisture target (between 15 and 25% RH *at the storage temperature*). Drying should occur at less than 30C to avoid damaging the seed. Drying rate depends on drying temperature and should be complete within 2 weeks (dry at 25C) to 2 months (dry at 5C). To achieve moisture target at storage temperature, final RH of seed should not be less than 15%. Upper limit of drying RH is temperature dependent: 34% at 25C, 30% at 15C, and 25% at 5C. Samples should be dried closer to the lower RH range as the risk of storage temperature failure increases. Monitor RH and temperature of drying space continuously, and send automatic alarms when beyond range. Confirm target moisture level is achieved by spot checking the RH within storage containers *at the storage temperature* before completely sealing for 5% of seed samples. If slight adjustments are needed, add calculated quantities of an innocuous water-absorber, such as polished rice, at a pre-adjusted water content. Control RH of seed storage environment using water-impermeable packaging or humidity controls if seeds are not stored in water-impermeable packaging. WVTR (water vapor transmission rate) is the standard to measure water impermeability of packaging and should range from 0.003 to 0.006 g H_2O/m^2/day, depending on ambient RH (lower WTVR needed for ambient RH > 80% and higher WTVR is appropriate when ambient RH is <40%). Metal foil thicknesses of 25 and 10 μm meet the minimum standards for seed banks located in areas of high and low ambient RH, respectively, as long as seal is strong and foil layer is undamaged. Glass containers also have specified WTVR if gasket is frequently replaced. Monitor RH of storage environment surrounding seed packages and spot check RH within storage container in 1% of samples. Replace laminated bags and gaskets when seeds are monitor-tested or after about 30–40 years of freezer storage.

(continued)

Table 10.4 (continued)

Standard	Explanation for difference from current FAO standard	Alternative best practice
	Ideally, standards or best practices would be based on achieving specific longevities, such as 20 years for medium term and 100 years for long term. Because longevities can't be known in advance, standards and best practices are defined by storage temperature that is anticipated to provide the desired outcomes. Revised standards should accommodate possibilities of cryogenic storage, which has been accommodated here by allowing temperatures to be lower than the recommended range.	*Storage temperature to achieve required longevity* Store seeds maintained for use or distribution at 5 ± 3C (i.e., refrigerated or medium-term storage) or below. Store seeds maintained for safety duplicate samples using long-term storage conditions at −18 + 3C (i.e., freezer storage) or below. Record date that seeds were placed at storage temperature. Monitor storage temperature continuously, and have system wired to send automatic alarms when out of range. Prepare incident reports for anomalies documenting dates of malfunction and return to normal as well as temperature deviations. Keep samples away from the heating element of the defroster. Samples in long-term storage should experience temperature fluctuations very infrequently. It is best to remove just a subsample for viability monitoring and distribution while at temperature, so that the entire sample doesn't warm. If warming the entire sample is unavoidable, the sample retrieved from long-term storage should be held in a refrigerator and experience no more than 10 h of ambient room temperature per year. Check RH before resealing, and adjust as needed by slight drying or adding a moisture absorber, as described above.
		Use freezer conditions for archival and safety-duplication Most original samples should also be stored at −18 °C or below. Maintain more than 1500 seeds in safety-duplicate samples (FAO 2014). Wear proper protection, and use a buddy system or a "man-down" alarm system when entering freezer rooms. Follow all OSHA regulations.

Assessing seed quality	Of all seed banking procedures, best practices for initial viability tests and subsequent viability monitoring carry the greatest uncertainty and highest risks for unnecessarily depleting samples or not detecting deterioration in time for regeneration. It must be clear that the standards and best practices are for seeds that are known to be orthodox. If effects of drying and temperature are unknown, preliminary tests are required to ensure the appropriateness of these practices. Current standards attempt to define acceptable quality of seeds. But this can be tricky for wild-collected samples where quality can range considerably and valid methods to assess quality are usually unknown. Our aim here is to assess quality relative to existing knowledge about the species.	*Initial viability testing* Test seed viability soon after receipt at genebank using methods that will allow valid comparisons in subsequent monitor tests. Test the sample after it has been cleaned and weighed and no longer than 8 months after receipt. The earlier the viability test is conducted after harvest, the more information is gleaned about distribution of dormancy and maturity within sample. Do not delay putting seeds at storage temperature until viability has been tested. If viability is not tested immediately upon receipt, a small subsample for germination testing should be kept dry and cold (<30% RH in a refrigerator) during the lag time. Record the date the test was initiated. Seed number in initial test should be sufficient to have a statistical sampling but not so much as to deplete the sample. We suggest about 6% of the sample is used, not to exceed 200 seeds or be fewer than 15 (Guerrant and Fiedler 2004). Record the number of seeds in the assay. Record the conditions used in a germination test. When deciding the conditions to germinate seeds, there is a balance between getting an accurate count of viable seeds and gaining insights on how to induce or synchronize germination. The former informs seed banking, and the latter informs propagation and use strategies. For seed banking purposes, it is best to have standardized procedures for testing seed germination so that the conditions can be replicated in monitor tests (i.e., AOSA rules for seed testing). Viability tests should record counts for normal, abnormal, and ungerminated seeds. It is useful if ungerminated seeds are examined further to determine number of broken, empty (i.e. lack embryo), viable according to additional assessments such as vital staining, and lacking physical integrity (i.e. become mushy during imbibition). Report samples with low viability to collector or donor. Low viability samples have higher than normal proportions of damaged, empty, or mushy seeds. "Normal" proportions are determined from average values obtained from other accessions of the species reported in the literature or by genebanks.

(continued)

Table 10.4 (continued)

Standard	Explanation for difference from current FAO standard	Alternative best practice
	In current standards, flags for management decisions such as regeneration are at 85% of initial viability, which is near the detection limits of change for most seed lots. We believe this revision encourages a more statistically relevant comparison that circumvents arguments that the 85% threshold is arbitrary. Unresolved is whether large decreases in viability occur between sampling intervals.	*Viability monitoring tests* Monitor seed viability using the same conditions as initial viability assessments. Fewer seeds can be used in monitor tests; we suggest about one third of the number of seeds used in viability assessments, not to exceed 60 seeds or be fewer than 15 (Guerrant and Fiedler 2004). Record the number of seeds in the assay. Monitor samples stored under long-term conditions at intervals commensurate with their anticipated longevities: Every 40 to 50 years for long-lived species, 25–30 years for medium lived species, and 15–20 years for short-lived species. Monitor samples stored under medium-term conditions every 4–8 years depending on anticipated longevity. When the species is known to produce orthodox seed but there is no a priori knowledge of longevity, we suggest a subset of about 120 seeds be stored at 25C and 20% RH (main sample is kept at −18 °C). The subset should be monitored four times in a 3-year interval (30 seeds each). Assuming a Q10 = 2, estimate longevity achieved at −18 °C as 16 times the longevity measured in the 25C simulation and set monitoring intervals accordingly (FAO 2014).
Distributing samples	Uses of germplasm differ considerably, and efforts should be made to distribute the needed number of seeds or plant material, without overly depleting the sample.	Median number of viable propagules distributed per request should be 20–50 depending on seed size (fewer for larger seeds) and estimates of viability. Distribute seed numbers based on the users' research needs. A researcher needing an exemplar of the species should be given 5 viable propagules, and someone looking for a rare gene might get 100–200 seeds. If a researcher requires genetic material and not a viable sample, per se, consider sending them leaf tissues arising from a germination assay.

Regenerating samples	We want to stress that a germplasm bank delivers viable samples that are representative of the source population. Hence, there is an inevitable need to regenerate dying or depleted samples. Regeneration introduces high risk that genetic composition will shift. Future users must be provided with some mechanism to account for the genetic changes that have occurred in the population as a consequence of regeneration.	Regeneration plans are fundamental to germplasm banking efforts, because it is essential that live samples are provided in perpetuity. The rigor of regeneration procedures depends on the stated conservation target of the sample, with maintaining the genetic identity of samples representing populations requiring high standards in when regeneration should occur as well as selection and number of parents. Set alerts for regeneration based upon the risk of degrading the conservation target. When the goal is to maintain genetic identity of a heterogeneous population, regeneration should be scheduled when decreased viability is first detected or the sample contains fewer than 200 seeds, which ever comes first. In contrast, regeneration for a genetically homogeneous sample or a species exemplar should be based on probabilities of establishing sufficient number of plants to yield at least 2500 seeds. For wild-collected samples, recollection at the same locale can substitute for regeneration; however, it is likely that samples collected from the same wild population years apart will differ. So as not to deplete the most original sample archived in long-term storage, use it in every third or fourth regeneration, and use F1 and F2 seed for regeneration in between those cycles. If the conservation target is a representative sample of a population, a genetic analysis of the most original sample should be conducted before it is depleted. Report the number of seed-bearing plants used in a regenerated sample. Current standards recommend regenerations should incorporate a minimum of 30 (outcrossing) or 60 (selfing) plants. However, the number of parental lines can be reduced by genetic analyses that identify parental lines that will maximize capture of alleles. Use and document barriers to prevent contamination from other pollen sources.

Best practices are based on FAO genebanking standards for agriculture (FAO 2014, Chap. 4) and standards used by the Millennium Seed Bank, Kew for collecting wild species for conservation purposes (http://www.kew.org/sites/default/files/MSBP%20Seed%20Conservation%20Standards_Final%2005-02-15.pdf). Best practices suggested here are intended to address particular challenges of seeds collected from wild species as well as resolve some scientific uncertainties included in the current FAO standards

usefulness of the sample, especially if the conservation target is a rare allele or an accounting of the population at a particular site and time (e.g., Franks et al. 2008). Plant genebanks can invoke certain activities to forestall, or at least understand, the extent of genetic erosion in curated samples.

10.5.1 Recollect from the Same Wild Population Over Time

Complementation of *in situ* reserves and *ex situ* collections provides a reservoir for replenishing genetic resources from wild populations (Maschinski and Haskins 2012; Guerrant et al. 2004; PCA 2015) as well as an opportunity for identifying traits of interest and how they are distributed in a natural population (e.g., Franks et al. 2008). Probabilities of mutation and fixation through drift are predicted to be lower in natural populations, compared to preserved samples, according to theoretical models (Schoen and Brown 2001). Resampling natural populations also provides the opportunity to measure the extent of genetic changes that have occurred through natural forces and those imposed by the genebank (Thormann et al. 2016; Greene et al. 2014). Programs to collect and resample after 15 years have been recently instituted and include species that are widely distributed in North America (Franks et al. 2008), some of which are priority species according to Khoury et al. (2013).

10.5.2 Regenerate Collected Seeds During Early Phases of Genebanking

Major limitations of seed accessions collected directly from wild populations are low seed number and poor seed quality. As the few available seeds age quickly, situations arise where a sample is regenerated from fewer than ten individuals, resulting in a significant bottleneck. An alternative management practice might be to immediately regenerate a newly collected sample. This would maximize the number of parents contributing to the regenerated sample as well as provide more seeds from a better growth environment and so presumably longer-lived. Studies are underway, using wild-collected germplasm of *Limnanthes*, *Humulus,* and *Artemisia*, to test this hypothesis and gain greater understanding of the interaction between storage time, seed degradation, and shifts in allelic richness for original and offspring populations created before storage and after notable degradation (unpublished; Walters, Richards, Hill, Jenderek).

Sometimes there are simply too few individuals to regenerate a population from seed. Under these circumstances, increasing sample size through clonal propagation can be effective (Pence 2013). When possible, it is important to identify the absolute number of propagules used during regeneration, as well as the number of founders from which they came: 100 individual seeds from a single maternal plant will provide a different regenerated population than one seed each from 100 different plants.

10.5.3 Use Seeds and Pollen to Back Up Wild-Collected Germplasm of Clonally Propagated Crops.

We estimate that over 30,000 accessions are maintained in living collections of the NPGS, collectively called clonal repositories. These field collections are expensive and vulnerable (Volk et al. 2015). Approximately one third of NPGS clonal accessions are CWR (Volk and Walters 2004). With current cryopreservation technologies and resources, we estimate that it will take 50–100 years to backup these accessions in preserved collections, which might not even begin until the higher priority cultivars are backed up – some 100 to 200 years from now. Clearly rapid and effective strategies to preserve these vulnerable collections are needed.

The conservation targets of CWR may be exemplars of species, snapshots of populations, or particular genes for crop improvement, but usually not the specific genotype of the collected plant. In most respects, CWR of clonal crops can be treated analogously to CWR of crops in general. Backing up these collections can be accomplished if there are seeds remaining from wild plant explorations that fit standards for quantity and quality (FAO 2014, Chap. 4 with adjustments as suggested in Table 10.4). When there are not adequate quantities of viable original seed available, trees planted in the field can serve as parents for regenerated populations. The diversity extant in these field collections can be captured through appropriate parental combinations. Feasibility of maximizing diversity with fewest crossing parents using a maximization algorithm was demonstrated (Richards et al. 2004, 2007; Volk et al. 2005), and a detailed genetic analysis shows high efficiency in capturing alleles with only minor introgression from neighboring pollen (Volk et al. 2016). Storing pollen may also be a promising method to capture and backup genetic diversity within collections (Hoekstra 1995; Volk 2011). Some CWR produce seeds that are not as amenable to conventional genebank storage conditions (i.e., they are not orthodox seeds) (Table 10.3). Methods to preserve these materials are available (Walters et al. 2013) but usually labor intensive and associated with some mortality (Wesley-Smith et al. 2014). No shifts in genetic composition were measured in recovering embryos of a high-priority CWR, *Zizania texana*, after cryoexposure (Richards et al. 2004).

10.6 Summary

Genebanks are tasked with ensuring safe preservation of genetic resources so they are available for future use. It is often difficult to predict the eventual use or the timeline for use. The challenge for plant genebanks is to provide viable germplasm that is unaffected by the genebanking experience (i.e., no genetic erosion). This mandate is harder to achieve for natural populations collected from the wild. Some wild-collected materials are likely to resist the extremely controlled conditions of the genebank and will die. Others might adapt and through drift or inadvertent

selection become more domesticated. By first identifying the causes for changes in quality of germplasm in preserved collections and then offering strategies to slow down, or at least quantify, the effects of the genebanking experience, wild-collected germplasm can successfully be conserved in genebanks.

References

Aubry C, Shoal R, Erickson VJ (2005) Grass cultivars: their origins, development and use on national forests and grasslands in the Pacific Northwest, U.S. Forest Service: 50

Ballesteros D, Pence VC (2017) Survival and death of seeds during liquid nitrogen storage: a case study on seeds with short lifespans. CryoLetters 38:278–289

Brockway LH (1979) Science and colonial expansion: the role of the British Royal Botanic Gardens. Am Ethnol 6:449–465

Burton PJ, Burton CM (2002) Promoting genetic diversity in the production of large quantities of native plant seed. Ecol Restor 20:117–123

Charlesworth D, Charlesworth B, McVean GA (2001) Genome sequences and evolutionary biology, a two-way interaction. Trends Ecol Evol 16:235–242

Clerkx EJ, El-Lithy ME, Vierling E, Ruys GJ, Blankestijn-De Vries H, Groot SP, Vreugdenhil D, Koornneef M (2004) Analysis of natural allelic variation of Arabidopsis seed germination and seed longevity traits between the accessions Landsberg erecta and Shakdara, using a new recombinant inbred line population. Plant Physiol 135:432–443

Colville L, Bradley EL, Lloyd AS, Pritchard HW, Castle L, Kranner I (2012) Volatile fingerprints of seeds of four species indicate the involvement of alcoholic fermentation, lipid peroxidation, and Maillard reactions in seed deterioration during ageing and desiccation stress. J Exp Bot 63:6519–6530

Dafni A, Firmage D (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. In: Pollen and pollination. Springer Vienna, Vienna pp 113–132

Daws MI, Lydall E, Chmielarz P, Leprince O, Matthews S, Thanos CA, Pritchard HW (2004) Developmental heat sum influences recalcitrant seed traits in Aesculus hippocastanum across Europe. New Phytol 162:157–166

Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. Ann Bot 45:13–30

Endresen DTF, Knüpffer H (2012) The Darwin Core extension for genebanks opens up new opportunities for sharing genebank datasets. Biodivers Inform 8:12–29

Engelmann F (2011) Use of biotechnologies for the conservation of plant biodiversity. In Vitro Cell Dev Biol Plant 47:5–16

Falk D, Richards CM, Montalvo A, Knapp E (2006) Population and ecological genetics in restoration ecology. In: Falk D, Palmer M, Zedler J (eds) Foundations of restoration biology. Island Press, Washington, DC, pp 14–41

FAO (2001) International treaty on plant genetic resources for food and agriculture. Food and Agriculture Organisation of the United Nations. Available at: http://planttreaty.org

FAO (2014) Genebank standards for plant genetic resources for food and agriculture. Rev. ed, Rome

Fleming MB, Richards CM, Walters C (2017) Decline in RNA integrity of dry-stored soybean seeds correlates with loss of germination potential. J Exp Bot 68:2219–2230

Franchi GG, Piotto B, Nepi M, Baskin CC, Baskin JM, Pacini E (2011) Pollen and seed desiccation tolerance in relation to degree of developmental arrest, dispersal, and survival. J Exp Bot 62:5267–5281

Franks SJ, Avise JC, Bradshaw WE, Conner JK, Etterson JR, Mazer SJ, Weis AE (2008) The resurrection initiative: storing ancestral genotypes to capture evolution in action. Bioscience 58:870–873

Gilligan DM, Frankham R (2003) Dynamics of genetic adaptation to captivity. Conserv Genet 4:189–197

Greene SL, Kisha TJ, Yu L-X, Parra-Quijano M (2014) Conserving plants in gene banks and nature: investigating complementarity with Trifolium thompsonii Morton. PLoS One 9.: art. no. e105145

Guerrant EO Jr, Fiedler PL (2004) Accounting for sample decline during ex situ storage and reintroduction. In: Guerrant EO Jr, Havens K, Maunder M (eds) Ex situ plant conservation: supporting species survival in the wild. Island Press, Covelo, pp 365–385

Guerrant EO Jr, Havens K, Maunder M (eds) (2004) Ex situ plant conservation: supporting species survival in the wild, vol 3. Island Press, Covelo

Guerrant EO Jr, Havens K, Vitt P (2014) Sampling for effective ex situ plant conservation. Int J Plant Sci 175:11–20

Haidet M, Olwell P (2015) Seeds of success: a national seed banking program working to achieve long-term conservation goals. Nat Areas J 35:165–173

Harding K (2004) Genetic integrity of cryopreserved plant cells: a review. CryoLetters 25:3–22

Havens K, Guerrant EO Jr, Maunder M, Vitt P (2004) Guidelines for *ex situ* conservation collection management: minimizing risks. In: Guerrant EO Jr, Havens K, Maunder M (eds) Ex situ plant conservation: supporting species survival in the wild. Island Press, Covelo, pp 454–473

Hay FR, Probert RJ (1995) Seed maturity and the effects of different drying conditions on desiccation tolerance and seed longevity in foxglove (Digitalis purpurea L.). Ann Bot 76:639–647

Hay FR, Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. Conserv Physiol 1. https://doi.org/10.1093/conphys/cot030

Hay FR, Mead A, Manger K, Wilson FJ (2003) One-step analysis of seed storage data and the longevity of Arabidopsis thaliana seeds. J Exp Bot 54:993–1011

Hoban S, Schlarbaum S (2014) Optimal sampling of seeds from plant populations for ex-situ conservation of genetic biodiversity, considering realistic population structure. Biol Conserv 177:90–99

Hoekstra FA (1995) Collecting pollen for genetic resources conservation. In: Guarino L, Rao VR, Reid R (eds). IPGRI/FAO/UNEP/IUCN Collecting plant genetic diversity: technical guidelines. CABI Publishing, Wallingford, pp 527–550

Hokanson SC, Szewc-McFadden AK, Lamboy WF, McFerson JR (1998) Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a Malus×domestica Borkh. core subset collection. Theor Appl Genet 97:671–683

ISBER (International Society for Biological an Environmental Repositories) (2012) Best practices for repositories: collection, storage, retrieval, and distribution of biological materials for research. Biopreserv Biobank 10:79–161

Kahler AL, Kern AJ, Porter RA, Phillips RL (2014) Maintaining food value of wild rice (Zizania palustris L.) using comparative genomics. In: Genomics of plant genetic resources. Springer, Dordrecht, pp 233–248

Khoury CK, Greene S, Wiersema J, Maxted N, Jarvis A, Struik PC (2013) An inventory of crop wild relatives of the United States. Crop Sci 53:1496–1508

Khoury CK, Castañeda-Alvarez NP, Achicanoy HA, Sosa CC, Bernau V, Kassa MT, Norton SL, van der Maeseng LJG, Upadhyaya HD, Ramírez-Villegas J, Jarvis A, Struik PC, Jarvis A (2015) Crop wild relatives of pigeonpea [Cajanus cajan (L.) Millsp.]: distributions, ex situ conservation status, and potential genetic resources for abiotic stress tolerance. Biol Conserv 184:259–270

Kilian B, Graner A (2012) NGS technologies for analyzing germplasm diversity in genebanks. Brief Funct Genomics 11:38 elr046

Kochanek J, Steadman KJ, Probert RJ, Adkins SW (2009) Variation in seed longevity among different populations, species and genera found in collections from wild Australian plants. Aust J Bot 57:123–131

Lawrence MJ, Marshall DF, Davies P (1995) Genetics of genetic conservation. I. Sample size when collecting germplasm. Euphytica 84:89–99

Li D-Z, Pritchard HW (2009) The science and economics of ex situ plant conservation. Trends Plant Sci 14:614–621

Lockwood DR, Richards CM, Volk GM (2007a) Probabilistic models for collecting genetic diversity: comparisons, caveats, and limitations. Crop Sci 47:861–866

Lockwood DR, Richards CM, Volk GM (2007b) Wild plant sampling strategies: the roles of ecology and evolution. Plant Breed Rev 29:285–313

Marshall DR, Brown AHD (1975) Optimum sampling strategies in genetic resources conservation. In: Frankel OH, Hawkes JG (eds) Crop genetic resources for today and tomorrow. Cambridge University Press, Cambridge, UK, pp 53–80

Maschinski J, Haskins KE (2012) Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC

Mazur P, Leibo SP, Seidel GE Jr (2008) Cryopreservation of the germplasm of animals used in biological and medical research: importance, impact, status, and future directions. Biol Reprod 78:2–12

Mead A, Gray D (1999) Prediction of seed longevity: a modification of the shape of the Ellis and Roberts seed survival curves. Seed Sci Res 9:63–73

Menard KP (2008) Dynamic mechanical analysis: a practical introduction. CRC press, Boca Raton

Meyer RS, DuVal AE, Jensen HR (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. New Phytol 196:29–48

Mira S, Hill LM, González-Benito ME, Ibáñez MA, Walters C (2016) Volatile emission in dry seeds as a way to probe chemical reactions during initial asymptomatic deterioration. J Exp Bot 67:1783–1793

Mondoni A, Orsenigo S, Donà M, Balestrazzi A, Probert RJ, Hay FR, Petraglia A, Abeli T (2014) Environmentally induced transgenerational changes in seed longevity: maternal and genetic influence. Ann Bot 113:1257–1263

Nagel M, Börner A (2010) The longevity of crop seeds stored under ambient conditions. Seed Sci Res 20:1–12

Nagel M, Rosenhauer M, Willner E, Snowdon RJ, Friedt W, Börner A (2011) Seed longevity in oilseed rape (Brassica napus L.)–genetic variation and QTL mapping. Plant Genet Resour 9:260–263

PCA (Plant Conservation Alliance) (2015) National Seed Strategy for Rehabilitation and Restoration 2015–2020. www.blm.gov/seedstrategy (visited 18 Feb 2017)

Pence VC (2011) Evaluating costs for the in vitro propagation and preservation of endangered plants. In Vitro Cell Dev Biol Plant 47:176–187

Pence VC (2013) Tissue cryopreservation for plant conservation: potential and challenges. Int J Plant Sci 175:40–45

Pence VC, Philpott M, Culley TM, Plair B, Yorke SR, Lindsey K, Vanhove A-C, Ballesteros D (2017) Survival and genetic stability of shoot tips of *Hedeoma todsenii* after long-term cryostorage. In Vitro Cell Dev Biol Plant 53:328–338

Probert RJ, Adams J, Coneybeer J, Crawford A, Hay F (2007) Seed quality for conservation is critically affected by pre-storage factors. Aust J Bot 55:326–335

Probert RJ, Daws MI, Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. Ann Bot 104:57–69

RBG (Royal Botanic Gardens Kew) (2017) Seed Information Database (SID). Version 7.1. Available from: http://data.kew.org/sid/ (February 2017)

Richards CM, Reilley A, Touchell D, Antolin MF, Walters C (2004) Microsatellite primers for Texas wild rice (Zizania texana), and a preliminary test of the impact of cryogenic storage on allele frequency at these loci. Conserv Genet 5:853–859

Richards CM, Antolin MF, Reilley A, Poole J, Walters C (2007) Capturing genetic diversity of wild populations for ex situ conservation: Texas wild rice (Zizania texana) as a model. Genet Resour Crop Evol 54:837–848

Richards CM, Lockwood DR, Volk GM, Walters C (2010) Modeling demographics and genetic diversity in ex situ collections during seed storage and regeneration. Crop Sci 50:2440–2447

Righetti K, Vu JL, Pelletier S, Vu, BL, Glaab E, Lalanne D Pasha A, Patel RV, Provart NJ, Verdier J,. Leprince O Buitink J (2015) Inference of longevity-related genes from a robust coexpression network of seed maturation identifies regulators linking seed storability to biotic defense-related pathways. Plant Cell 27: 2692–2708

SCBD (2010) Strategic plan for biodiversity 2011–2020. Secretariat of the convention on biological diversity. Available at: http://cbd.int/sp

SCBD (2014) Global strategy for plant conservation. Secretariat of the convention on biological diversity. Available at: http://cbd.int/gspc

Schoen DJ, Brown AHD (2001) The conservation of wild plant species in seed banks. Bioscience 51:960–966

Schwember AR, Bradford KJ (2010) Quantitative trait loci associated with longevity of lettuce seeds under conventional and controlled deterioration storage conditions. J Exp Bot 61:4423–4436

Soulé M (1991) Conservation: tactics for a constant crisis. Science 253:744–750

Tarquis AM, Bradford KJ (1992) Prehydration and priming treatments that advance germination also increase the rate of deterioration of lettuce seeds. J Exp Bot 43:307–317

Thormann I, Reeves P, Thumm S, Reilley A, Engels JMM, Biradar CM, Lohwasser U, Börner A, Pillen K, Richards CM (2016) Genotypic and phenotypic changes in wild barley (Hordeum vulgare subsp. spontaneum) during a period of climate change in Jordan. Genet Resour Crop Evol 64:1–18. https://doi.org/10.1007/s10722-016-0437-5

Towill LE, Forsline PL, Walters C, Waddell JW, Laufmann J (2004) Cryopreservation of Malus germplasm using a winter vegetative bud method: results from 1915 accessions. CryoLetters 25:323–334

Tweddle JC, Dickie JB, Baskin CC, Baskin JM (2003) Ecological aspects of seed desiccation sensitivity. J Ecol 91:294–304

Urban MC (2015) Accelerating extinction risk from climate change. Science 348:571–573

Van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010) Genetic erosion in crops: concept, research results and challenges. Plant Genet Resour Charact Util 8(01):1–15

Volk G (2011) Collecting pollen for genetic resources conservation. In: Guarino L, Ramanatha Rao V, Goldberg E (eds) Collecting plant genetic diversity: technical guidelines—2011 update

Volk GM, Walters C (2004) Preservation of genetic resources in the national plant germplasm clonal collections. Plant Breed Rev 23:291–344

Volk GM, Richards CM, Reilley AA, Henk AD, Forsline PL, Aldwinckle HS (2005) Ex situ conservation of vegetatively propagated species: development of a seed-based core collection for Malus sieversii. J Am Soc Hortic Sci 130:203–210

Volk GM, Waddell J, Bonnart R, Towill L, Ellis D, Luffman M (2008) High viability of dormant Malus buds after 10 years of storage in liquid nitrogen vapour. CryoLetters 29:89–94

Volk GM, Richards CM, Forsline PL (2009) A comprehensive approach toward conserving Malus germplasm. In international symposium on molecular markers in horticulture 859: 177–182

Volk GM, Chao CT, Norelli J, Brown SK, Fazio G, Peace C, Mcferson J, Zhong G, Bretting P (2015) The vulnerability of US apple (Malus) genetic resources. Genet Resour Crop Evol 62:765–794

Volk GM, Henk AD, Forsline PL, Szewc-Mcfadden AK, Fazio G, Aldwinckle H, Richards CM (2016) Seeds capture the diversity of genetic resource collections of Malus sieversii maintained in an orchard. Genet Resour Crop Evol 64:1513. https://doi.org/10.1007/s10722-016-0450-8

Walters C (1998) Ultra-dry technology: perspective from the National Seed Storage Laboratory, USA. Seed Sci Res 8(suppl 1):11–14

Walters C (2015a) Genebanking seeds from natural populations. Nat Areas J 35:98–105

Walters C (2015b) Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. Planta 242:397–406
Walters C, Hanner R (2006) Platforms for DNA banking. In: de Vicente MC, Andersson MS (eds) DNA banks: providing novel options for Genebanks. Bioversity International
Walters C, Wheeler L, Stanwood PC (2004) Longevity of cryogenically stored seeds. Cryobiology 48:229–244
Walters C, Wheeler LM, Grotenhuis JM (2005) Longevity of seeds stored in a genebank: species characteristics. Seed Sci Res 15:1–20
Walters C, Volk GM, Richards CM (2008) Genebanks in the post-genomic age: emerging roles and anticipated uses. Biodiversity 9:68–71
Walters C, Ballesteros D, Vertucci VA (2010) Structural mechanics of seed deterioration: standing the test of time. Plant Sci 179:65–573
Walters C, Berjak P, Pammenter N, Kennedy K, Raven P (2013) Preservation of recalcitrant seeds. Science 339:915–916
Wesley-Smith J, Berjak P, Pammenter NW, Walters C (2014) Intracellular ice and cell survival in cryo-exposed embryonic axes of recalcitrant seeds of Acer saccharinum: an ultrastructural study of factors affecting cell and ice structures. Ann Bot 113:695–709
Wieczorek J, Bloom D, Guralnick R, Blums, Döring M, Giovanni R, Robertson T, Vieglais D (2012) Darwin Core: an evolving community-developed biodiversity data standard. PLoS One 7(1):e29715
Zheng GH, Jing XM, Tao K-L (1998) Ultradry seed storage cuts cost of gene bank. Nature 393:2