Techniques and key issues in collecting crop wild relatives

Michael Way, Royal Botanic Gardens, Kew, UK

1 Introduction

Much of the scientific basis for collecting germplasm from wild plants developed in the 1970s as the potential role for seed banking to confront global threats to plant diversity became clearer. This applied to both the preservation of plant genetic resources for agriculture and conservation of seed for recovery of threatened wild plants. Of the more than 7.4 million germplasm collections estimated by FAO (2010) to be held in gene banks worldwide, Castañeda-Álvarez et al. (2016) reported a critical under-representation in gene banks of crop wild relatives (CWR), which are increasingly recognised as essential for future breeding and research efforts. This chapter reviews the main techniques for collecting seed of CWR, with an emphasis on current scientific challenges faced by CWR collectors, concluding with research issues that influence the quality and genetic diversity of a collection, and therefore its ultimate use by researchers.

Although much work remains to be done to define optimal collecting, handling, storage, and germination conditions, and also to improve the genetic

http://dx.doi.org/10.19103/AS.2020.0085.08

and resource efficiency of programmes, the essential conditions required to keep seeds with orthodox seed storage behaviour viable in conventional seed bank storage are now well established. The seed biology knowledge applied to collecting and conservation of seed, as reviewed by Hay and Probert (2013) and updated by Hay in this volume, has now been tested in practice across all taxonomic groups and environments, this is informed by several decades of large-scale practical seed banking of crop and wild species, for example, by US Agricultural Research Service at National Laboratory for Genetic Resources Preservation (NLGRP), and of wild species by Royal Botanic Gardens, Kew, at the Millennium Seed Bank (MSB). Detailed guidance is now accessible for practitioners in manuals such as Collecting Plant Genetic Diversity (Guarino et al. 2011) and standards such as the FAO gene bank standards (FAO 2014), MSB Partnership collections standards (Breman and Way 2018) and the CPC Best Plant Conservation Practices (Center for Plant Conservation (undated)).

From the collectors' point of view, there are two major research constraints that have impacted our ability to make sufficient high-quality, genetically diverse CWR collections over the last two decades:

First, in contrast to the relatively uniform and well-documented flowering and fruiting of crops, populations of CWR typically retain important traits that have not been narrowed by crop breeding; indeed the collecting and use of CWR is required precisely to overcome that genetic bottleneck (Tanksley and McCouch 1997). Collectors therefore have needed to better understand the natural variation of CWR populations, in order to make good decisions about seed quality (Section 6), genetic sampling (Section 7) and seed handling (Section 8) resulting in viable germplasm with high genetic diversity that is representative of the source population.

Secondly, the detailed knowledge about relative importance, distribution, identification, collecting and handling techniques for CWR remained patchy and fragmented prior to the development of online tools including the prioritised Harlan and de Wet CWR Inventory (Vincent et al. 2013), digitised specimen data through GBIF https://www.gbif.org/ and a range of catalogues and botanical databases reviewed by Garruccio (2011). One approach to integrating the essential information required by collectors in a consistent and useful form for fieldwork has been development of printed CWR target collecting guides covered in Section 3, which have proved useful in collecting regions where access to information is poor.

The main focus of this chapter is the collecting of seed, which has been shown to provide the most cost-effective (Li and Pritchard 2009) ex situ response to the erosion of genetic diversity at species level, for taxa-producing orthodox seed, that is, that tolerate conventional dry and cold storage conditions according to FAO & MSBP standards (FAO 2014; Breman and Way 2018). Wyse et al. (2018) estimated that 90% of 4,888 species on the CWR

inventory are capable of conventional seed bank storage; accordingly there are nearly 500 that require alternative techniques for germplasm collection and preservation, so the protocols for handling 'exceptional species' (Pence 2013) through cryopreservation and recovery need continual development to be able to extend taxonomic coverage in a cost-effective way.

In this chapter the collecting of other germplasm such as pollen, meristems, tubers, cuttings or live plants will not be covered in detail, but resources are suggested (see Section 10) to cover this material, which is generally more difficult and more costly to collect, transport, preserve and use than orthodox seeds, and also requires sampling from more individuals to capture equivalent genetic diversity. Future work needs to determine which exceptional species (Pence 2013) can be best preserved ex situ through use of these methods.

Collecting techniques and issues are explored through the example of the 'Adapting Agriculture to Climate Change: collecting, protecting and preparing crop wild relatives' (CWR Project) https://www.cwrdiversity.org/ managed by the Global Crop Diversity Trust and the Millennium Seed Bank Partnership (MSBP) of the Royal Botanic Gardens, Kew. The CWR Project was launched in 2011 with US$50 million in funding from the Government of Norway, and has been implemented in partnership with national and international gene banks and plant breeding programmes around the world, to find and collect seed of wild relatives of 28 of the world's most important crop gene pools. In 2019, having completed nearly 3,000 collecting-days in the field in 25 countries, the CWR Project reported the collection of seed from an unprecedented 4,644 collections of 371 CWR species, with particular impact on CWR of *Solanum, Vicia, Medicago, Hordeum* and *Oryza* (Global Crop Diversity Trust 2019).

2 Targeting species and regions for collecting

The ongoing and predicted loss of wild CWR populations due to climate change (Jarvis et al. 2008) cannot be effectively countered by opportunistic specimen-collecting as an extension to existing field activities, as populations of potentially useful taxa will be overlooked and genetic diversity may be lost. Instead, the collection of high-quality, genetically diverse seed from populations of priority CWR requires thorough preparation, logistical support and capable personnel to be deployed into the field, often for remote expeditions which need to be planned in a cost-effective manner. Following global level commitments to CWR conservation recognised by the FAO International Treaty (FAO 2001) and the Global Strategy for Plant Conservation (CBD 2010), prioritisation of CWR conservation has successfully made advances at two critical levels. First, the taxa falling within the three accepted gene pools of major crops have been compiled and published as the prioritised Harlan and de Wet CWR Inventory (Vincent et al. 2013) available at www.cwrdiversity

.org/checklist/. Secondly, the coverage of existing germplasm collections from across recorded native distribution of CWR taxa has been analysed following the Gap Analysis procedure demonstrated for CWR by Maxted et al. (2008), subsequently enhanced for *Phaseolus* beans (Ramírez-Villegas et al. 2010), then extended to model the global distribution of 1 076 CWR taxa related to 81 crops by Castañeda-Álvarez et al. (2016). See Maxted in this volume for further developments of this important technique.

To enable conservation actions to proceed at the necessary scale without detailed genetic diversity data, the proxy of 'ecogeographical representativeness' is used (e.g. Parra-Quijano et al. 2011) to maximise the likely capture of population level genetic diversity. We know that there are exceptions where genetic diversity is not always partitioned equally across geographical range, for example, the high proportion of diversity of the lentil, *Lens culinaris*, held in the western part of its range, cited in Maxted et al. (2011). In practice, the CWR Project set as an initial objective the sampling of ten new populations per target taxon within each region of collection. If sufficient well-identified and georeferenced specimens exist, a map of potential distribution can be prepared for each species such as shown in the Brazil CWR Seed Collecting Guide (Embrapa and Kew 2016). Where specimens' predate use of GPS or taxonomic identifications are in doubt then additional field survey should attempt to both relocate and encounter new populations, such as that achieved for wild barley, *Hordeum chilense*, shown in Fig. 1. Sampling of genetic diversity from populations is explored further in Section 7.

As updates were compiled in 2011 to the Collecting Plant Genetic Diversity technical guidelines, the use of digital data sources was already becoming essential for the planning of germplasm collection (Garruccio 2011). Access to geographical and botanical information through online databases, maps, image libraries, citizen science portals and social media has continued to expand, although care needs to be taken with the quality of georeferencing and of naming which is particularly variable from sources that aggregate massive datasets, for example, GBIF https://www.gbif.org/. The checking of raw specimen data during compilation of a research database is therefore essential. In general, one should prioritise georeferenced records that are supported by herbarium voucher specimens determined by a competent botanist. For fieldwork planning, probably the most helpful additional online tool for collectors has been Google Earth which has proved consistently valuable to flag locations of existing collections and to identify remnant vegetation patches and potential access points to habitats for sampling.

Collaboration and consultation at several levels are essential pre-requisites for collecting of CWR. Although the growing breadth of digital data available to collectors through desktop and mobile devices is compelling, this is not a substitute for strengthening cooperation with botanists and communities

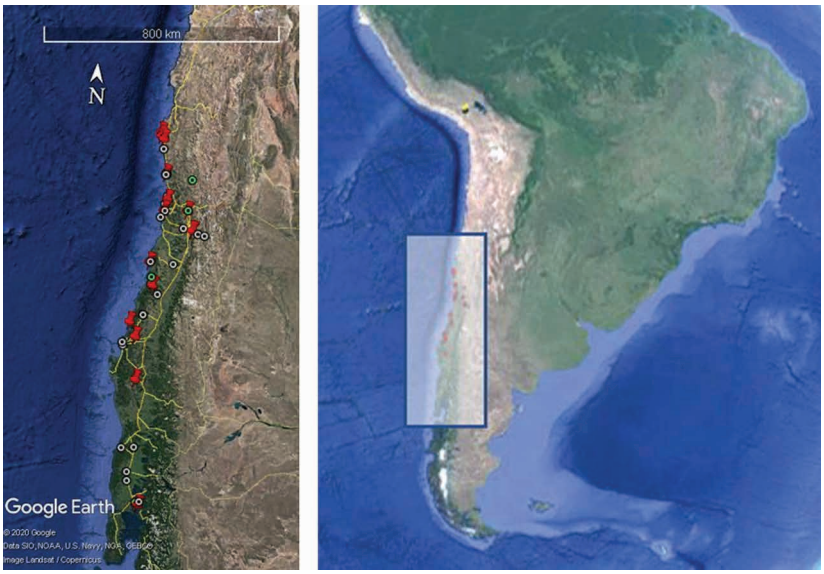

Figure 1 Locations of *Hordeum chilense* specimens compiled by the Chile CWR Project (RBG Kew 2016). Not all historic populations could be relocated for seed collecting, although some new populations were discovered and seed sampling has extended widely across the known distribution of *Hordeum chilense* in Chile. **Grey circles**: Geo-referenced specimens provided by the SGO herbarium, prior to the CWR Project. Collection date ranges from 1854 to 1996 (median 1943). **Green circles**: herbarium specimens from new populations located by the CWR Project. **Red pins**: New seed collections made by the CWR Project.

from the collecting region who can offer local advice and participate in fieldwork. Indeed, they may be able to offer a logistical or staging base for holding botanical collections safely during longer expeditions and could be collaborators for the in situ conservation of plant genetic resources

National legislation implementing the access and benefit-sharing obligations of the Convention on Biological Diversity (CBD) and specifically the Nagoya Protocol usually requires collectors to obtain research and collection permits from national agencies, permit authorities and protected area managers, in consultation with local communities. Specific prior-informed consent may need to be obtained from local providers of genetic resources or for access to traditional knowledge. For ambitious projects operating across multiple administrative boundaries, this consultation and application process is a significant task which needs to be started well in advance of the field season. Projects also need to allow sufficient resources to deliver on the many conditions that attach to collection permits including the deposit of labelled voucher specimens in national facilities, and distribution of reports and publications.

Assuming that permit conditions have been complied with, a set of collections can often be duplicated at research partner facilities in another country under one of the following access routes, which should be determined at the start of a project. Collections of wild species required for biodiversity research (including herbarium vouchers of CWR collections) are often transferred under the terms of the CBD through a bilateral agreement or access contract. In contrast, the supply of germplasm listed within Annex 1 of the International Treaty (FAO 2001) and intended for 'Food and Agriculture' purposes would normally be under the standard Material Transfer Agreement (sMTA) of the IT, following advice from the national focal point for the IT via fao.org/plant-treaty/ (see Louafi, this volume). In addition, the transfer of CWR collections between countries needs to follow plant health regulations and may require inspection of the collections for pest and diseases, a separate phytosanitary certificate and additional packaging for export, and a period of quarantine for germplasm on arrival at the destination.

3 Identifying target CWR taxa successfully in the field

Without up-to-date and accurate botanical keys and descriptions for target taxa, it will be difficult for a field team to be confident that the collecting effort in the field is focussed on the priority species. Even where a team is equipped with field computers and reliable power source, a challenge for collecting CWR taxa is often the scarcity of geolocated, fully identified, digitised collections with accompanying high-quality images either from field or from herbarium, and thus the CWR seed-collecting guides described below provide a practical and consistent way for collectors to locate and identify target species. The content and format of seed-collecting guides continually evolves, but through the use of Brahms database (© University of Oxford) it has been possible to compile essential information to support successful exploration and collecting of seed within the CWR Project. The current format summarises each target taxon on a pair of laminated pages: the first page includes the botanical description, identification notes, and potential distribution maps overlaid with existing specimens, together with habitat and locality information from herbarium labels. The second page, as illustrated in Fig. 2 contains selected images of representative herbarium specimens, and flower, fruit, and mature plant of *Hordeum chilense,* compiled for the Chile seed collecting guide. Additional examples of this approach for CWR Project taxa *Oryza, Solanum, Ipomoea* and *Eleusine* can be reviewed in *Brazil Seed Collecting Guide* (Embrapa and RBG Kew 2016).

Although such guides take several weeks to prepare using all available references and knowledge available to a research team, it is an invaluable resource to collectors in the field and provides all key information required

Figure 2 *Hordeum chilense* excerpt modified from Chile Collecting Guide (RBG Kew 2016). Image top left: Crop Trust – LM Salazar. Other main images: RBG Kew. Inset images at foot: from L to R show: seed; seed collecting method; habit and approximate size of plant; conservation status; phenology information derived from specimens. RBG Kew.

to make collections for a germplasm bank or research project, irrespective of digital connectivity in the field.

In practice, the provision of seed collecting guides has helped facilitate the collection of 94% of the CWR Project's 3 713 priority collection targets (Global Crop Diversity Trust 2019). Continual innovation of this approach is required as

digital data integration improves and as new mobile devices come to market. Already the capacity to post a photograph with provisional name and receive a confirmed identification within minutes from specialists on platforms such as *iNaturalist* https://www.inaturalist.org/ enables collectors to respond to opportunities during fieldwork and to be confident that they have identified the target taxon. In turn the most reliable records submitted to *iNaturalist* are accepted as 'research grade' records by GBIF for wider use, including for Gap Analysis.

4 Understanding seed development, ripening and dispersal

Knowledge of the development of seeds in key families is helpful in order to plan seed collecting expeditions at the correct time and to make good decisions about seed harvesting.

4.1 Biology of seed development

After fertilisation of the ovule, seeds form within the fruit and develop to a point of mass maturity at which the storage reserves of protein, lipid and carbohydrate reach a maximum level. The four stages of seed formation; reserve accumulation; ripening and dispersal are illustrated in the graph (Fig. 3) below. At seed mass maturity, abscission stops the transfer of fluid or nutrients from the vascular tissue of the parent plant and the fruit then undergoes ripening to prepare the seed for dispersal. Ripening is characterised by texture and colour changes, both of the fruit and of the seed tissues. The ripening of dry fruits is accompanied by loss of moisture and hardening of seeds before dispersal. See Section 5 for an overview of common ripening cues.

In terms of seed physiology, a seed collection capable of use following storage in conventional seed banks needs to have three quality traits:

1 high initial viability (i.e. high % germination of fresh seed, excluding empty or infested seeds);
2 full desiccation tolerance (i.e. ability to germinate following drying to 15% equilibrium relative humidity);
3 maximum potential longevity under dry cold storage conditions.

In Fig. 3, these three traits are typically achieved successively in wild species with orthodox seeds, although it should be noted that seed of domesticated cereals like wheat, *Triticum aestivum*; barley, *Hordeum vulgare*; and rice, *Oryza sativa* do not follow this pattern (Hay and Smith 2003).

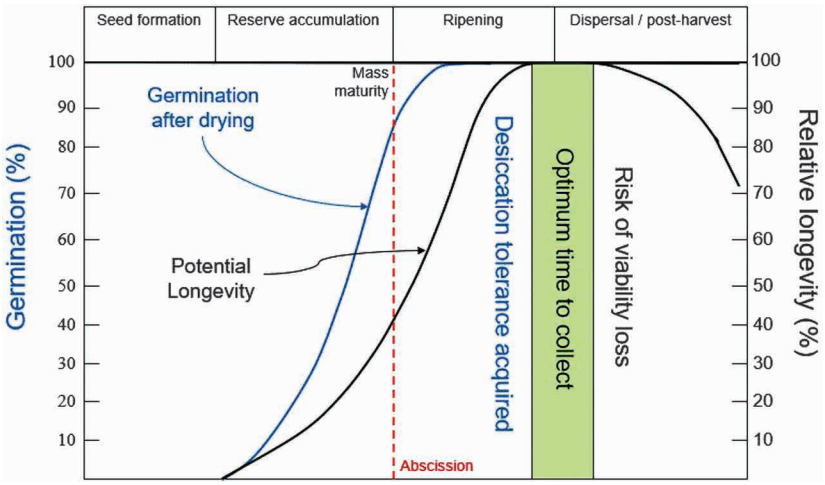

Figure 3 The acquisition of seed quality traits including desiccation tolerance and potential longevity in storage, across four seed development stages, adapted from Hay and Probert (2013) and Way and Gold (2014a). The optimum time to collect seed from wild plant populations for long-term conservation is illustrated with a green column at the boundary of the 'ripening' and 'dispersal' phases, that is, at the onset of natural seed dispersal. Image: RBG Kew. During the final dispersal/post-harvest stage, there is a risk of viability loss if seed is left open to hot and/or humid conditions, and seeds could also be subject to additional predation in this stage especially if within fleshy fruits or dispersed on the ground (see Section 8). For these reasons, the correct timing of seed harvesting is a key factor determining initial seed viability of a collection.

4.2 Predicting timing of fieldwork for seed collecting

In practice, there are two steps that collectors can take to predict optimum time for fieldwork. First, well in advance, herbarium specimen data and literature can be analysed to provide an indicative flowering and fruiting window as shown in the seed collecting guide extract at the foot of Fig. 2. Secondly, it is recommended that one member of the collecting team visit the region for a scouting or survey visit, ideally during the flowering stage of the target species.

To assess phenology of multiple taxa, a review of 111 European species estimated the interval between flowering and seed dispersal as a mean of 3.5 months (range 0.5–12 months) for woody species, and as a mean of 1.8 months (range of 1.0–8.5 months) for herbaceous species. This data is drawn from literature and botanic garden observations (Appendix 3 in ENSCONET 2009) and is an indicative starting point for fieldwork planning. The speed of seed development and ripening in a population depends primarily on plant family and fruit type, described in Section 5 but the altitude, relative humidity and exposure of the population to prevailing weather conditions are also

important factors. Weather reports and forecasts can now be accessed during most expeditions to adjust itineraries while in the region.

Priority tasks during a scouting or survey visit are to confirm the identification, including through making a set of herbarium specimens and recording the geolocation with GPS, and assessing the size and extent of populations. If taxon identification mainly relies on flower features, small markers such as paper jewellers' tags discretely attached to stems can help in relocating the targets later in the season. Additional tasks are to contact local landowners, park authorities and local communities to update them on plans and offer opportunities to participate in fieldwork, and complete logistics including bookings of vehicles, accommodation and equipment.

5 Collecting seed at the correct time

5.1 Variation in flowering and fruiting

Flowering of wild plants in a population is not completely synchronous, so collectors should expect variations between individual plants in the onset, peak and completion of flowering and fruiting. Examples of the variation encountered in flowering and seed development of wild plants are detailed in Hay and Smith (2003), and in the same volume Dickie and Stuppy (2003) provide a helpful review of fruit and seed types. For taxa with indeterminate inflorescences such as *Solanum*, a single individual may bear flowers and fruit at various development stages, for example, as shown for *Solanum asperolanatum* in Fig. 4, and CWR collectors must decide which of the ripening fruit can be successfully harvested. Variation is found across all wild plant groups, for

Figure 4 *Solanum asperolanatum* (Ruiz and Pav) showing flower, ripe fruit and abscission scars from previously dispersed fruit. Image: INIAP Ecuador, Edwin J. Naranjo.

example in wild grasses Poaceae; the individual tillers (reproductive units) can exhibit variations in development between inflorescences, between flowering and dispersal (shattering) within the spike.

In contrast to crops, the development and ripening of seed from herbaceous wild plants is rarely studied in detail, but collecting teams develop experience in estimating the date to return for collecting of ripe seed. An exhaustive study reported by Ison and Wagenius (2014) showed that in a restored population of *Echinacaea angustifolia*, a common insect-pollinated prairie perennial within the Asteraceae tribe Heliantheae, the onset, peak and end of flowering varied markedly between individuals both within and between seasons. For example, the authors showed that although position within the population affected seed production, the timing of flowering had a larger and more consistent effect on output. Flowering was also variable between years, for example, the total number of individuals plants recorded in flower varied successively from 204, 572 to 529 over the three years, and the median flowering date differed by around 10 days between years.

As a consequence of this natural variation, collectors should prepare to collect and handle a range of seed ripeness encountered, and they should use cut tests of a random sample of fruits from the population (which primarily assess physical seed quality, detailed in Section 6), to also confirm the seed development, ripening and dispersal stage.

5.2 Cues for natural seed dispersal

Collectors need to use indicators for fruit ripeness and seed dispersal in order to cope with a large diversity of species in the field. According to Spjut (1994) there are actually as many as 95 botanical types of fruits, but for the collection of CWR the three main categories of fruit (fleshy; dry dehiscent; or dry indehiscent) can guide essential seed collecting and handling in the field.

- **Fleshy fruits** include the berry (*Vitis, Solanum*), drupe (*Prunus*) and hesperidium (citrus, Rutaceae). The relatives of potato, tomato and eggplant, *Solanum* spp., produce berries that are attractive to birds and other fauna that may also play a role in dispersal. Fruit traits that consistently indicate ripening in crops and in wild species include fruit colour and texture. However, fruit size and seed coat colour are less reliable as predictors in wild species (Hay and Smith 2003). Entire, whole, fleshy fruits may need to be collected a few days ahead of natural dispersal in order to harvest before seed is predated or damaged, but they will require careful handling and monitoring as they ripen (see Section 8).
- **Simple dehiscent fruits** include follicles (*Asclepias*), many legumes (Fabaceae), capsules (some Iridaceae) and siliqua (Brassicaceae). As

these fruits progressively ripen, they dry and open, sometimes explosively along weak margins or sutures, often with an additional cue that seeds are audibly free and rattling in their fruits. If the team have easy access to the target population, it is possible to tie small mesh or gauze bags around a sample of the ripening dehiscent fruit, on completion of the population and physical seed quality assessments, ready for return as seed is dispersing. Such bags need to be monitored frequently and seed removed promptly from dehisced fruits before it is subject to predation or ageing.

- **Dry indehiscent fruits** include achenes (*Carex, Ranunculus*), cypsela (*Helianthus*) and caryopsis (Poaceae including CWR of *Oryza, Hordeum, Aegilops, Avena, Secale* and *Triticum*). Note that *Arachis* and some *Medicago* (Fabaceae) have indehiscent pods. At dispersal, wild grasses tend to shatter (i.e. disperse seed progressively by abscission and disarticulation of individual seed dispersal units) from the spike, and gentle pressure from a gloved hand will release the proportion of seed that is at natural dispersal. Ideally, collectors would return on several occasions through the season, but if that is not possible, an approach to consider is to collect a sample (maximum of 10%) of entire grass spikes, complete with a short length of stem, checking that most seeds have reached abscission point and are close to dispersal. If the spikes are protected from fast drying for a few days in a ventilated and shaded tray or box, it is likely that the seed will progressively reach dispersal point and acquire the desiccation tolerance and longevity required for transport and preservation.

In addition to these visual cues for dispersal, the moisture levels of seed within dry fruit types can be assessed by use of a portable hygrometer in the field. By measuring the relative humidity of air that is in equilibrium with seed which almost completely fills a hermetic container as shown in Fig. 5, the equilibrium Relative Humidity (eRH) can be quickly determined (Gold and Manger 2014). Before abscission, seeds are moist, that is, close to 100% eRH. A lower measured seed eRH, that is close to ambient RH, indicates that seeds are close to dispersal point (Hay and Smith 2003), but there are relatively few studies of the moisture relations of wild species' seed, and its significance for seed quality.

Areas for future research include the better prediction and remote monitoring of fruit-ripening indicators. For example, Elliot (2019) reported experiments to identify seed-ripening cues in tree crowns via unmanned aerial vehicles (drones). There is a need to better document indicators of ripening in wild species and much scope to explore the use of cameras, loggers, hygrometers and either drones or remote sensing to monitor seed ripening at the population level, especially within forest habitats.

Figure 5 Use of a portable hygrometer to assess the moisture status of a fully ripe seed collection at harvest. Note that at 62% equilibrium relative humidity, this seed collection can be moved to drying conditions to further reduce viability loss. Image: RBG Kew.

6 Assessing seed quality and quantity

Wild plants do not consistently produce healthy, filled seed available for sampling, indeed the Poaceae and Asteraceae are notorious for the high frequency (sometimes over 50%) of seed that at dispersal is found to be either predated or without embryo (the latter is categorised as 'empty'). Evidence at family level from European seed collections with empty or infested seeds are in the Appendix of the ENSCONET (2009) Manual. In the seed lab a quantitative assessment of seed quality can be made precisely using X-ray (Fig. 6), estimating the % of seeds not expected to be viable, and to use that % to calculate usable seed numbers in a collection.

In the field, collectors need to carry out 'cut tests' of a random sample of around 50 seeds from the target population using secateurs, scissors or other cutting tools, assessed with a minimum of ×10 magnification hand lens, before proceeding with a collection. An example of the cut test is shown in Fig. 7, and this simple step is central to the guidelines in Way and Gold (2014a). In addition to recording the proportion of empty and infested seeds, it also helps to confirm indicators for ripening and dispersal such as abscission points, seed tissue and seed coat colour prior to collecting as described in Section 5. Cut test data from the field is not routinely transferred to gene banks for databasing at present, but there are opportunities to inform

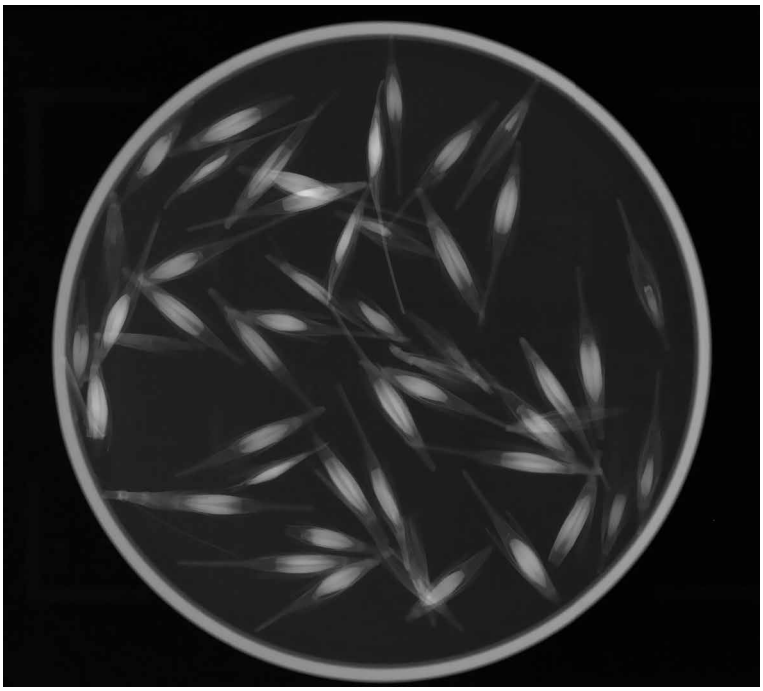

Figure 6 X ray image assessment of 50 seeds from Poaceae, *Hordeum spontaneum*, indicating 41 fully filled, 6 empty and 3 part filled seeds. Image: RBG Kew. Seed provided by Lebanese Agricultural Research Institute.

Figure 7 Cut test through a single cone of Engelmann Spruce *Picea engelmannii*, Oregon 2016, showing at least four filled, hard seeds ready for collecting. Image: RBG Kew.

future seed biology, ecology and plant health studies by publishing these observations.

Cut test data is used to calculate the potentially viable, filled seed available for harvesting from the population, and to ensure that a normal harvesting limit of 20% of seed available on the day of collection is not exceeded (Breman and Way 2018). Although seed production and demographic data is not documented for the majority of wild plants, a review by Menges et al. (2004) is the basis for the comparable CPC Best Practices guideline to generally limit collection to no more than 10% of the whole season's seed crop. In the event that seed availability in a population falls far short of objectives, collectors may choose to make the first of several partial collections from the population, or can continue to explore for more productive populations within the region.

How many seed should be sampled? For two decades the MSB Partnership has recommended that a collection of 10 000 viable seeds is ideal for providing curators, researchers and sister gene banks (i.e. those receiving a duplicate collection for safe storage) with enough seed to meet essential needs for the 50–100 year target lifespan of a typical seed collection (Way and Gold 2014a). If sampled randomly from 50 well-spaced individuals across the full extent of the population, it would also be likely to far exceed the Brown and Marshall (1995) genetic sampling objectives. It is important that any specific demographic or reproductive biology information on the species be used to modify the general approach and to reset the safe harvesting limit if appropriate. With these caveats, this general guideline has been adopted (10 000+ seed objective set by Bureau of Land Management 2015) or adapted for other programmes (e.g. the 5 000 seed objective set by ENSCONET 2009).

Allowing for empty and infested seeds in a collection, a well-resourced collection programme targeting widespread species for restoration can routinely achieve 10 000 viable seeds (Carvey and Way 2016), but for species of restricted distribution or under threat these ideal quantities will not often be met. For example, the global median collection size within the CWR Project is approximately 1 000 seed (RBG Kew unpublished) that could be due to lower seed quality and smaller populations of many CWR taxa.

7 Sampling genetic diversity effectively from populations

7.1 Development of the guidance for population genetic sampling

The sampling of plant genetic resources in recent decades has been guided by research aiming to collect samples of high genetic diversity, representative of the target population, that are intended primarily for research, breeding and preservation in gene banks (Brown and Marshall 1995), as updated by Parra-Quijano et al. (2011) (Crossa and Vencovsky 2011). To achieve capture of 95%

of the non-rare alleles (i.e. those at a frequency of more than 5%) in a typical population, key tenets have been the random sampling across a population from at least 25 individuals (in a predominantly outcrossing species) and from all populations or sub-populations in which there is any evidence of genetic structure. A particular interest has been to capture any possible local adaptations (i.e. locally abundant at population level) that are of potential value for breeders.

Progressively the guidelines from Guerrant et al. (2004) and the CPC Rare Plant Academy https://academy.saveplants.org have continued to refine the genetic sampling recommendations for small or rare plant populations. For example, to enable evaluation, propagation and re-introduction to the wild as part of conservation strategies, it is recommended to keep seed from each maternal line separate when sampling from threatened plants. This approach has also been adopted for programmes confronting specific pest and disease threats, for example, the UK National Tree Seed project (Kallow 2014), and if accompanied by DNA tissue samples collected from the population, provides high resolution for studies of genetic diversity, geneflow and presence of traits such as disease resistance in the population.

In future, research on optimising population sampling must continue rapidly, as it has major resource implications for large-scale ex situ collection programmes supporting food security, species conservation and ecological restoration worldwide. Certainly any knowledge of the reproductive biology and connectivity of populations are central to determining sampling guidance for a species, but as shown by Hoban (2019) it is also important to first agree at programme level the relative value of capturing the rarest of alleles, that is, those present at less than 5% frequency in a population, and how many copies of these alleles are desired in the final seed collection. For collecting of CWR that are thought to have traits of interest to crop breeders, it is especially important to capture locally common alleles by ensuring that populations are sampled across all of the eco-geographic diversity known for the species.

7.2 Determining a plan for genetic sampling within a population

Hoban and Strand (2015) reviewed 15 sets of published seed sampling guidelines, mostly drawing on the underlying work of Brown and Marshall (1995), and drew attention to two factors in particular which were not given weight in the 1995 guidelines: first, the significance of spatial clustering of related individuals in a population, as a result of dispersal and recruitment patterns, and secondly the relatedness of seeds from a single maternal plant as a result of a limited number of pollen donors, especially where pollinated by insects.

In practice, how should collectors plan to sample, given the often spatially heterogeneous (patchy) nature of individuals within a population? Taking into

account the best collecting technique (Section 8) and observations on ripeness (Section 5) and physical seed quality (Section 6), collectors need to use a random sampling approach from the full accessible extent of the population. Hoban and Strand (2015) illustrate the likely superior genetic capture modelled from a truly random or grid pattern of sampling, compared to collecting either by transect or from one location on the perimeter of the population (the latter is termed 'points' sampling, evaluated as the worst of all the sampling options). The separation distance between sampled individuals is also critical to minimise relatedness of seed, and typical recommendations would be a minimum of 50 m between mature trees (Kallow 2014), or 10 m between herbaceous plants as shown in Fig. 8.

The research by Ison and Wagenius (2014) has revealed that in the case of *Echinacea angustifolia* a combination of spatial and flowering variation can significantly affect the distance that pollinators travel, and therefore the seed produced from longer-distance pollination events at the beginning and end of flowering could be especially valuable for sampling. For insect-pollinated species in particular, collectors do not need to therefore concentrate effort entirely at the peak of seed production, but may be able to capture higher genetic diversity by sampling randomly on two or more dates during the season and pooling ('bulking') the samples.

Figure 8 A team collecting seed from a large population of *Hordeum chilense* near a windfarm at Talinay, Chile. Collectors are maintaining an agreed separation distance between harvested individuals, using multiple transects as the sampling approach. Image: Crop Trust – LM Salazar.

8 Techniques for collecting and post-harvest handling

A high-quality collection will comprise sufficient ripe seed of high viability, handled with care in the field and accompanied by a set of herbarium vouchers, associated botanical collections and comprehensive field data. The possible transfer of pests or pathogens between sites and collections will be minimised by good plant health practices in the field.

8.1 Seed collecting

Seed collecting from CWR in the field is usually by hand, selecting the most effective of the techniques summarised in Way and Gold (2014b) that will successfully harvest agreed ripeness and quality of seed from randomly selected individuals as described above. In brief, techniques such as plucking individual indehiscent or fleshy fruit, or gently shaking stems with dehiscent fruit or wind dispersed seed, are more likely to harvest a single cohort of seed ripeness, and accordingly post-harvest handling will be straightforward. In contrast, the pruning of clusters of mixed ripeness fruits or entire spikes or racemes may introduce a wider range of seed ripeness into the collection. Ventilated cloth bags are the material of choice for collectors but paper envelopes are preferable for awned seeds or hooked fruits, and plastic is required for handling fleshy fruits. All containers are labelled inside and out with the unique field collection number that is also used for associated botanical collections and data. Seed samples are bulked together from the population unless individual maternal lines have been specifically requested for multiplication, genetic analysis or trait screening.

Compared to some botanical inventory in which dozens of specimens can be quickly collected at a single location, collection of seed of high genetic diversity and quality usually requires more time to be carried out correctly. For example, summary data from the CWR Project (Global Crop Diversity Trust 2019) indicates that a global average of 1.5 seed collections was made per field day (n = 4 644 samples) across the 371 taxa sampled from four continents. Allowing for travel time, taxonomic identification and population assessment, this is consistent with a long-term average that from one to two hours is often needed for the practical collection of seed of wild species with accompanying herbarium vouchers and field data (e.g. Way 2003).

Note that although mechanised seed harvesting from wild populations is often attempted in large-scale restoration programmes for immediate sowing of seed, it is not recommended for CWR because of the likelihood of harvesting some seed before abscission and also for the additional physical damage likely to the seed coat (described by Dickie and Stuppy 2003 after seed scarification) which could affect viability and longevity of CWR collections. Such techniques

also increase the risk of exceeding the conservation limits to seed collecting from rare or threatened taxa, and could increase collateral damage to other species in the community.

8.2 Post-harvest handling of seed collections

The post-harvest seed handling recommendations of Gold (2014) rely on management of ripeness and speed of drying of a collection, while always avoiding hot, humid and anoxic conditions which would reduce seed viability and longevity. Measurements of seed equilibrium Relative Humidity (eRH) using a hygrometer as described in Section 5 can continue to guide post-harvest handling with reference to safe moisture thresholds, that is, above 85% eRH, seeds are thought to be capable of repair, and below about 60% eRH, seed ageing is significantly reduced (Hay and Smith 2003). For example, Fig. 5 shows a recently sampled seed collection; if the surrounding air is at 40% RH, the reading of 62% eRH indicates that the seed is in the ripening phase just prior to natural dispersal and should be allowed to continue gently drying in a ventilated, shaded tray or box with frequent monitoring.

If the ambient conditions are humid (i.e. the daytime relative humidity is over 50%) then fully ripe, dry collections at risk of re-absorbing moisture can be placed in a hermetic barrel with a little dried silica gel to absorb excess moisture, as shown in Fig. 9. As ambient humidity rises at night, collections should preferably be placed on open newspaper in an air-conditioned room for checking and for ventilation. If this is not possible during remote expeditions, then seed collections can be placed in polythene bags overnight, but must be ventilated as soon as possible in the morning. Under no circumstances should freshly harvested seed, especially if over 50% eRH moisture status, be left in an unventilated or stationary vehicle in the sun – the high temperatures and humidity could be lethal to any fresh germplasm collection.

Mixed ripeness collections present a challenge. If the ripe and unripe portions of the collection cannot be separated for individual handling, then the mixed ripeness collection should be handled as though unripe, that is, not yet desiccation tolerant. Examples are given in Gold (2014) of fruit colour indicators used in practice to make decisions about handling of this material. **Recalcitrant seeds** will not acquire desiccation tolerance during handling in the field and will need to be held in moist, ventilated conditions during fieldwork and transferred promptly to the nursery or gene bank for the appropriate next ex situ preservation step.

8.3 Herbarium vouchers and associated botanical collections

The set of pressed plant (herbarium) specimens taken at the time of seed collection will be used to provide an updatable botanical name for the seed

Figure 9 A hermetically sealed barrel with dried silica gel in the base can be used in the laboratory to actively dry incoming seed collections before processing or storage. In the field, partially dried collections can be held and transported in the barrel to ensure that they do not absorb moisture and enter the 60–85% eRH moisture zone which will be especially damaging. For recently harvested seed collections, significant drying can be achieved by placing an equal mass of silica gel to seed in the barrel. Image: RBG Kew.

collection, and are the first point of reference for any concerns about the identity of the collection. It is important that these 'vouchers' are truly representative of the population and individuals that were harvested. For perennials, it may be possible to take a set of four specimens from an individual that has also been sampled for seed. Annuals that have similar appearance and phenology to the nearby sampled individuals may be collected as vouchers, in all cases sharing the unique field collection number which may follow an individual botanist's number series, or a team's series created for a series of expeditions under a single project or organisation.

The minimum requirements for a quality herbarium specimen, as well as family-specific guidance on material to harvest and prepare have been well

documented, for example, in the Herbarium Handbook (Bridson and Forman 2000, 3rd edition) and in the well-illustrated Southern African herbarium user manual (Victor et al. 2004). Rapid drying of herbarium specimens is needed within 48 h of collection to preserve flower and leaf characters and ideally this can be done in ovens or driers at a collaborating facility while the main collecting is underway. If not, portable driers can be used with care on the expedition, or the bundles of specimens can be preserved with ethanol in strong plastic bags to prevent deterioration in transit. The presence of ripe fruit on specimens is usually of interest to botanists, but there is a high chance that the physical pressing and heat of drying will cause any dry fruits to be disarticulated and separated, if not lost, from the herbarium material. Fruits and/or seeds should be placed in a separate envelope, labelled and inserted within the main specimens.

The most common additional material to also sample on a CWR collecting expedition is tissue for DNA analysis. The protocol provided in the CPC Best Plant Conservation Practices (CPC undated) provides advice on material selection and rapid drying with silica gel. Once in silica, the individual labelled packets can be stacked in a rigid box and are quite robust for transport to a herbarium or gene bank. Other germplasm such as tubers, or cuttings, are very vulnerable to transport and storage conditions and should be handled as carefully as recalcitrant seeds, maybe using an insulated coolbox for travel in the hottest part of the day and keeping in shade at all times.

8.4 Documenting the CWR collection in the field

The essential passport data common to germplasm collection programmes is largely defined by Darwin Core (Darwin Core Task Group 2009) with extensions of particular interest and need for programmes. Most collecting organisations have explored the use of tablets and field computers for field data capture on germplasm expeditions. Although mobile applications with positioning and data capture are widespread, paper data forms are always carried as a backup in the event that devices fail or power is lost during the collecting trip. The hoped-for benefits of access in the field to full botanical catalogues and help-lists to speed up and streamline data entry has not yet been demonstrated with resilience in a field setting. However, other sectors are consolidating GIS, GPS and data-form entry for their applications, so collectors need to continue developing workflows and testing systems for field botany and germplasm collecting. Use of pre-printed or locally generated barcodes or QR codes which can speed collection data transfer and specimen sorting and increase the accuracy of record keeping would also be valuable.

There have been advances in integration of GIS base maps, GPS positioning and geolocated image capture successfully in the field, for example, the

value of GPS-enabled data recording during seed collecting was reported by Mayzlish Gati et al. (2018). Nowak et al. (2020) review the leading mobile applications available for field mapping based on various workflows, including the ability of the Open Data Kit software to collect data offline from remote sites, followed by aggregation of data centrally once connections are restored. Also within their scope is the interaction with specialist identifiers on citizen science platforms such as iNaturalist and PlantNet https://plantnet.org/ which could potentially expand the community of people that may be willing to send in records of distribution, flowering or fruiting of target species to support a collecting programme.

Field data that is comprehensive and accurate will be of immediate value to the gene banks receiving the associated material, but as soon as possible data will be accessible to a wide range of users through online portals such as GBIF https://www.gbif.org/, Genesys-PGR https://www.genesys-pgr.org/ and the MSB Partnership Data Warehouse http://brahmsonline.kew.org/msbp/SeedData/DW. Samples from collections that meet minimum seed and data quality requirements will be promoted to user groups and partners subject to access agreements. For example, the CWR Project has sent samples of 3 218 collections to eight international gene banks. They will be used to identify traits of value in crop breeding such as tolerance of heat, drought, salinity and waterlogging, resistance to pests and diseases, resistance to root rot and improved yield. For this reason field data on geology, soil type, exposure and habitat will be of value for selection of potential breeding material.

8.5 Plant health practices

Many CWR genera are listed in national or regional plant health regulations because of the possible risk to agriculture, horticulture and forestry from inadvertent introduction of pests and diseases through plant material movement. This places a duty on programmes to be well informed in advance of fieldwork, using sources cited in Macfarlane et al. (2011), and to maintain cleanliness of clothing and equipment between sites and between collections. Kallow (2014) includes disinfection instructions that the collecting team follow to clean equipment between sites, but the knowledge about likely presence of a pest or pathogen on a particular site is often poor; therefore programme risk assessments and mitigating actions are typically very general. Rapid pest and pathogen prediction or assessment tools with capability on and offline would be valuable to manage these risks in the field.

During post-harvest handling and transport of collections to the gene bank, it is equally important for collections to be labelled and not contaminated by nearby collections. For example, bags should not be re-used for other taxa, and newspaper should be discarded after collections have been ventilated

overnight. Controlled (quarantine) collections may need to be triple-bagged within a larger shipment in order to control spread of pests or pathogens, and should be handled to quarantine standards until inspected by a plant health specialist.

9 Future research directions

For orthodox-seeded species, the underpinning sciences of seed biology, taxonomy and ecology continue to inform the essential requirements for the collecting, handling and conservation of germplasm from CWR species. These guidelines are widely available in literature such as the FAO gene bank standards (FAO 2014) the MSBP standards (Breman and Way 2018), the ENSCONET (2009) manual and the CPC Best Plant Conservation Practices. Although effective in most cases to deliver viable seed to gene banks the past guidance has not always been optimal in two respects (a) for sampling of genetic diversity and (b) for achieving target longevity in storage of all species.

9.1 How can knowledge of genetic sampling be strengthened?

Current ex situ conservation targeting has limitations. For example, Griffiths et al. (2014) showed that the large ex situ MSBP collection resulting from priority given to threatened and useful species does not represent optimal sampling of phylogenetic diversity in the Leguminosae. Within the botanic garden community, Hoban et al. (2020) showed that for 11 woody taxa across five genera, the ex situ collections were not optimal for preserving genetic diversity of the taxa, and the minimum collection requirements were not consistent within genera, for example, *Zamia decumbens* would require almost twice the number of conserved individuals to maintain diversity compared to its congener *Z. lucayana*. Simulations by Hoban (2019) indicate that more extensive sampling of as many as 1 000 individuals across the natural range may be advisable, with an increasing emphasis on locating additional populations and individuals for sampling to maximise the genetic diversity that can be captured. It is time to expand research across ex situ networks to assess the diversity of germplasm held in terms of ecogeography, genotypes and traits. Resulting knowledge will enable gap analysis for future collecting, and lead to a greater coordination of in situ and ex situ conservation efforts, and between collection holders and germplasm users. Within ex situ collection holders the concept of the multi-institutional meta-collection (Griffith et al. 2019) is encouraging curators to think about appropriate, but not excessive duplication of genotypes between organisations curating collections.

Given the threats to CWR wild populations, for example, that reported by Jarvis et al. (2008), and specifically identified for inventories of Mexican CWR (Contreras-Toledo et al. 2018) and North American CWR (Frances et al. 2018),

we cannot wait for completion of species-specific genetic diversity studies before undertaking conservation actions, rather we have to use the best available evidence to guide sampling before wild populations are lost. Where the need is urgent, there is scope to carry out a rapid 'genetic risk assessment' for seed collection and subsequent use, such as that reported by Gargiulo (2019) for 44 native woody plants of the UK in preparation for seed sampling by the UK National Tree Seed Collecting Project. This could be one way that sampling opportunities can be based on minimum evidence without delay or excessive research cost.

9.2 Which taxa are sensitive to standard handling methods and what more research is needed?

The most recent review of seed desiccation tolerance by Wyse and Dickie (2016) showed that there is a high agreement between desiccation sensitivity status of species within the same genus, and so programmes can predict behaviour in most genera using online sources such as the Seed Information Database https://data.kew.org/sid/. Seed longevity knowledge is also expanding, as Colville and Pritchard (2019) have reviewed several decades of seed performance in gene banks. They note the underlying importance of phylogeny (e.g. Poaceae grasses and Asteraceae are generally shorter lived than Fabaceae and Malvaceae) to determine lifespan in storage, and compare the affect of different gene bank conditions. Building on this and on research reviewed by Hay and Probert (2013), further work is needed to identify taxa for which collecting and handling protocols are not currently optimal, for example, to determine for wild grasses whether the occasional ex situ ripening of whole spikes, as described in Section 5, affects the longevity of seed in storage.

Gene banks responded quickly to evidence (Ballesteros and Pence 2017) that harvested seed of very small seeded orthodox taxa such as Salicaceae were not maintaining viability under conventional conditions and they introduced rapid transport to gene banks for checking and cryopreservation of part of the sample. Further research will alert collectors to other plant groups that need special handling. Research must also continue into the exceptional species (Pence 2013) for which embryo rescue, cryopreservation, in vitro cultivation and micropropagation may be recommended as the only way to safely preserve germplasm that may be sensitive to dry or cold conditions.

9.3 Deploying research knowledge in the field

Ultimately it is the skills and knowledge of the field team that determine the success of a collecting expedition. The core competencies of a collector continue to rely on botanical identification skills, thorough knowledge of seed development and ripening, and attention to detail in preparation for fieldwork,

in data recording and the correct handling of seed and herbarium vouchers. Often this is combined with a talent for exploration and the curiosity to research the ecology and distribution of plants and associated species in the target region, together with an ability to engage local botanists and communities to play an active role in conservation of these genetic resources. Collectors continue to experiment with tablets and other field devices to help with field retrieval of the wealth of digital data now available, and to improve capture of their own collecting data, but new workflows resilient to field conditions need to be developed, tested and published.

In conclusion, our objective is not purely to deliver viable, adequately representative germplasm collections of CWR to gene banks and programmes, but to increasingly demonstrate that the collections are directly meeting recognised sampling gaps and research needs. Through application of new knowledge and technology in the field, we need to collect and handle seed of all target species in ways to maximise the longevity in storage and increase the usefulness of samples for research and conservation.

10 Where to look for further information

Guidelines

Collecting Plant Genetic Diversity Technical guidelines, 2011 update, edited by L. Guarino, V. Ramanatha Rao and E. Goldberg, Bioversity International https://cropgenebank.sgrp.cgiar.org/index.php/procedures-mainmenu-242/collecting

MSB Partnership standards online http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/MSBP-Seed-Conservation-Standards.pdf

MSBP technical information sheets http://brahmsonline.kew.org/msbp/Training/Resources

Center for Plant Conservation Best Plant Conservation Practices to Support Species Survival in the Wild (undated) https://academy.saveplants.org/best-practices/cpc-best-practice-guidelines-table-contents

ENSCONET (2009) Seed Collecting Manual for Wild Species ISBN: 978-84-692-3926-1 Citation: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/ENSCONET_Collecting_protocol_English.pdf

Other useful resources

Research on Exceptional species: Pence, V.C. (2013) "In Vitro Methods and the Challenge of Exceptional Species for Target 8 of the Global Strategy for Plant Conservation," *Annals of the Missouri Botanical Garden* 99(2):

214–220, (13 December 2013). https://doi.org/10.3417/2011112 www.jstor.org/stable/42703725
Review of seed conservation research. Hay F.R.; Probert R.J. (2013) Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology* 1. doi:10.1093/conphys/cot030 Open Access
Crop Wild Relative global inventory https://www.cwrdiversity.org/checklist/
Seed Information Database (seed storage behaviour and germination) https://data.kew.org/sid/
Genesys-PGR the online platform for access to information on PGR collections https://www.genesys-pgr.org/

11 Acknowledgements

This chapter draws extensively on research and guidelines developed by the Millennium Seed Bank Partnership, ENSCONET, and the Center for Plant Conservation. Images and data have been kindly provided by RBG Kew, the Global Crop Diversity Trust and CWR Project Partners. Particular thanks are due to Chris Cockel (RBG Kew), Luis M Salazar (Global Crop Diversity Trust), Pedro León Lobos and Sergio Ibañez Browne (INIA Chile), Edwin J. Naranjo (INIAP Ecuador) and to an anonymous reviewer for comments on an earlier version of the manuscript.

12 References

Ballesteros, D. and Pence, V. C. (2017). Survival and death of seeds during liquid nitrogen storage: a case study on seeds with short lifespans. *Cryo Letters* 38(4): 278–289.
Breman, E. and Way, M. (2018). Safe for the future: seed conservation standards developed for the Millennium Seed Bank Partnership. In: *Proceedings of the EuroGard VII Congress*. 6–10 July 2015. Paris, France. pp. 267–274. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/MSBP-Seed-Conservation-Standards.pdf.
Bridson, D. and Forman, L. (2000). *The Herbarium Handbook* (3rd edn.). RBG Kew, UK.
Brown, A. H. D. and Marshall, D. R. (1995). A basic sampling strategy: theory and practice. In: *Collecting Plant Genetic Diversity: Technical Guidelines. 2011 Update* Guarino, L., Ramanatha Rao, V. and Reid, R. (Eds). CABI Publishing, Wallingford, UK, pp. 75–91.
Bureau of Land Management. (2015). *Seeds of success collecting protocol*. Available at: https://www.blm.gov/sites/blm.gov/files/program_nativeplants_collection_quick%20links_technical%20protocol.pdf.
Carvey, N. and Way, M. (2016). *Exploring millennium seed bank collections from North America- a case study*. Millennium Seed Bank Data Warehouse, RBG Kew. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/MSBP_Data_Warehouse_USA_study.pdf.
Castañeda-Álvarez, N. P., Khoury, C. K., Achicanoy, H. A., Bernau, V., Dempewolf, H., Eastwood, R. J., Guarino, L., Harker, R. H., Jarvis, A., Maxted, N., Müller, J. V., RamirezVillegas, J., Sosa, C. C., Struik, P. C., Vincent, H. and Toll, J. (2016). Global

conservation priorities for crop wild relatives. *Nature Plants* 2: 16022. https://doi.org/10.1038/nplants.2016.22.

Center for Plant Conservation. (undated). *CPC Best Plant Conservation Practices to Support Species Survival in the Wild*. Available at: https://academy.saveplants.org/best-practices/cpc-best-practice-guidelines-table-contents.

Colville, L. and Pritchard, H. W. (2019). Seed life span and food security. *New Phytologist* 224(2): 557–562. https://nph.onlinelibrary.wiley.com/doi/epdf/10.1111/nph.16006.

Contreras-Toledo, A. R., Cortés-Cruz, M. A., Costich, D., Rico-Arce, M., Brehm, J. M. and Maxted, N. (2018). A crop wild relative inventory for Mexico. *Crop Science* 58(3): 1292–1305. https://doi.org/10.2135/cropsci2017.07.0452.

Convention on Biological Diversity. (2010). *Global strategy for plant conservation*. The Secretariat of the Convention on Biological Diversity, Montreal. Available at: https://www.cbd.int/undb/media/factsheets/undb-factsheet-gspc-en.pdf.

Crossa, J. and Vencovsky, R. (2011) Chapter 5. Basic sampling strategies: theory and practice. In: *Collecting Plant Genetic Diversity: Technical Guidelines. 2011 Update* Guarino, L., Ramanatha Rao, V. and Goldberg, E. (Eds). Bioversity International. Available at: https://cropgenebank.sgrp.cgiar.org/index.php/procedures-mainmenu-242/collecting.

Darwin Core Task Group. (2009). *Simple Darwin Core*. Biodiversity information standards (TDWG). Available at: http://rs.tdwg.org/dwc/terms/simple/.

Dickie, J. B. and Stuppy, W. H. (2003). Seed & fruit structure. In: *Seed Conservation: Turning Science into Practice* Smith, R. D., Dickie, J. D., Linington, S. H., Pritchard, H. W. and Probert, R. J. (Eds). pp. 253–279. Royal Botanic Gardens, Kew, UK.

Elliot, S. (2019). *Droning for seeds, Samara 33*, p. 4. RBG Kew. Available at: https://www.kew.org/sites/default/files/Samara%2033%20web.pdf.

Embrapa and RBG Kew. (2016). *Brazil Seed Collecting Guide*. Allen, R. and Heiden, G. (Compilers). 46 pp. https://doi.org/10.13140/rg.2.2.34706.25289.

ENSCONET. (2009). *Seed collecting manual for wild species*. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/ENSCONET_Collecting_protocol_English.pdf. ISBN: 978-84-692-3926-1.

Food & Agriculture Organisation. (2001). The international treaty on plant genetic resources for food and agriculture, adopted by the thirty-first session of the conference of the food and agriculture organization of the United Nations, 3 November 2001.

Food & Agriculture Organization. (2010). *The second report on the state of the world's plant genetic resources for food and agriculture*. Rome. Available at: http://www.fao.org/3/i1500e/i1500e.pdf.

Food & Agriculture Organisation. (2014). *GeneBank standards for plant genetic resources for food and agriculture* (rev. edn.). Rome. Available at: http://www.fao.org/3/a-i3704e.pdf.

Frances, A. L., Smith, A. B. and Khoury, C. K. (2018). Conservation status and threat assessments for North American crop wild relatives. In: *North American Crop Wild Relatives (Vol. 1)* Greene, S. L., Williams, K. A., Khoury, C. K., Kantar, M. B. and Marek, L. F. (Eds). Springer, Cham. Available at: https://link.springer.com/chapter/10.1007%2F978-3-319-95101-0_7. https://doi.org/10.1007/978-3-319-95101-0_7.

Gargiulo, R. (2019). *Pyrus Cordata Desv. Genetic Risk Assessment*. Research report. Kew Research Repository. Available at: https://kew.iro.bl.uk/work/278bd662-d8af-4017-992d-5a7d26f84ec4. doi: 10.34885/17.

Garruccio, M. (2011), Chapter 13. Published information resources for plant germplasm collectors. In: *Collecting Plant Genetic Diversity: Technical Guidelines. 2011 Update* Guarino, L., Ramanatha Rao, V. and Goldberg, E. (Eds). Bioversity International. Available at: https://cropgenebank.sgrp.cgiar.org/index.php/procedures-mainmenu-242/collecting.

Genesys (undated). *Global Portal on Plant Genetic Resources*. Available at: https://www.genesys-pgr.org/, Accessed 1 May 2020.

Global Crop Diversity Trust. (2019). *A Global Rescue: Safeguarding the Worlds Crop Wild Relatives*. Available at: https://www.cwrdiversity.org/.

Gold, K. (2014). *Post harvest handling of seed collections.* RBG Kew Technical Information Sheet 4. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/04-Post-harvest-handling.pdf.

Gold, K. and Manger, K. R. (2014). Measuring seed moisture status using a hygrometer. RBG Kew Technical Information Sheet 5,. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/05-eRH-moisture-measurement.pdf.

Griffith, M. P., Beckman, E., Callicrate, T., Clark, J., Clase, T., Deans, S., Dosmann, M., Fant, J., Gratacos, X., Havens, K., Hoban, S., Lobdell, M., Jiménez-Rodriguez, F., Kramer, A., Lacy, R., Magellan, T., Maschinski, J., Meerow, A. W., Meyer, A., Sanchez, V., Spence, E., Toribio, P., Walsh, S., Westwood, M. and Wood, J. (2019). *Toward the Metacollection: Safeguarding Plant Diversity and Coordinating Conservation Collections*. Botanic Gardens Conservation International-US, San Marino, CA. Available at: https://www.bgci.org/resources/bgci-tools-and-resources/toward-the-metacollection-coordinating-conservation-collections-to-safeguard-plant-diversity/.

Griffiths, K. E., Balding, S. T., Dickie, J. D., Lewis, G. P., Pearce, T. R. and Grenyer, R. (2014). Maximising the phylogenetic diversity of seed banks. *Conservation Biology* 29: 370–381. https://doi.org/10.1111/cobi.12390.

Guarino, L., Ramanatha Rao, V. and Goldberg, E. (Eds.) (2011). *Collecting Plant Genetic Diversity: Technical Guidelines. 2011 Update*. Bioversity International, Rome, Italy. ISBN 978-92-9043-922-6. Available at: https://cropgenebank.sgrp.cgiar.org/index.php/procedures-mainmenu-242/collecting.

Guerrant, E. O., Fiedler, P. L., Havens, K. and Maunder, M. (2004). Revised genetic sampling guidelines for conservation collections of rare and endangered plants. In: *Ex Situ Plant Conservation: Supporting Species Survival in the Wild* Guerrant, E. O., Havens, K. and Maunder, M. (Eds). Island Press, London.

Hay, F. R. and Probert, R. J. (2013). Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology* 1(1). doi: 10.1093/conphys/cot030 Open Access.

Hay, F. R. and Smith, R. D. (2003). Seed maturity: when to collect seeds from wild plants. In: *Seed Conservation: Turning Science into Practice* Smith, R. D., Dickie, J. D., Linington, S. H., Pritchard, H. W. and Probert, R. J. (Eds). Royal Botanic Gardens, Kew, UK, pp. 97–133.

Hoban, S. (2019). New guidance for ex situ gene conservation: sampling realistic population systems and accounting for collection attrition. *Biological Conservation* 235: 199–208. https://doi.org/10.1016/j.biocon.2019.04.013.

Hoban, S., Callicrate,T., Clark, J., Deans, S., Dosmann, M., Fant, J., Gailing, O., Havens, K., Hipp, A. L., Kadav, P., Kramer, A. T., Lobdell, M., Magellan, T., Meerow, A. W., Meyer, A., Pooler, M., Sanchez, V., Spence, E., Thompson, P., Toppila, R., Walsh, S., Westwood, M., Wood, J. and Griffith, M. P. (2020). Taxonomic similarity does not predict necessary

sample size for ex situ conservation: a comparison among five genera. *Proceedings of the Royal Society B* 287(1926). http://doi.org/10.1098/rspb.2020.0102.

Hoban, S. and Strand, A. (2015). Ex situ seed collections will benefit from considering spatial sampling design and species' reproductive biology. *Biological Conservation* 187(July): 182–191. https://doi.org/10.1016/j.biocon.2015.04.023.

Ison, J. L. and Wagenius, S. (2014). Both flowering time and distance to conspecific plants affect reproduction in Echinacea angustifolia, a common prairie perennial. *Journal of Ecology* 102(4): 920–929. https://doi.org/10.1111/1365-2745.12262.

Jarvis, A., Lane, A. and Hijmans, R. J. (2008). The effect of climate change on crop wild relatives. *Agriculture, Ecosystems and Environment* 126(1–2): 13–23. https://doi.org/10.1016/j.agee.2008.01.013.

Kallow, S. (2014). *UK national tree seed project seed collecting manual*. RBG Kew. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/UK-national-tree-seed-project-manual.pdf.

Li, D. Z. and Pritchard, H. W. (2009). The science and economics of ex situ plant conservation. *Trends in Plant Science* 14(11): 614–621. https://doi.org/10.1016/j.tplants.2009.09.005.

Macfarlane, R., Jackson, G. V. H. and Frison, E. A. (2011). Chapter 17. Plant health & germplasm collectors. In: *Collecting Plant Genetic Diversity: Technical Guidelines. 2011 Update* Guarino, L., Ramanatha Rao, V. and Goldberg, E. (Eds). Bioversity International. Available at: https://cropgenebank.sgrp.cgiar.org/index.php/procedures-mainmenu-242/collecting.

Maxted, N., Castañeda Álvarez, N. P. and Vincent, H. A. (2011) Chapter 41. Gap analysis: A tool for genetic conservation. In: *Collecting Plant Genetic Diversity: Technical Guidelines. 2011 Update* Guarino, L., Ramanatha Rao, V. and Goldberg, E. (Eds). Bioversity International. Available at: https://cropgenebank.sgrp.cgiar.org/index.php/procedures-mainmenu-242/collecting.

Maxted, N., Dulloo, E., Ford-Lloyd, B. V., Iriondo, J. M. and Jarvis, A. (2008). Gap analysis: a tool for complementary genetic conservation assessment. *Diversity and Distributions* 14(6): 1018–1030. doi:10.1111/j.1472-4642.2008.00512.

Mayzlish Gati, E., Beerman, A., Singer, A., Faraj, T., Lifshitz, D., Bar, D. and Golan, S. (2018). *A new GPS-based system for seed collection at the Israeli Gene Bank. Samara 33*. RBG Kew. Available at: https://www.kew.org/sites/default/files/Samara%2033%20web.pdf.

Menges, E. S., Guerrant, Jr., E. O. and Hamze, S. (2004). Effects of seed collection on the extinction risk of perennial plants. In: *Ex Situ Plant Conservation: Supporting Species Survival in the Wild* Guerrant, E. O., Havens, K. and Maunder, M. (Eds). Island Press, London.

Nowak, M. M., Dziob, K., Ludwisiak, Ł and Chmiel, J. (2020). Mobile GIS applications for environmental field surveys: a state of the art. *Global Ecology and Conservation* 23. https://doi.org/10.1016/j.gecco.2020.e01089.

Parra-Quijano, M., Iriondo, J. M. and Torres Lamas, E. (2011). Chapter 6. Strategies for the collecting of wild species. In: *Collecting Plant Genetic Diversity: Technical Guidelines. 2011 Update* Guarino, L., Ramanatha Rao, V. and Goldberg, E. (Eds). Bioversity International. Available at: https://cropgenebank.sgrp.cgiar.org/index.php/procedures-mainmenu-242/collecting.

Pence, V. C. (2013). In vitro methods and the challenge of exceptional species for Target 8 of the global strategy for plant conservation 1. *Annals of the Missouri*

Botanical Garden 99(2): 214–220. Available at: www.jstor.org/stable/42703725. doi: 10.3417/2011112.

Ramírez-Villegas, J., Khoury, C., Jarvis, A., Debouck, D. G. and Guarino, L. (2010). A gap analysis methodology for collecting crop genepools: a case study with phaseolus beans. *PLOS ONE* 5(10): e13497. https://doi.org/10.1371/journal.pone.0013497.

RBG Kew. (2016) *Chile Seed Collecting Guide*, Allen, R. (Compiler). 57 pp. Unpublished.

Spjut, R. W. (1994). A systematic treatment of fruit types. *Memoirs of the New York Botanical Garden* 70: 182.

Tanksley, S. D. and McCouch, S. R. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277 (5329): 1063–1066. https://doi.org/10.1126/science.277.5329.1063.

Victor, J. E., Koekemoer, M., Fish, L., Smithies, S. J. and Mössmer, M. (2004). *Herbarium essentials: the Southern African herbarium user manual*. Southern African Botanical Diversity Network Report No. 25. SABONET, Pretoria. Available at: http://www.sanbi.org/sites/default/files/documents/documents/sabonet-report-no-25-herbarium-essentials-southern-african-herbarium-user-manual.pdf.

Vincent, H., Wiersema, J., Kell, S., Fielder, H., Dobbie, S., Castañeda-Álvarez, N. P., Guarino, L., Eastwood, R., Leόn, B. and Maxted, N. (2013). A prioritized crop wild relative inventory to help underpin global food security. *Biological Conservation* 167: 265–275. https://doi.org/10.1016/j.biocon.2013.08.011.

Way, M. J. (2003). Collecting seed from non-domesticated plants for long term conservation. In: *Seed Conservation: Turning Science into Practice* Smith, R. D., Dickie, J. D., Linington, S. H., Pritchard, H. W. and Probert, R. J. (Eds). Royal Botanic Gardens, Kew, UK, pp. 163–201.

Way, M. J. and Gold, K. (2014a). *Assessing a population for seed collection*. Technical Information Sheet _02. RBG Kew. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/02-Assessing-population.pdf.

Way, M. J. and Gold, K. (2014b). *Seed collecting techniques*. Technical Information Sheet _03. RBG Kew. Available at: http://brahmsonline.kew.org/Content/Projects/msbp/resources/Training/03-Collecting-techniques.pdf.

Wyse, S. V. and Dickie, J. B. (2016). Predicting the global incidence of seed desiccation sensitivity. *Journal of Ecology* 105(4): 1082–1093. https://doi.org/10.1111/1365-2745.12725.

Wyse, S. V., Dickie, J. B. and Willis, K. J. (2018). Seed banking not an option for many threatened plants. *Nature Plants* 4(11): 848–850. https://doi.org/10.1038/s41477-018-0298-3.