Potato Genetic Resources
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Abstract
A very narrow genetic base of cultivated potato has permitted the collection and conservation of its landraces and wild relatives as sources of resistant and agronomically desirable traits. Despite its importance for identifying genetic materials and understanding diversity, potato taxonomy is complex and problematic due to the crossability, polyploidy, and reproductive nature of the potato species. Currently, the collected germplasm is preserved in gene banks around the world and distributed to potato researchers with accompanying data. Many gene banks maintain potato collections in vitro, and technological innovations have been developed to assure the long-term preservation of potato genetic resources. Release of the first draft sequence of the potato genome reaffirms the importance of potato genetic resources. Collaboration between potato researchers and gene bank curators promotes the effective use of the genetic resources. Application of next generation sequencing (NGS) technologies would accelerate germplasm management, the evaluation of genetic diversity in situ and ex situ, and conservation planning of potato genetic resources.

2.1 Introduction
The potato (Solanum tuberosum L.) is one of the most important staple crops in the world. It is a New World crop and was unknown to the rest of the world until the sixteenth century. Within the six following centuries, potato cultivation had spread from its center of origin, in the high Andes region in South America to the rest of the world. Potato is currently the fourth most important staple crop after rice, wheat, and maize.
(de Haan and Rodriguez 2016) and provides a substantial part of the world’s food supply, but it is susceptible to a wide range of pests and diseases. The genetic diversity is harbored in landraces and wild relatives considered to be valuable sources of variation for genetic enhancement and crop improvement, because the genetic base of the modern cultivated potato is very narrow (Hawkes 1979; Hanneman 1989; Jansky et al. 2013, 2015). Numerous expeditions have collected important potato genetic resources and conserved them in gene banks. Their effective collection, characterization, conservation and use will be an important key to future sustainable crop production and adaptation under climate change scenarios. At the same time, the evolution of genetic diversity is on-going in situ and in farmers’ field in its center of origin (de Haan and Rodriguez 2016). In this chapter, potato taxonomy, history, current conservation status and future challenges in the genomic era are described in relation to recent biotechnological and genomic technologies.

2.2 Potato Taxonomy: Recent Updates

Species names are an important key to identifying genetic materials, understanding levels of diversity, linking studies across different publications, and identifying germplasm to be used for breeding programs. However, the taxonomy of wild and cultivated potatoes seem to be problematic, because of their interspecific crossability, auto- and allopolyploidy, mixture of sexual and asexual reproduction, possible recent species divergence, phenotypic plasticity, and the consequent high morphological similarity among species (Spooner and Berg 1992; Spooner 2009). Many potato species maintain their sexual compatibility, making it difficult to distinguish the boundary of a species.

Comprehensive taxonomic treatment by Hawkes (1990) reported that there are 235 potato species in total, 228 wild and 7 cultivated potato species. Since then, taxonomical studies have been carried out. Various studies, implementing advanced molecular tools with a large number of samples covering a wide range of species, have suggested that a reconsideration of the taxonomic classification is needed (Jacobs et al. 2008, 2011; Spooner 2009). Lately, combinations of molecular and morphological studies have reduced the number of species to 107 wild and 4 cultivated (Spooner et al. 2014). A detailed historical overview and updates of the taxonomical descriptions of wild and cultivated potatoes were reviewed by Spooner et al. (2014).

2.3 Cultivated Potato Landraces

The distribution of cultivated potato landraces ranges over the upland Andes from western Venezuela to northern Argentina and in the lowlands of south-central Chile (Contreras et al. 1993), adapting to middle to high elevations (3000–4000 m above sea level, masl). Potato landraces are highly diverse in skin and flesh colors, and tuber shapes and it is assumed that perhaps 3000 landraces of potato are still grown by indigenous farmers in South America in semi-traditional and market-oriented production systems (de Haan and Rodriguez 2016; Spooner et al. 2014).

Cultivated potato species have a base chromosome number of n = 12 and include diploid (2n = 2x = 24), triploid (2n = 3x = 36), tetraploid (2n = 4x = 48), or pentaploid (2n = 5x = 60) types. Spooner et al. (2007) classified the cultivated potatoes into four species: (1) Solanum tuberosum, with two cultivar groups (the Andigenum Group of upland Andean genotypes containing diploids, triploids, and tetraploids and the Chilotanum Group of lowland tetraploid Chilean landraces); (2) Solanum ajanhuiri (diploid); (3) Solanum juzepczukii (triploid); and (4) Solanum curtioleum (pentaploid).

Solanum tuberosum is the original species from which modern cultivars were selected (Ames and Spooner 2008). The species comprises the Andigenum Group of upland Andean genotypes (containing diploids, triploids, and tetraploids) and the Chilotanum group of lowland tetraploid Chilean landraces. Andean genotypes are difficult to distinguish from each other due to
extensive gene flow. Furthermore, differentiation from the Chilean tetraploids is slight and incomplete (Gavrilenko et al. 2010). Current distribution of the Chilotanum group is restricted to the Chiloe Island of south-central Chile (Spooner et al. 2010). The Chilotanum group has contributed to the establishment of the current European and North American gene pool as well as global crop improvement (van den Berg and Groendijk-Wilders 2014).

A cultivated diploid species, S. ajanhuiri, was formed by hybridization between diploid cultivars of the S. tuberosum Andigenum Group (2x = S. stenotomum) and the diploid wild species S. boliviense (= S. megistacrolobum) (Rodríguez et al. 2010). Two major morphotypes, Ajawiri (bitter type) and Yari (non-bitter type), are present.

Solanum juzepczukii Bukasov is a triploid (2n = 3x = 36) cultivar, formed by hybridization between diploid cultivars of the S. tuberosum Andigenum Group (2x = S. stenotomum) and the tetraploid wild species S. acaule (Rodríguez et al. 2010). Its distribution range is from central Peru to southern Bolivia, and can be grown at an altitude of 4000 masl (Spooner et al. 2010) and in the frost-affected areas of the Altiplano (Hijmans et al. 2007; Spooner 2009; Spooner et al. 2014). Wild potatoes are distributed in a broad latitudinal range from the South-Western U.S. to Central Chile and Argentina (Hijmans et al. 2002; Spooner et al. 2014).

Two centers for diversity in wild potatoes have been recognized: one in North and Central America, with its center in Mexico, and the other in South America, with its center in the Andes, extending from Venezuela to Chile. This broad area of distribution, along with a broad range of altitudinal distribution, from sea level up to 4500 masl, indicates a wide range of adaptation (Hijmans and Spooner 2001; Hijmans et al. 2002, 2007). Their natural habitats are quite diverse, including cloud forests, cultivated fields, cliffs, as epiphytes, deserts, forests, and on the Pacific islands. This wide range of adaptation has also been reflected in a diversity of morphological traits (Hanneman 1989).

Adaptations to a wide range of habitats have made the wild species tolerant of different environmental stresses, and resistant to a broad range of pests and diseases, and other agricultural interests (Bamberg and Rio 2005; Barker 1996; D’hoop et al. 2008; Hawkes 1990, 1994; Hijmans et al. 2003; Jansky 2010; Ochoa 1999; Spooner and Bamberg 1994). These sources of breeding interests have been screened, identified, and listed by several authors. Genetic diversity, the availability of the germplasm, and usefulness have been the drive to incorporate wild genes into cultivated ones (Bamberg and Rio 2005).

2.5 The Potato Gene Pool and Crossability

The success of the use of wild relatives for genetic improvement relies a lot on their crossability with cultivated species. Although some barriers to cross-species compatibility in potato are known, such as differences in the endosperm balance number (EBN) and ploidy level, potato researchers have developed methods to overcome these hybridization barriers.

2.4 Wild Potato

As aforementioned, potato species are highly complex in taxonomic classification. Currently, 107 wild species are recognized, including diploids, tetraploids, and hexaploids (2n = 6x = 72), without including triploids and pentaploids (Hijmans et al. 2007; Spooner 2009; Spooner et al. 2014). Wild potatoes are distributed in a broad latitudinal range from the South-Western U.S. to Central Chile and Argentina (Hijmans et al. 2002; Spooner et al. 2014).
The gene pool is the most commonly used concept defining the degree of relatedness between species in Harlan and de Wet’s (1971) study. It is based on the degree of crossability among species and it is classified as following: (1) primary; cultivated taxa and wild or weedy forms of the crop that cross easily with the crop and possess total fertility; (2) secondary; less closely related species from which gene transfer to the crop is possible with conventional breeding techniques; and (3) tertiary; species from which gene transfer to the crop is impossible, or if possible, requires sophisticated techniques. Although Harlan and de Wet’s gene pool concept has been widely accepted, it has not been applied to all crops, including the potato. The taxonomic classifications of the crop genus can be used as a proxy for relative crossability, as in the taxon group concept (Maxted et al. 2006).

Attempts to apply the gene pool concept to the potato have been made (Bradeen and Haynes 2011; Veilleux and De Jong 2007). Spooner et al. (2014) proposed a concept especially for the potato, applying five crossability groups based on EBN and self-compatible/self-incompatible systems. The primary gene pool of potato includes *S. tuberosum* ssp. *tuberosum* with all landraces and cultivars. All the cultivated potatoes are tetraploid (2n = 4x = 48) with 4EBN (Johnston et al. 1980). Potato has an extremely large secondary gene pool consisting of related wild species which provides a rich, unique, and diverse source of genetic variation.

The EBN is a model determining the success of interspecific crosses. It was first proposed by Johnston et al. (1980) to explain the success or failure of intraspecific crosses. It relates to a strong isolating mechanism present in the section Petota that affects the endosperm development, but the genetic basis is still under investigation (Ehlenfeldt and Hanneman 1988; Camadro and Masuelli 1995). The EBN classification reported in potato are 2x (1EBN), 2x (2EBN), 4x (2EBN), 4x (4EBN), and 6x (4EBN) (Spooner and Hijmans 2001). Hybridization within each group is expected to be successful rather than hybridization across groups, and thus the success of hybridization can be predicted. Whereas the gene pool concept and the EBN model offer guidance in the utilization of wild genetic resources, they also provide insight into phylogenetic relationship and taxonomy (Bradeen and Haynes 2011). Nevertheless, attempts to directly test species crossability are always important to provide concrete evidence (Jackson and Hanneman 1999).

Potato researchers have developed methods to overcome the hybridization barrier to transfer genes from wild species of the secondary and even tertiary gene pool (Jansky 2006). Manipulation techniques to modify the ploidy level have already been reported (Hermundstad and Pelquin 1985; McHale and Lauer 1981; Camadro and Espinillo 1990; Iwanaga et al. 1989). Even genes from the tertiary gene pool can be introduced using bridge crosses (Hermsen and Ramanna 1973; Jansky and Hamernik 2009), mentor pollinations, and embryo rescue (Iwanaga et al. 1991; Watanabe et al. 1995), and somatic hybridization (Chen et al. 2013; Fock et al. 2001). The transgenic approach has been used for disease resistance (Missiou et al. 2004; van der Vossen et al. 2003; Wu et al. 1995; Zhu et al. 2012) and abiotic stresses in potato (reviewed by Kikuchi et al. 2015). Once commercial hybrids are obtained with valuable genes from wild species, they can be maintained clonally as tubers.

### 2.6 Domestication and Dissemination of the Potato

The center of origin of the cultivated potato is believed to be the Andes region of southern Peru and northern Bolivia, where they still grow wild relatives (Hawkes 1994). Archeological evidence and other data suggest that the potato was domesticated between 10,000 (Ovchinnikova et al. 2011) and 7000 years ago, in the southern Andes of Peru, north of Lake Titicaca (Hawkes 1990). Characters selected for domestication of wild potato involved producing larger tubers, with lower glycoalkaloid content, shorter stolons, and attractive colors of tuber skin and tuber flesh (Gavrilenko et al. 2013). Although the evolution
of the cultivated potato has not reached a conclusive result, phylogenetic and biogeographic patterns have been studied for each cultivated species (de Haan and Rodriguez 2016). The DNA sequence data of orthologous nuclear genes have elucidated the allopolyploid origin of \textit{S. tuberosum} (Spooner et al. 2008, 2010; Rodriguez and Spooner 2009; Rodriguez et al. 2009).

The tetraploid cultivar groups of \textit{S. tuberosum Chilotanum}, which contributed to most modern cultivars cultivated in Europe and North America, are still controversial (Spooner et al. 2012). The group appears to have hybridized with a closely related species as well as the wild Chilean species \textit{S. maglia} (Ugent et al. 1987) or hybrids of \textit{S. tarjiense} (Hosaka 2003; Spooner et al. 2014). However, there still remains a possibility of an Andean origin with the early introduction into Chile (Hawkes 1990, 1999).

The domestication process of the \textit{S. tuberosum} Andigenum Group is believed to have started from wild progenitors of the \textit{Solanum brevicaule} complex (\textit{S. bukasovii}, \textit{S. canasense} and \textit{S. multissectum}) in southern Peru (Spooner et al. 2005a). These species would have an ancestral relationship to the diploid \textit{S. tuberosum} Andigenum Group (= \textit{S. stenotomum}), which is believed to be the most primitive form of cultivated potato (Hawkes 1990). Multiple origins (Grun 1990; Hawkes 1994; Huaman and Spooner 2002) and hybrid origins (Rodriguez et al. 2010) of cultivated potatoes have also been suggested. The progenitor species have developed into the current cultivated potato through repeated sexual polyploidization processes in different cultivation zones.

Since its first appearance in Europe, the potato has rapidly spread worldwide. A detailed history of the potato introduction from its origin to the rest of the world is described by de Haan and Rodriguez (2016). Molecular studies have revealed that the origin of the European potato was dominated by the Andean potato in the 1700s, and later the Chilean potato was introduced into Europe and became predominant long before the late blight epidemics (Ames and Spooner 2008; Rios et al. 2007; Spooner et al. 2005a, 2005b). The successful introduction of South American materials into higher latitudes involved an adaptation to long-day circumstances (Hawkes 1994). Cultivated potatoes were introduced to North America in 1691 from Bermuda, where they had been grown from an earlier introduction from England since 1613. The potato was taken to India and China in the seventeenth century by British missionaries and at about the same time, potatoes were introduced to different parts of Africa, and New Zealand in 1769 (Hawkes 1994).

### 2.7 Potato Genetic Resources

Genetic resources are a strategic resource for sustainable crop production. Their efficient conservation and use are critical to keep feeding increasing world populations. Gene banks play a key role in the conservation and distribution of germplasm for crop improvement and research for sustainable food production. An intensive and systematic potato germplasm collection was initiated in Central and South America in the 1920s by Russian scientists and formed the basis of the potato germplasm collection of the N.I. Vavilov Institute of Plant Industry in St. Petersburg (Ovchinnikova et al. 2011). Since then, much effort has been invested in collecting, maintaining, exchanging, and evaluating these collections. As a result, currently potato genetic resources are preserved in gene banks around the world and are available for potato breeders and researchers.

#### 2.7.1 Germplasm Conserved in Gene Banks

The Food and Agriculture Organization of the United Nations (FAO) (2010) reported there were approximately 98,000 accessions currently conserved ex situ and 80% of them are maintained in 30 key collections. Within these, 25,727 potato accessions are registered in GENESYS (https://www.genesys-pgr.org/ accessed March 17, 2017). A list of major genebank collections
of the Solanaceae species (nightshade, including
tomato, eggplant and potato) is available
(Machida-Hirano 2015).

Cultivated potatoes are conserved mainly as
clonal collections, such as tuber, in vitro and
cryopreservation; on the other hand, wild potato
species are primarily collected and conserved in
the form of botanical seeds (Salas et al. 2008).
Preservation by botanical seed reduces the
maintenance cost, increases the conservation
period (20+ years) and eliminates systemic
viruses such as the Potato Spindle Tuber Viroid
(Bamberg and Rio 2005), therefore, preservation
of botanical seeds should be considered an option
for potato conservation.

Potato germplasm, including wild and culti-
vated potatoes, is conserved in gene banks
throughout the world. The Global Crop Diversity
Trust (GCDT) (2006) reported that at least 23
gene banks have a total of nearly 59,000 acces-
sions of potato germplasm with a considerable
number of duplications. The report classified the
collection into four categories; (1) wild relatives;
(2) native cultivars; (3) modern cultivars of the
common potato (Solanum tuberosum
susp. tuberosum); and (4) other germplasm (e.g.
inter-specific hybrids, breeding clones, etc.).
Wild species are the largest group present in the
collections, followed by native cultivars collected
from centers of diversity in Latin America. The
most important collections are in Latin America,
Europe, and in North America and a few coun-
tries in Asia. Recently, ex situ conservation sta-
tus was assessed to identify potato crop wild
relatives (CWR) in need of conservation. A total
of 49,164 records for 73 species of CWR was
found, with 76% of them possessing geographi-
cal coordinates, which corresponds to 11,100
germplasm accessions.

Most of the gene banks have web-searchable
databases of their gene bank holdings. Passport
information, taxonomy, phenotypic and evalua-
tion data of agronomical traits are available to
help gene bank users search and identify acces-
sions to be ordered, for example:

- the Centre for Genetic Resources, the
  Netherlands, http://cgngenis.wur.nl/;
- the Leibniz Institute of Plant Genetics and
  Crop Plant Research, https://gbis.ipk-
gatersleben.de/GBIS_I/detail.jsf;
- the U.S. National Plant Germplasm System,
  https://npgsweb.ars-grin.gov/gringlobal/
  search.aspx;
- the Genebank Project, National Agriculture
  and Food Research Organization https://
  www.gene.affrc.go.jp/databases_en.php?
  section=plant.

The GENESYS database (https://www.
genesys-pgr.org/) is a comprehensive database
of plant genetic resources for food and agricul-
ture supported by the GCDT. This database can
cross-search accessions conserved in different
gene banks. An inter-gene bank network has also
been developed to coordinate activities on potato
genetic resources conserved in different gene
banks. The Association of Potato Intergenebank
Collaborators (APIC) has produced a global
inventory of wild potato genetic resources collab-
oration with gene banks in Europe, the United
States, Peru, and Argentina. The database was
first developed with about 12,000 entries and
contained more than 5300 accessions identified
with a collector number (Huamán et al. 2000a).
Now the database is hosted by the International
Potato Center (CIP) and can be found online
(http://germplasmdlb.cip.cgiar.org/index.jsp),
containing passport, taxonomical, and evaluation
data. The Working Group on Potato of the
European Cooperative Programme for Crop
Genetic Resources Networks (ECP/GR) is
working to coordinate and extend the potato
genetic resources conservation in the European
Union. The network has developed a central
database of both cultivated and wild potatoes.
The databases can be found on http://ecpgr.cgn.
wur.nl/eupotato/ and the European Cultivated
Potato Database https://www.europotato.org/
menu.php.

As a precaution, duplication is recommended
to safeguard the collection from partial or total
loss caused by natural or human-made catastro-
phes (Engels and Visser 2003; FAO 2014). Some
sister gene banks have already made a back-up of
their holdings. Germplasms of Solanaceae seeds
have been deposited in the Svalbard Global Seed Vault (https://www.croptrust.org/our-work/svalbard-global-seed-vault/). The vault was donated by Norway to the international community and is being supported by the GCDT.

### 2.7.2 In Vitro Collections of Potatoes

Many gene banks around the world are maintaining potato genetic resources including wild types. The current accession number of potato in these gene banks is as follows:

- International Potato Center (CIP), Peru, 6768;
- Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)/The Groß Lüsewitz Potato Collection (GLKS), Germany, 6124;
- Northern Region 6 (NR6), USA, 5808 (Niino and Arizaga 2015);
- Vavilov Institute of Plant Industry, Russia, 9000;
- Central Potato Research Institute (CPRI), India, 3500;
- Potato Research Institute, Czechoslovakia, 2225 (Kaczmarczyk et al. 2011).

The preservation of potato genetic resources (GRs) in gene banks is mostly in field collections, by vegetative propagation due their allogamous nature. Vegetatively maintained potato GRs are vulnerable to loss from natural disasters and damage caused by pests and diseases. Also, this way of preserving potato GRs requires a sufficient area of land, funding and continuous maintenance.

#### 2.7.2.1 Short-Term and Mid-Term Storage

In vitro gene banks are a means to overcome the disadvantage of field gene banks. In vitro cultures can easily be propagated and regenerated in plantlets with the latest progress achieved in plant tissue culture techniques. The advantages of tissue cultures are their maintenance in a sterile and pathogen-free environment and growth in a controlled environment. For the establishment of in vitro gene banks, tissue culture systems and subculture systems for species are needed without contamination of materials. Some rare and endangered plants with no previous information on tissue culture establishment have required the development of new tissue culture protocols (Niino et al. 2014). Several slow growth (minimal growth) methods have been established for short-term (3 months) to mid-term (3 years) storage using low temperature, minimal nutrition, growth retardants, and so on, alone or in combination (Oka and Niino 1997). The main disadvantage of an in vitro gene bank is the induction of genetic variation or somatic mutations during subculturing. Maki et al. (2015) pointed out that DNA mutation occurs due to the long period of subcultures of the plants, while stored shoot tips in super-low temperature retained their original genetic structure. For this reason, a minimal growth method is desirable for preservation of in vitro materials in order to reduce the subculture numbers. The frequency of subculturing can be reduced by incubating them at low temperature, under low light intensity and varied photoperiods, and growing the micro plants on the Murashige and Skoog medium (MS medium, Murashige and Skoog 1962) supplemented with growth retardants or osmotic stress-inducing polyols (Gopal and Chauhan 2010).

The CIP maintains 4062 accessions in vitro under slow growth conditions. The MS medium used contains 40 g/l sorbitol, 20 g/l sucrose. Cultures are maintained at 6–8 °C under 22 µmol/m²/s illumination and 16-h light. This allows in vitro plantlets to be stored for approximately two years without subculturing (Niino and Arizaga 2015). The IPK maintains 2855 potato accessions in vitro at 4 °C as microtubers. The cycle of slow growth maintenance consists of a warm phase with long-day at 20 °C for 2–3 months, a microtuber induction phase with short-day at 9 °C for 2–4 months and a microtuber storage at 4 °C for 12–15 months (Keller et al. 2006; Niino and Arizaga 2015). At the CPRI, more than 1500 parental lines and potato varieties are maintained in vitro on an MS medium supplemented with 40 g/l sucrose and 20 g/l mannitol at 6–8 °C and 16-h photoperiod.
(Gopal and Chauhan 2010). Besides these institutes, in vitro storage of potato GRs is conducted at many other institutes around the world, such as in Mexico, Chile, Korea, Japan by their adjusted techniques (Fig. 2.1).

### 2.7.2.2 Long-Term Storage

Cryopreservation techniques using in vitro shoot tips are recognized as a long-term storage tool for plant genetic resources (PGR). Cryopreservation is based on the reduction and subsequent interruption of metabolic functions of biological materials by freezing at a super-low temperature, while maintaining viability. At liquid nitrogen (LN) temperature (−196 °C), almost all the cellular metabolic activities are quiescent and the cells can be preserved in such a state for a long time. Preservation of in vitro shoot tips at cryogenic temperatures is considered to be a suitable alternative that can ensure the long-term security of vegetatively propagated plants. Once stored in LN, PGR can be kept for almost unlimited periods as a base collection. Almost all cryopreservation protocols are combined with tissue culture techniques, because the cryopreservation procedure is usually preceded by tissue culture (Niino and Arizaga 2015).

The current status of the main cryo-stored PGR encompasses orthodox seeds, some non-orthodox seeds, pollen, dormant buds of some temperate woody plants and in vitro cultures. Large-scale cryo-storage of in vitro shoot tips has been accomplished at several institutes by optimizing cryopreservation protocols. The International Network for the Improvement of Banana and Plantain (INIBAP) has been maintaining the *Musa* spp. cryo-bank collection of over 700 accessions by the droplet vitrification method (Panis et al. 2005; Panis 2008). Other crops (except for potato which have been cryopreserved in cryo-banks), include but are not limited to cassava (Escobar et al. 1997), garlic (Kim et al. 2004a, 2004b; Keller 2005), mat rush (Niino et al. 2013), mint (Senula et al. 2007), pear (Reed 1990), and raspberry (Reed 1988) among others.

The potato cryo-banks of in vitro-grown shoot tips have been established at several institutes around the world. IPK and CIP are two of the largest potato gene banks, which have been applying cryo-storage to potato and have achieved large cryo-bank collections, with over 1456 and 869 accessions, respectively (Niino and Arizaga 2015; Fig. 2.1). The cryopreservation methods used in both Institutes are DMSO droplet and droplet vitrification methods. These methods perform the ultra-rapid cooling and warming by direct immersion in LN and in the rewarming solution of shoot tips on aluminum foil strips. Cooling and warming rates are about 4000–5000 °C/min and about 3000–4000 °C/min, respectively (Niino et al. 2013).

The DMSO droplet method was developed at IPK (Schäfer-Menuhr et al. 1994). The explants (2–3 mm) are incubated in the medium overnight at 22 °C and treated with cryoprotectant solution with 10% DMSO for 1–3 h at room temperature (RT), followed by transfer into droplets of 2.5 μl cryoprotectant solution one by one on aluminum foil. Afterwards, the aluminum foil is immersed directly into the cryotube filled with LN. The explants are rewarmed quickly by putting
aluminum foils in a liquid MS medium with 30 g/l sucrose at RT for regeneration (Kaczmarczyk et al. 2009, 2011; Keller et al. 2008). Currently 1436 potato accessions are stored at IPK using this protocol with a mean regeneration rate of 46% (Kaczmarczyk et al. 2011; Niino and Arizaga 2015).

The droplet vitrification method was developed for banana cryopreservation at first (Panis et al. 2005). This method was applied to in vitro potato shoot tips, resulting in a regrowth of 46–51% (Halmagyi et al. 2005), 8–47% (Panta et al. 2006) and 64–94% (Kim et al. 2006). The latest procedure for droplet vitrification in CIP is as follows. The shoot tips (1.3–2.5 mm) are dissected from the shoots, preconditioned and incubated at RT for about 1 h on a potato meristem medium. Osmoprotection is performed by loading a solution (LS, 2 M glycerol and 0.4 M sucrose; Matsumoto et al. 1994) for 15 min at RT. After that, the shoot tips are dehydrated by a plant vitrification solution 2 (PVS2, Sakai et al. 1990) for 50 min. at 0 °C, and 3 min. before the end of each PVS2 treatment, the shoot tips are transferred to a PVS2 drop (10–15 µl) on an aluminum foil strip (0.5 × 2 cm). Then, the strips holding the shoots are rapidly immersed in an LN-filled cryotube. For regeneration, the strips are rewarmed quickly by dropping them into a liquid MS medium with 1.2 M sucrose at RT and then incubated for 20 min for regeneration. Post-cryo cultures are kept in the dark on the medium with a progressive decrease in sucrose levels and then incubated at 22 °C under standard conditions (Panta et al. 2014). This protocol was successfully applied to four genotypes showing different reactions to abiotic stress, which had high regrowth levels ranging from 23 to 76% (Panta et al. 2014; Niino and Arizaga 2015). The modified droplet vitrification protocol was applied to the Korean in vitro potato collection at the National Agrobiodiversity Center for cryo-storage of 130 accessions. The National Center for Genetic Resources USA has also adopted this protocol with slight modifications for potato cryo-storage of 247 accessions (Niino and Arizaga 2015). Kaczmarczyk et al. (2011) emphasized that a critical aspect in potato cryopreservation is the diverse response between different genotypes in terms of their regeneration capacities after cryopreservation. To overcome this issue, it is crucial not only to make uniform, healthy and robust shoot tips tolerable to cryopreservation procedures but also to develop the regeneration system. Also, it is important that the cryopreservation method should be simple, modifiable and a suitable protocol for different genotypes.

Recently, two novel vitrification methods using aluminum plates, called the V cryo-plate and the D cryo-plate methods, have been developed in order to establish a simple, reproducible and reliable protocol (Yamamoto et al. 2011; Niino et al. 2013). The procedure of the V cryo-plate and the D cryo-plate for potato (Yamamoto et al. 2015) is as follows. The shoot tips (about 1.5–2.0 mm) are excised from the preconditioned shoots and precultured on an MS medium containing 0.3 M sucrose at 25 °C overnight. The shoot tips are placed on aluminum cryo-plates and embedded in calcium alginate gel. Osmoprotection is performed by immersing the cryo-plates for 30 min. at 25 °C in an LS solution (2 M glycerol and 0.8 or 1.0 M sucrose). In the V cryo-plate method, dehydration is performed for 30 min. at 25 °C in PVS2. In the D cryo-plate method, dehydration is performed by placing the cryo-plates for 2.0 h under an air current in a laminar flow cabinet after osmoprotection. Then, the cryo-plate is transferred into an uncapped 2 ml cryotube and directly plunged into LN. For regeneration, the cryo-plate is retrieved from the cryotube in LN and immersed in a 2 ml cryotube containing 2 ml MS basal medium with 1 M sucrose, in which it is incubated for 15 min. at RT. Rewarmed shoot tips are placed on the solid MS medium and cultured under standard conditions. These protocols were successfully applied to 16 cultivars and 4 wild potato accessions, resulting in high regrowth rates of cryopreserved shoot tips in V cryo-plate and D cryo-plate, 96.7% and 93.3%, respectively. The Genetic Resource Center, National Agriculture and Food Research Organization, Japan, has started cryo-storage of
in vitro potato shoot tips using the V cryo-plate method (Fig. 2.2). The National Genetic Resources Center in Mexico has adopted the D cryo-plate method with slight modifications for potato cryo-storage of 13 accessions (Valle Arizaga et al. 2016).

To date, many molecular, biochemical, and morphological studies have been conducted in order to evaluate the genetic stability of the cryopreserved plants. No significant differences have been observed in the regenerated material (Maki et al. 2015; Matsumoto et al. 2013). However, the possibility of some genetic changes may occur in cryopreserved plants, therefore, it is necessary to constantly monitor the genetic stability of regenerated plants. Cryo-storage of potato germplasm is at the cutting edge of cryopreservation research. Many experiences obtained from potato cryo-banking have facilitated the cryo-banking of other plant species. In particular, such a huge diversity of potatoes needs to have many choices of protocol for cryopreservation. Cryopreservation should be considered a back-up to field collections to insure against loss of plant germplasm (Niino et al. 2007). To realize comprehensive cryo-storage of PGR, future further refinement of cryopreservation techniques will be needed.

2.7.3 In Situ and on-Farm Conservation

Supporting physical and biological processes which drive the evolulutional process of life, in situ conservation is valued as complementary to ex situ conservation. This complementary approach is essential to ensure the availability of genetic resources for current and future uses. Recently, the importance of crop wild relatives (CWR) has been addressed for sustainable food production (Maxted and Kell 2009). The importance of potato landraces and their wild relatives has largely contributed to improving the agronomically desirable traits of the cultivated potato (Bradshaw et al. 2006), and will continue to be critical to develop cultivars especially adapted to climate change (Jansky et al. 2013). However, their habitats are threatened by human-mediated habitat destruction (Maxted et al. 2012) and climate change (Schafliteiner et al. 2011).

The importance of population dynamism in their natural habitat has been emphasized for in situ conservation planning (Castañeda-Álvarez et al. 2015; Jansky et al. 2013; Maxted and Kell 2009), but only a limited number of studies have been carried out on the wild potato species (Cadima Fuentes et al. 2015; Marfil et al. 2015). In situ conservation of wild species largely encompasses population genetics in their natural habitat, whereas in situ conservation of crops (landraces) involves research on cultural and socio-economic aspects (Maxted et al. 1987). Scientific evidence of loss of genetic diversity and genetic erosion of landraces has emphasized the need for conservation strategies on farms (Brush et al. 1992; de Haan and Thiel 2004; de Haan et al. 2013), therefore, research on farmer-driven conservation has become a key to the conservation of potato landraces. In Peru, on-farm conservations of potato landraces have been studied.
using different approaches (Asociación ANDES 2016; de Haan 2009; Pradel 2013; Scott 2011; Zimmer 1998). Together with knowledge obtained from the perceptions of farmers on genetic diversity management, including seed exchange, incorporation of new varieties and farmers’ selection (Meldrum et al. 2017), applications of the genomic tools could be useful for understanding the status of the genetic diversity of materials conserved in situ and on farm.

### 2.8 Next Generation Sequencing (NGS) Technologies for Potato Genetic Resources

A highly heterozygous and complex genome of the potato has hampered the genomic understanding of potatoes. Various molecular tools have been intensively applied to elucidate taxonomic or phylogenetic relationships, and identify genes for breeding interests (D’hoop et al. 2008; Jacobs et al. 2008; Spooner et al. 2007; Watanabe 2015). Recent advances in high-throughput sequencing technologies and next generation sequencing (NGS) have greatly contributed to aid analysis of the genomic information of various species, including potato.

The original genome sequence was generated from a homozygous doubled monoploid, which was derived from a heterozygous clone of the Phureja group of cultivated potato (The Potato Genome Sequencing Consortium 2011). Publications of the potato reference genome sequencing have led to the development of a wide range of genomic and transcriptomic resources (Massa et al. 2011; The Potato Genome Sequencing Consortium 2011; Sharma et al. 2013; Hardigan et al. 2016). Recent updates and the data resources were reviewed by Gálvez et al. (2017) and Hirsch et al. (2014, 2016). These genomic information studies and tools have facilitated the discovery of genes contributing to breeding interests, such as disease resistance (reviewed by Ramakrishnan et al. 2015) and stress tolerance (reviewed by Kikuchi et al. 2015).

Recently, the genome sequence of potato wild relatives, *S. commersonii*, has been reported (Aversano et al. 2015). Further sequence release and assembly with cultivated potato will shed light on the phylogenetic relationship and taxonomy of potatoes. Comparative studies of the genome sequence with related crop species (The Tomato Genome Consortium 2012; Kim et al. 2014; Sierro et al. 2014; Bombarely et al. 2016) will increase the ability to identify genes of interests shared with the Solanaceae family.

### 2.9 Challenges for Conservation and Utilization of Potato Genetic Resources

#### 2.9.1 The Conservation Gap

The world potato collection exhibits some gaps and most institutes intend to organize future collecting missions or acquisition from other gene banks to fill the gaps (GCDT 2006). A gap analysis compares the natural distribution range and documented inventories of germplasm conserved in the gene bank. This analysis identifies underrepresented collections in gene banks and provides direction for further germplasm collection (Maxted et al. 2008; Ramirez-Villegas et al. 2010).

The state of ex situ conservation of 73 species of potato wild relatives revealed that 32 species were not yet represented in ex situ collections and identified as high priority species for further collections. Four species (*S. ayacuchense*, *S. neovavilovii*, *S. olmosense* and *S. salasianum*) were identified as missing from internationally available gene bank collections and their collection and conservation are urgently needed (Castañeda-Álvarez et al. 2015). Most of the high priority species for collecting are concentrated in the north-central Andes, particularly in Peru, although a long history of collecting missions has been conducted in the center of the species diversity (Castañeda-Álvarez et al. 2015).

#### 2.9.2 Taxonomy and Inter-Gene Bank Cross-References

Updating taxonomic classification and the re-evaluation of the materials stored in the gene
bank are urgently needed to assure the consistency of their genetic materials. In the case of potato, a practical concern exists for the correct identification of germplasm conserved in different gene banks, because potato gene banks around the world use different taxonomic classifications (Machida-Hirano 2015). This situation makes cross-referencing accession difficult. Van den Berg and Groendijk-Wilders (2014) suggested recording the name under which the material was originally collected and adding later taxonomic options in the additional fields in the database to make possible the cross-reference species names on the databases. Recently, the germplasm collection of the Vavilov Institute of Plant Industry, Russia, was assessed for their collection based on modern taxonomy, including morphological characters, chromosome number, and SSR genotyping (Gavrilenko et al. 2010).

Taxonomic nomenclatural issues also affect the distribution of germplasm. Annex list 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) provides a list of crops covered by the multilateral system. For potato, it is described as “Solanum section tuberosa, excludes S. phureja” (FAO 2009). The interpretation seems to depend on the gene bank. For example, CGN uses the system of Hawkes (1990) to distinguish some of the tomato-related species which are not in Annex 1, and applied the regulations of the Convention of Biological Diversity (CGN website, accessed March 13, 2017). As described above, current taxonomical treatment includes S. phureja in S. tuberosum sbsp. Andigenum Group (Spooner et al. 2007). In the CIP database, 37 accessions of S. phureja are listed and all of them are included in the materials under ITPGRFA and can be distributed using Standard Material Transfer Agreement (the accessions data is available on the GENESYS website) (accessed March 4, 2017, https://www.genesys-pgr.org/). Breeders and researchers should be aware of the issues regarding international legislation on genetic resources.

2.9.3 Maintaining Genetic Diversity in Gene Banks

The level and representativeness of originally introduced germplasm seem to be a key to successful diversity conservation. Having a sampling strategy is one of the most important factors to avoid unrepresented sampling, especially for heterogeneous populations (Camadro 2012). Information on the in situ genetic structure of sampling populations will be useful when planning a collection, however, such information is usually not available. Studies of germplasm conserved in situ and in gene banks revealed genetic changes in some accessions due to genetic drift and contamination. Accessions conserved in gene banks might not represent the genetic diversity that exists in the natural populations because of the sampling effects, mode and types of reproduction, natural hybridization and other reasons. Broadening the sampling area and resampling from a single site would capture the genetic diversity of in situ population and increase the representativeness of ex situ collections (Bamberg and Rio 2005; Cadima Fuentes et al. 2015; Camadro 2012).

Regeneration at the gene banks level involves challenges to minimize selection, genetic drift, gene flow and handling errors resulting in genetic changes and/or loss of genetic diversity of seedling progeny during the process (Börner et al. 2000; Chebotar et al. 2003; Van Hintum et al. 2007; Soengas et al. 2009; Cadima Fuentes et al. 2015). Although the genetic diversity of the potato germplasm has not demonstrated significant changes in both auto- and outcrossing potato species (del Rio et al. 1997a, 1997b), Cadima Fuentes et al. (2015) demonstrated that the regeneration process has affected genetic diversity and the integrity of some wild potato accessions conserved in gene banks. Regeneration methods, particularly for wild potato, have been described by Salas et al. (2008). However, the genetic variation of outcrossing species is
vulnerable to genetic changes during regeneration (Bamberg and del Rio 2004; Chebotar et al. 2003) and maintaining genetic diversity and the genetic integrity of the original collections is always a challenging task due to the heterogeneous and outbreeding nature of potatoes.

2.9.4 Phenotypic Variation and Plasticity

Recent discussions on the phenotypic variation and plasticity of potato have led to a re-consideration of how to manage potato germplasm under gene bank conditions. Jansky et al. (2015) reported considerable tuber phenotypic variation in an F2 population, as much as all the phenotype found in a large landrace collection, derived from self-pollinated diploid F1 hybrid potato derived from a single inbred-line. In contrast, insufficient morphological variation sometimes makes it difficult to correctly identify the species (van den Berg and Groendijk-Wilders 2014). Phenotypic plasticity also is a problem in potato morphological characterization (van den Berg and Groendijk-Wilders 2014). Since most of the wild species are maintained as true seed, segregation during regeneration is a considerable problem to certify the true-to-type materials and identify the correct species. Considering the natural occurrence of introgression and natural hybridization among distinct species, maintaining the genetic component of the original materials introduced into a gene bank collection is an extremely challenging task. Consistent phenotyping strategies need to be developed because phenotypic characterization is the key to NGS data exploitation.

2.10 Sustainable Conservation and Utilization of Potato Genetic Resources in the NGS Era

The main functions of gene banks are the comprehensive collection, the long-term conservation, distribution, organization and dissemination of germplasm information to all interested scientists (Bamberg and del Rio 2007). Recent progress in NGS technologies has led to a significant decrease in the sequencing costs (van Dijk et al. 2014; Goodwin et al. 2016) and it will make genotyping with NGS technologies more affordable and feasible. Massive genotyping of the gene bank collections and sharing the information would be a way to demonstrate the potential use of germplasm collections in gene banks. Some gene banks have already started distribution of germplasm collections together with the genotyping data by NGS datasets (Ellis 2014). The International Maize and Wheat Improvement Center is distributing a set of maize inbred line collections, consisting of 538 accessions, and the agromorphological data and the more than 360 k filtered Single Nucleotide Polymorphisms datasets are publicly available on their website (Wu et al. 2016).

Genomic information obtained by NGS can be expected to have an impact on its user-oriented services, such as the preparation of the core collection (CC) (van Treuren and van Hintum 2014). Core collection is a subset which represents genetic variation in the collection and serves as a reference for the characterization of its biological diversity (Brown 1989). The CC should be chosen for specific objectives, and should be made publicly, quickly and cheaply available, and the genomic and phenotypic characterization should be accessible to a broad range of the research community (Glassman et al. 2010; Odong et al. 2013; van Treuren and van Hintum 2014). The CC also needs to be maintained and conserved for reference purposes, comparative studies, future re-analysis and integrative genomic analysis (Hawkins et al. 2010).

Currently, several CCs are available for both tetraploid cultivated potato (based on morphological or geographical data, and disease and pest resistance, Huamán et al. 2000b) and diploid CRW (based on molecular markers, Bamberg and del Rio 2014; Bamberg et al. 2016). The main challenge of potato CC selection is mixed ploidy within species (Ghislain et al. 2006), which induces complications with the
interpretation of genotyping data. Another challenge is obtaining homogeneous/stabilized genetic stocks which are taxonomically classified in the correct way (Kilian and Graner 2012). Although heterogeneity may reflect the original genetic state of potato wild relatives and landraces, heterogeneity within an accession will be a considerable problem in designing a CC because it seriously can impair its molecular characterization and its subsequent use for research and breeding. The usefulness of CC-produced data will be highly dependent on the quality and generation of the corresponding phenotyping data (Furbank and Tester 2011; Cobb et al. 2013; Jansky et al. 2015). Strengthening the cooperation between gene banks and the users will allow the efficient identification of specific alleles of interest, and the collection of evaluation data. Consequently, it will lead to a more efficient use of the germplasm preserved in its collection.

NGS technologies will also be useful for different fields of study such as conservation, evolution, domestication, ecology, and taxonomy (Egan et al. 2012; Jansky et al. 2015; van Treuren and van Hintum 2014; Kilian and Graner 2012). NGS will also be beneficial for potato taxonomy which relies greatly on the herbarium specimen made from wild plants growing under stressful conditions. Environmental effects on phenotype have made direct comparison difficult between gene bank collections and their wild counterparts because their growing habitats may be very different (Bamberg and Rio 2005), but genomic information and NGS techniques will make this comparison possible. Understanding genetic diversity in situ is key to developing strategies to identify genotypes that possess desirable traits, and to understanding the processes that create and maintain such useful variations for functional traits. The data produced by NGS can potentially be used to understand the genetic diversity conserved both ex situ and in situ/on farm.

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