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Long-term conservation of potato genetic resources: Methods and status of conservation

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Abstract

Plant genetic resources (PGRs) play an important role in agriculture, environment protection, cultural property and trade; they need to be conserved. There are two fundamental approaches for the conservation of PGRs: *in situ* and *ex situ. In situ* conservation is the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings. *Ex situ* preservation is the storage of seeds or plant materials under artificial conditions to maintain their long term viability and availability for use. Genebanks employ seed storage, field collections of living plants and *in vitro* storage (tissue culture or cryopreservation) for *ex situ* preservation of PGR. Storage of orthodox seeds, which are tolerant to low moisture content and low temperatures at appropriate temperature and humidity, is the most convenient *ex situ* conservation method. Plants that produce recalcitrant seeds or non-viable seeds are conserved in field genebanks as well as *in-vitro* in slow growth media for short-to-medium term and cryopreservation in liquid nitrogen at -196^{0}C for long-term periods. Cryopreservation is very expensive and needs trained personnel; this could explain why this method is rarely used for conservation of plant genetic resources in most developing countries. Potato tubers are bulky and highly perishable; the crop is generally conserved as clones either in field genebanks (with annual replanting), *in-vitro* conservation in slow growth media for short-to-medium term and cryopreservation for long term. Field genebanks are expensive to maintain and the crop is exposed to many dangers; hence, cryopreservation is the only feasible method for long term conservation. However, given the high cost of cryopreservation, long-term conservation of potato genetic resources is poorly developed in most resource-poor countries leading to high rates of genetic erosion. This paper looks into the various methods that that can be applied to conserve potato genetic resources and the status of conservation of potatoes in major genebanks and some countries.

Keywords: Plant genetic resources; *in situ* conservation; *ex situ* conservation; *in vitro* conservation; cryopreservation; potatoes.

Introduction

Genetic resources support the maintenance of biological diversity, promote sustainable agricultural production and contribute to the sustainable development and diversification of agricultural production. Plant genetic resources contain the natural gene pool responsible for yield, natural pests' resistance, adaptation to environmental changes and people's future needs among other desirable traits. Plant genetic resources play an important role in agriculture, environment protection, cultural property and trade. However, pressure from the rapidly expanding population, overgrazing, overuse of the farm land, the needs for agricultural development and climate change are destroying the natural resources at an alarming rate; this calls for conservation of plant genetic resources. There are two fundamental approaches for the conservation of plant genetic resources (PGR): *in situ* and *ex situ* (Maxted et al., 1997a). *In situ* is the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings (CBD, 1992). *Ex situ* preservation of PGRs is the storage of seeds or plant materials under artificial conditions to maintain their long term viability and availability for use. Genebanks employ seed storage, field collections and *in vitro* storage (tissue culture or cryopreservation) for *ex situ* preservation of PGR. Storage of orthodox seeds, which are tolerant to low moisture content and low temperatures at appropriate temperature and humidity, is the most convenient *ex situ* conservation method. Field genebanks maintain living plants; field genebanks are used to conserve plants which produce non-orthodox seeds or no seeds and are vegetatively propagated. The two basic methods of *in-vitro* conservation are slow growth for short-to-medium term and cryopreservation for the long-term (Scowcroft, 1984). Cryopreservation is based on the reduction and subsequent interruption of metabolic functions of biological materials by decreasing the temperature with liquid nitrogen (–196°C), while maintaining viability (Niino and Arizaga, 2015). Preservation of *in vitro* shoot tips and somatic embryos at cryogenic temperatures is considered to be a suitable alternative that can ensure the long-term security of

vegetatively maintained germplasm. Once stored in liquid nitrogen, germplasm can be kept for apparently almost unlimited periods, and as a result, cryopreservation is the most appropriate for long term storage of base collections.
This review paper looks into the various methods that can be applied to conserve plant species whose seeds lose viability upon drying (recalcitrant seeds) as well as vegetatively propagated plants. In addition, the status of conservation of potato genetic resources in major genebanks and some countries is explored.

Approaches for conserving plant genetic resources

Conservation and sustainable use of biological diversity is of critical importance for meeting the food, health and other needs of the ever-growing human population (Carlos et al., 2015). The conservation of plant genetic resources for food and agriculture (PGRFA), which represent the basis of global food security, has occupied a prominent position in the efforts undertaken by the international community. Plant genetic resources for food and agriculture must be conserved not only as biological diversity but also as the cultural heritage of Mankind. Conservation and sustainable use of plant genetic resources for food and agriculture is important for breeding new crop varieties, agricultural research and crop production in general. Plant genetic resources for food and agriculture include varieties of agricultural and horticultural crops, breeding materials, traditional cultivars, species and forms. The PGRFA comprise, chronologically ordered, the diversity of genetic material contained in: (i) the crop wild relatives (CWR), which include crop progenitors and their close relatives, (ii) landraces or traditional varieties, and (iii) modern cultivars (Carlos et al., 2015).
The strategy of conserving genetic resources depends on: 1) The nature of the conserved material, 2) The objective of conservation, and 3) The scope of conservation (Sammour, 1993). The nature of the conserved material is defined by the length of the life cycle, the mode of reproduction, the size of individuals, and the ecological status (Frankel, 1947). The objective of conservation research, introduction, breeding, and others, may determine the degree of integrity, which it is essential or desirable to maintain. The scope of conservation is the time over which preservation is projected, and the area, or space, to which it relates a locality, a region, or the world (Simmonds, 1962).
There are two fundamental approaches for the conservation of plant genetic resources: *in situ* and *ex situ* (Maxted et al., 1997a). *In situ* conservation is the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings (CBD, 1992). It involves conserving plant genetic resources in their natural habitats such as wild communities (e.g. natural forests, rangeland, nature reserves and national parks) and on-farm conservation of crop land races. One particular advantage of *in situ* conservation is that it allows the maintenance of evolving populations in their natural habitats, permitting the preservation of gene frequencies and the generation of genetic variability during the dynamic and permanent interaction of target populations with biotic and abiotic factors. *Ex situ* conservation is the removal of germplasm from the place where it is found growing and storing it off-site as seeds in a genebank, vegetative material in *in vitro* storage, or plant accessions growing in a botanical garden or field genebank (Jarvis et al., 2000). *Ex situ* conservation is the most significant and widespread means of conserving PGRFA. The *ex situ* conservation method involves exploration, collection and maintenance of plant genetic materials in new sites outside their native habitat (Maxted and Kell, 2009). These new sites include field genebanks (e.g. plantations, orchards, and botanical gardens), seed banks as well as *in vitro* conservation. Seed banks are the most efficient and effective method of long-term storage of seeds which can be dried and stored under conditions of low temperature and low humidity for long periods without loss of viability (Ellis et al., 1985); such seeds are said to be orthodox.
Plants whose seeds lose viability upon drying are conserved either in field gene banks (plantations, orchards, botanical gardens etc.) or in natural reserves (*in situ* conservation). Problems encountered in maintaining these collections are changes in population structure through natural selection, genetic drift due to small sample sizes and out crossing, loss of ill-adapted genotypes, high cost of maintenance, potential loss through natural disasters (e.g. pests and diseases, drought) as well as manmade calamites such as arson and cutting (Simmonds, 1962; Withers, 1987). Some of these problems can be overcome by *in vitro* conservation (Withers, 1982; De Langhe, 1984). The two basic methods of *in-vitro* conservation are slow growth for short-to-medium term and cryopreservation for the long-term (Scowcroft, 1984). In slow growth storage, growth rate can be slowed through various methods such as incubation at reduced temperature and/or low light intensity, manipulation of nutritive elements in the culture media, use of growth retardants and osmoticums such as mannitol and sorbitol in the culture media (Withers, 1987). In slow growth, inputs in terms of labour and consumables are reduced, as also the risks of contamination at each transfer interval. In addition, slow growth cultures can be readily brought back to normal culture conditions to produce plants on demand. *In vitro* conservation is the most useful and efficient way of distributing clonal materials. It avoids the transfer of most pests and pathogens and allows virus eradication through meristem culture (Roca et al., 1979). Slow growth has its disadvantages; the stress imposed on a culture to retard its growth will, inevitably, affect its vitality. Consequently, a careful balance needs to be maintained to avoid general loss of viability or selective loss of viability that could lead to genetic drift. In addition, the need for frequent subculturing may pose problems such as contamination of cultures as well as imposition of selection pressure with subsequent change in genetic make-up due to genetic variation or somatic mutations during subculturing. Consequently, minimal growth method is desirable for preservation of *in vitro* materials in order to reduce the subculturing frequency. Furthermore, there is a knowledge gap in relation to genetic stability or even phenotypic stability in material exposed to slow growth conditions for extended periods of time. There is limited information from studies of callus cultures to suggest that there may either be selection or persistent physiological change brought about by slow growth at reduced temperatures (Hiraoka and Kodama, 1984; Withers and Alderson, 1986).

Slow growth has been used for a number of crops. In *Solanum* species, particularly in potato, Henshaw *et al.* (1980) reported that there was 14 % survival of meristems stored for one year at 22^0 C, whereas it was 61 % at 6^0 C for the same storage period. Survival rate could be increased to 83 % by alternating day and night temperatures (12^0 C and 6^0 C, respectively). At temperatures below 6^0 C, no success could be achieved. Modification of culture medium has also been used (Henshaw et al., 1979, 1980) to reduce the subculture period for meristem cultures. Survival of cultures maintained at -10^0 C for one year could be increased from 39 % to 56 % following elevation of sucrose from 3 % to 8 % and it could be further increased to 30 % by increasing culture volume from 3.5 to 6.0 ml. Incorporation of mannitol at 6 % or abscissic acid (5 mg l^{-1}) in the medium for cultures maintained at 22^0 C resulted in 63 % and 43 % survival respectively, compared with 14 % without supplements, after one year's storage.

Cryopreservation is the most promising method of *in-vitro* germplasm storage (Stushnoff and Fear, 1985). Cryopreservation techniques using *in vitro* shoot tips are recognized as a long-term storage tool for plant genetic resources (PGR). Cryopreservation of *in vitro* shoot tips at cryogenic temperatures is considered as suitable long-term storage of vegetatively propagated plants; once stored in liquid nitrogen, the materials can be kept for almost unlimited periods as a base collection. Cryopreservation is a long-term storage of biological materials in liquid nitrogen at -196^0C (Helliot and Boucaud, 1997). During cryopreservation, cell division as well as metabolic and biochemical processes are arrested (Niino et al., 1992; Niino et al., 1995); consequently, the cells retain their genetic properties unchanged for an indefinite period of time. Cryopreservation offers long-term storage with maximum stability of phenotypic and genotypic characteristics of the stored germplasm (Ashmore, 1997; Steponkus, 1985). Because all metabolism is suspended at the temperature of liquid nitrogen (-196^0 C), time is not a critical factor in the design and application of cryopreservation regimes. In cryopreservation, the most critical stages are the transition to and from the frozen state; this is a highly unnatural transition and measures must be taken to protect both cellular and tissue structure and the metabolic processes against deleterious effects. Cryopreservation is relatively convenient and economical, large number of genotypes and variants can be conserved and thus maximize the potential for storage of genetically desirable material.

Cryopreservation decreases material handling during storage; consequently, it minimizes contamination incidences, labour and risks of losing samples due to human errors. Although cryopreservation has many advantages, freezing and thawing injuries related to membrane structure and function may result in low survival rates (Ashmore, 1997). In addition, the inability to develop general guidelines for cryopreservation of all plants has made in impossible to develop a standard protocol as every plant has its own requirements for cryopreservation; consequently, there is no one single cryopreservation protocol (Lipavska and Vreugdenhil, 1996). Cryopreservation may be considered a back-up to field collections to insure against loss of plant germplasm (Niino et al., 2007).

The main cryo-stored plant genetic resources include orthodox seeds, some non-orthodox seeds, pollen, dormant buds of some temperate woody plants and *in vitro* cultures (Machida-Hirano and Niino, 2017). Cryopreservation has been successfully applied to callus, protoplast, pollen, meristems, zygotic and somatic embryos and suspension cultures of many crop species. In *Solanum tuberosum* subsp. *andigena,* frozen meristems exhibited 36 % survival whereas S.*tuberosum* subsp. *tuberosum* showed between 2 % and 32 % survival following recovery (Henshaw et al., 1979). Using improved procedures, Towill (1983) reported high survival (71 %) and regeneration of larger number of multiple plantlets following cryopreservation under liquid nitrogen. While studying cryopreservability of potato shoot tips, Benson et al. (1996) found that potato ploidy status was maintained and no chromosomal abnormalities were observed; this means genetic stability was maintained. Successful cryopreservation of meristems has also been reported in several other species, such as *Manihot esculenta, Pisum sativum, Fragaria ananassa* (Kartha et al., 1979, 1980, 1982), *Cicer arietinum* (Bajaj 1979; Kartha 1985) and *Arachis hypogoea* (Bajaj, 1983). For safe long-term conservation of important clonal crops such as potato, banana, cassava, sweetpotato, yam and other Andean root and tuber crops, international genebanks have been using various cryopreservation methods (Benson et al., 2011). 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 which have been cryopreserved include cassava (Escobar et al. 1997), garlic (Kim et al., 2004a, 2004b; Keller 2005) and mat rush (Niino et al., 2013) among others.

In *in vitro* conservation, organized tissues such as shoot tips are preferred over cell and callus cultures for preservation of germplasm of many plant species due to their high genetic stability, high survival and regrowth abilities (Reed et al., 1998); shoot tips, 1-3 mm long are the ones widely used. Shoot tips have small, dense and actively dividing cells which assure rapid multiplication rates. In addition, the low water content of shoot tip cells justifies their selection as a basic plant material for cryopreservation (Ashmore, 1997). Organs are not recommended for cryopreservation due to their large size and the fact that they contain different types of cells; these different cells require different protocols for conservation without damaging the organ (Ashmore, 1997). Maintaining viability and genetic stability during storage is important for cryopreserved *in vitro shoot* tips (Niino and Arizaga, 2015.

The potato genetic resources

The potato, *Solanum tuberosum* L. is a crop of global importance for food security; it is ranked third in food supply behind wheat and rice and it is fourth in terms of protein supply behind wheat, rice and maize, averaging 1.45 g/capita/day during the years 2002–2011 (FAOSTAT, 2014). Comprehensive taxonomic studies indicate that there are 235 potato species globally, 228 wild and 7 cultivated species (Hawkes, 1990). These species have been described and grouped in section *Petota* which occur in the Americas from south-western United States to central Argentina and

Table 1. Potato germplasm collections in major genebanks.

Genebank	Total no. of accessions	No. of *in vitro* accessions	Reference
International Potato Center (CIP), Peru	6768	4062	Niino and Arizaga, 2015
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)/The Groß Lüsewitz Potato Collection (GLKS), Germany,	6124	2855	Niino and Arizaga, 2015
Centre for Genetic Resources, the Netherlands	1471		
Northern Region 6 (NR6), USA	5808		Niino and Arizaga, 2015
Vavilov Institute of Plant Industry, Russia	9000	350	
Central Potato Research Institute (CPRI), India,	3500	1500	Gopal and Chauhan, 2010
National Institute of Agrobiological Sciences (NIAS) Genebank, Japan	1217	130	Machida-Hirano, 2015; Niino and Arizaga, 2015
Potato Research Institute, Czechoslovakia	2225		Kaczmarczyk et al., 2011
National Institute of Crop Science (NICS), Rural Development Administration (RDA), Rep. Korea		1223	

Source: Machida-Hirano and Niino, 2017.

Table 2. Ex situ storage status of potato genetic resources in selected genebanks

Institute, country	Total accessions	Number of accessions				Cryopreservation methods	Literature
		Field preservation	Seed storage	*In vitro* storage	Cryo-storage		
IPK/GLKS, Germany	6124 (2846)	89	2846 (2846)	2855	1456	DMSO droplet vitrification	Niino and Arizaga, 2015
CIP, Peru	6768 (2414)	3931	6125 (2289)	4062 (49)	869	Droplet vitrification & Vitrification	Niino and Arizaga, 2015
Northern Region 6, USA	5808					Droplet vitrification	Niino and Arizaga, 2015
NCGRP, USA					247	Droplet vitrification	Niino and Arizaga, 2015
NICS, RDA, Rep. Korea	1223	670		1223		Droplet vitrification	Niino and Arizaga, 2015
NAC, RDA, Rep. Korea					130	Droplet vitrification	Niino and Arizaga, 2015
NIAS, Japan	1964	1964		20	20	V cryo-plate	Yamamoto, 2013
NCSS, Japan				130		V cryo-plate	
KAES HRO, Japan	500	500				Encapsulation vitrification	Hirai, 2011
CAES HRO, Japan					100	Encapsulation vitrification	Hirai, 2011

( ) means number of wild potato accessions. IPK (Leibniz Institute of Plant Genetics and Crop Plant Research); GLKS (The Groß Lüsewitz Potato Collection); CIP (International Potato, Center); NR6 (The US Potato Center); NCGRP (National Center for Genetic Resources Preservation); NICS RDA (National Institute of Crop Science, Rural Development Administration); NAC, RDA (National Agrobiodiversity Center, Rural Development Administration); NIAS, (National Institute of Agrobiological Sciences); NCSS ((National Center of Seeds and Seedlings); KAES HRO (Kitami Agricultural Experiment Station, Hokkaido Research Organization); CAES HRO (Central Agricultural Experiment Station, Hokkaido Research Organization).

Chile, Uruguay, Paraguay and Brazil (Hawkes, 1990). Lately, combinations of molecular and morphological studies have reduced the number of species to 107 wild and 4 cultivated (Spooner et al., 2014). Potato crop wild relatives (CWR) are found in a wide range of ecogeographic habitats; from sea level to 4697 meters above sea level, from 96 to 3601 mm of annual precipitation and, from 5.6^0 C to 22.1^0 C of annual average temperature (Hijmans et al., 2002). Potato CWR have been successfully used to introduce bacteria, nematode, insect and virus resistance; improve culinary and processing qualities, enhance yield and tolerance to abiotic factors (Ross, 1966; Bradshaw and Ramsay, 2005; Bradshaw, 2009). Potato has the richest genetic diversity of any staple crop (Messer, 2000); in the Andean region, more than 4,500 potato landraces belonging to seven *Solanum* species exist (Panta et al., 2015). Around the world, there are hundreds of improved varieties, derived mainly from *Solanum tuberosum* spp. *tuberosum*. This wide diversity needs to be preserved to assure food security for future generations. The commonly cultivated potato is a highly heterozygous autotetraploid and the genetic constitution of cross-pollinated seeds is unpredictable. Thus, once a cultivar is bred, it is maintained in a clonal form so as to maintain its genetic integrity. Approaches used for conservation of potato genetic resources include field genebanks, *in vitro* methods, DNA genebanks and herbaria.

Status of conservation of potato genetic resources

Cultivated potatoes are conserved mainly as clonal collections such as tuber, *in vitro* and cryopreservation while the wild potato species are primarily collected and conserved in the form of botanical seeds (i.e. true potato seeds) (Salas et al., 2008). Generally, potato genetic resources are preserved in *ex situ* gene banks around the world mostly as field collections; many gene banks also maintain potato collections *in vitro* for short term (3 months) and medium term (3 years) periods (Machida-Hirano and Niino, 2017). In addition, about 190 CWR of the cultivated potato are conserved in botanical gardens globally (FAO, 2010). There were about 98,285 potato accessions conserved *ex situ* and 80% of them are maintained in 30 key collections (FAO, 2010). Within these, 25,727 potato accessions are registered in GENESYS data base. The GENESYS database (https://www. genesys-pgr.org/) is a comprehensive database of plant genetic resources for food and agriculture supported by the Global Crop Diversity Trust (GCDT). The GCDT reported that at least 23 gene banks have a total of nearly 59,000 accessions of potato germplasm with a considerable number of duplications (GCDT, 2006). Wild species are the largest group present in the collections followed by native cultivars collected from centres of diversity in Latin America. Landraces and wild relatives are found mostly in Latin American collections whereas modern cultivars and breeding materials are found mostly in collections of Europe and North America (FAO 2010). The major potato collections are in Latin America, Europe, and North America and a few in Asia (Table 1). It has been reported that the most useful potato genetic material has already been collected and there are only few significant gaps (FAO, 2010). Recently, an analysis on the state of *ex situ* conservation of 73 of the closest wild relatives of potato was conducted with the aim of establishing priorities for further collecting to fill important gaps in germplasm collections. The analysis showed that 32 potato species were assigned high priority for further collecting due to severe gaps in their *ex situ* collections. Such gaps are most pronounced in the geographic center of diversity of the wild relatives in Peru. In addition, 20 and 18 species were assigned as medium and low priority for further collecting, respectively, and only 3 species were determined to be sufficiently represented (Alvarez et al., 2015). The major holders of the potato genetic resources globally are Institut national de la recherché agronomique (INRA)-Rennes (France) which holds 11% of the global potato accessions followed by N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIRS001) which holds 9% of the global collection. Centro Internacional de la Papa (CIP) is third accounting for 8% while Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Germany is the fourth holding 5% of the global potato collection (FAO, 2010). Centro Internacional de la Papa (CIP) is the custodian of the world's largest *in vitro* potato collection; CIP genebank is one of the first genebanks to obtain ISO 17025 certification for safe and secure movement of germplasm (CIP, 2018). The CIP genebank maintains 4062 potato accessions *in vitro* under slow growth conditions; the *in vitro* plantlets can be stored for about two years without subculturing (Niino and Arizaga, 2015). Potato germplasm is preserved at CIP through *in-vitro* techniques using meristem and shoot tip culture for international exchange. Besides the major genebanks, *in vitro* storage of potato genetic resources is conducted at many other institutes around world such as in Mexico, Chile, Korea and Japan (Machida-Hirano and Niino, 2017). CIP also maintains a large number of potato accessions under cryopreservation for long term storage and supports a DNA bank. The CIP genebank has been suggested as a center of excellence for cryobanking (CIP, 2018). In addition, CIP carries out *in situ* conservation of potato germplasm in partnership with farmers and local organizations in the Andean highlands; the center of origin of native potato (CIP, 2011). The potato cryo-banks of *in vitro*-grown shoot tips have been established at several institutes around the world. The IPK and CIP are two of the largest potato gene banks and have cyro-banked over 1456 and 869 accessions, respectively (Niino and Arizaga 2015). The cryopreservation methods used in both Institutes are DMSO droplet and PVS2 droplet vitrification methods (Machida-Hirano and Niino, 2017; IPK, 2013). The IPK collection comprises mainly European commercial improved cultivars belonging to the species *Solanum tuberosum* subsp. *tuberosum*. The National Center for Genetic Resources Program in the United States (NCGRP) (ARS, 2013) and the National Agriculture Center (NAC) in the Republic of Korea (Kim et al., 2006) also apply the PVS2 droplet vitrification method to cryopreserve potato. The Crop Research Institute (CRI) in the Czech Republic is cryo-preserving potato using an alternative ultra-rapid freezing method (Kaczmarczyk et al., 2011) while the Central Agricultural Experiment Station, Hokkaido Research Organization (CAES HRO) in Japan uses the encapsulation vitrification method (Table 2).

Conservation of potato genetic resources in some countries

The Commonwealth Potato Collection (CPC) is the UK's genebank of landrace and wild potatoes held in trust by the

James Hutton Institute with the support of the Scottish Government. The collection comprises around 1500 accessions of about 80 wild and cultivated potato species (https://ics.hutton.ac.uk/germinate-cpc). *In vitro* preservation of parts of plants, such as meristem tips, buds or stem tips, is used infrequently in the UK and cryo-preservation is used to slow down growth rates of this material and enhance long term conservation. The Nordic Genetic Resource Center is responsible for conservation of Nordic potatoes (Solanum tuberosum L.). The *in vitro* collection includes clones of 76 potato accessions from Denmark, Finland, Iceland, Norway and Sweden. Modern cultivars, breeding lines and landraces are present in the collection (https://www.nordgen.org/en/plants/molecular-vitro-laboratory*).* In Estonia, the Department of Plant Biotechnology of Estonian Research Institute of Agriculture (EVIKA) has been collecting and preserving potato and horticultural crops *in vitro* (in slow growth) as meristem plants for more than 25 years. By 2009, the *in vitro* gene bank had 410 accessions of potato cultivars, breeding lines and land-races, and 865 potato meristem clones conserved in slow growth media (FAO, 2012) while by 2013, there were 598 accessions of potato and horticultural plants conserved in *in vitro* genebank (https://www.agri.ee/sites/default/files/.../program-genetic-resources-2014-2020).

The Argentinean Potato Germplasm Bank at the Experimental Station of Balcarce, INTA (Instituto Nacional de Tecnologia Agropecuaria), was created in the 1970s. The potato genetic resources are conserved *ex situ* at the Argentinean Genebank of INTA, Estacion Experimental Agropecuaria Balcarce (Active Genebank) and by the Base Genebank, located at the Instituto de Recursos Biologicos (INTA, Castelar). In the active genebank of Balcarce, accessions of wild and native potatoes are conserved as true potato seeds at 4-9% moisture content and 4°C for the short-term storage. At the Base Genebank, the seeds are stored at 3-7% moisture content and -20°C for the long-term preservation. However, the landraces produce highly heterozygous seeds and clonal maintenance is required for the conservation of specific genotypes (Ashmore 1997). Therefore, these are conserved *in vitro* in slow growth media (Clausen et al., 2010).

In Canada, the Canadian Potato Genetic Resources, or potato gene bank (which is part of Plant Gene Resources Canada (PRGC)), preserves nearly 180 breeding lines or potato varieties of importance to potato researchers and breeders http://agr.gc.ca/eng/news/scientific-achievements-in-agriculture/biodiversity-and-bioresources/keeping-potatoes-alive-we-ve-got-your-backup/?id=1496186643119. The potato gene bank is a living library comprised of *in vitro* plantlets in slow growth media, greenhouse/field grown tubers and microtubers. The collection is comprised of heritage varieties, modern Canadian-bred varieties, as well as strains known to show differential reactions to certain diseases and breeding lines with specific traits scientists are interested in studying. In addition to Canadian varieties, the collection also includes varieties from the US, Peru and many European countries including Ireland, the Netherlands and Estonia.

In Asian continent, India, Indonesia, Japan, Nepal, Pakistan and the Philippines use cryopreservation to conserve some of their plant genetic resources (FAO, 2010). Field genebanks predominate in the Pacific Islands countries, reflecting the regional importance of crops such as taro, coconut and banana that cannot be stored as seed (FAO, 2010). In China, *in vitro* conservation and cryopreservation for vegetatively propagated crop species is carried out by the National Genebank of China. They have stored more than 300 accessions *in vitro*, belonging to 35 crops, such as potato, sweet potato, banana, yam, taro, cassava, ginger, lily, strawberry among others (Zhang et al., 2014). In addition, the cryopreservation is also established by using shoot tips, dormant buds/twigs, and pollen. The cryopreserved shoot tips belong to potato, banana, lily, *Petunia*, carnation and so on. In Nepal, plants that produce recalcitrant seeds or sterile seeds such as citrus, banana, mango, sweet potato, sugarcane, cassava, yam, potato and taro are generally conserved in field genebanks. In addition, the National Agriculture Genetic Resources Center (NAGRC) in Nepal has conserved 6 accessions of potato, 1 banana, 1 cardamom, 1 sugarcane and 1 sweet potato accession in tissue bank using shoot tip explant; these accessions are being maintained at slow growth condition (Joshi, 2017).

In Africa, most countries have seed and field genebanks; seed genebanks are generally more important and widespread than field genebanks in the continent. Few African countries conserve plant genetic resources in slow growth *in vitro* state while very few, if any, have the ability to conserve germplasm cryogenically. In South Africa, potato accessions are mainly maintained *in vitro* in slow growth conditions at the Roodeplaat Vegetable and Ornamental Plant Institute of the Agricultural Research Council (ARC Roodeplaat-VOPI) (Kleynhans, 2013).

In Kenya, potato germplasm is conserved in *ex situ* field genebanks as well as *in vitro* plantlets in slow-growth media at the National Potato Research Centre, Tigoni. The field genebanks are planted out yearly, as potato is an annual species and cannot be conserved in the field for long. The tubers are kept in the cold room at 4^0C and 95 % relative humidity for 6 months to 1 year before replanting in the field. This conservation method is used to a limited extent, particularly for short-term storage of active breeding collections. *In vitro* conservation in slow-growth media is the main method employed for potato germplasm conservation at the National Potato Research Centre in Kenya. The conservation media (or slow growth media) consist of the standard propagation media (MS salts + sucrose) into which sorbitol or mannitol is added to increase osmotic potential of the culture and, hence, reduce the growth rate of the plantlets. The cultures are normally subcultured after 1 year (Muthoni et al., 2010).

Conclusion

Conservation of orthodox seeds in seedbanks is the most convenient means of *ex situ* conservation of plant genetic resources. Consequently, seedbanks are the most commonly used form of germplasm conservation in resource- poor countries especially in Africa. Plants that produce recalcitrant seeds or non-viable seeds are conserved in field genebanks as well as *in-vitro* conservation in slow growth media for short-to-medium term and cryopreservation in liquid nitrogen at -196^0C for long-term periods. Potato tubers are bulky and highly perishable; the crop is conserved as clones either in field genebanks (with annual replanting),

in-vitro conservation in slow growth media for short-to-medium term and cryopreservation for long term periods. However, cryopreservation is very expensive and needs trained personnel; this could explain why this method is rarely used for conservation of plant genetic resources in most developing countries. This could explain why long term conservation of potato genetic resources is poorly developed in most resource-poor countries leading to high rates of genetic erosion.

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