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INVITED REVIEW

Cryopreserved storage of clonal germplasm in the USDA National Plant Germplasm System

Maria M. Jenderek[1] · **Barbara M. Reed**[2]

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Abstract The US Department of Agriculture-Agricultural Research Service (USDA-ARS), National Plant Germplasm System (NPGS) plant collections are a critical source of genetic diversity for breeding and selection of improved crops, including vegetatively propagated plants. Information on these collections is readily accessible to breeders and researchers on the internet from the Germplasm Resources Information Network (GRIN). The clonal collections are at risk for loss due in part to their genetic diversity that makes growing them in one location a challenge, but also because it is difficult to have duplicate collections without incurring great expense. The development of cryopreservation techniques during the last two decades provides a low maintenance form of security backup for these collections. National plant collections for vegetatively propagated crop plants and their wild relatives are maintained by the USDA-ARS, NPGS at 15 sites across the country. These sites include various combinations of field, greenhouse, screenhouse, and *in vitro* collections. Cryopreserved backup collections in liquid nitrogen storage were instituted in the 1990s, increased greatly in the 2000s with the advent of new techniques, and are continuing today. Collections of dormant buds of temperate trees, shoot tips of *in vitro* cultures of many crops, and embryonic axes of some large seeded or recalcitrant seeded plants are all part of the clonal backup storage system.

Keywords Cryopreservation · Dormant buds · Germplasm · *In vitro* shoot tips

✉ Maria M. Jenderek
maria.jenderek@ars.usda.gov

[1] USDA-ARS Plant and Animal Genetic Resources Preservation Unit, 1111 S. Mason, Fort Collins, CO 80521, USA

[2] USDA-ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333-2521, USA

Introduction

Clonal plant germplasm collections in the USA are managed at nine Clonal Germplasm Repositories and six other sites of the USDA-ARS, National Plant Germplasm System (NPGS) organization (http://www.ars-grin.gov/npgs/collections.html). Since their inception from 1980 to 1987, the repositories have collected and identified over 41,500 clonally propagated accessions. In these collections, approximately 31,070 accessions (from 4425 species) are maintained, characterized, and available for distribution (Germplasm Resources Information Network (GRIN); http://www.ars-grin.gov/npgs/collections.html 2016). These collections include the genetic diversity of temperate, subtropical and tropical, vegetatively propagated fruit, industrial crops, nuts, ornamentals, specialty crops, vegetables, and wild relatives. Genera and species included in the NPGS holdings are listed for each germplasm collection on the GRIN (http://www.ars-grin.gov/npgs/collections.html , select "Genebank location" and then "Species held at site"). Before the clonal repositories were established, these plant materials existed in plant breeder collections, or other *ex situ* locations, or in their native habitats. These collections were not widely available and often were destroyed when breeding programs were discontinued or lost due to disease or weather (Jahn and Westwood 1982). Plants in clonal collections must be maintained in an actively growing state to preserve the selected genotypes or because they are sterile or produce seed that cannot be stored. The original outline of procedures for setting up the repository system was described by Westwood (1989). A description of the clonal system and the individual repositories was compiled at the 25th anniversary of

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creating the first repositories (Postman *et al.* 2006). Over the past 35 yr, the collections at these institutions have become some of the most complete collections in the world of the genetic resources of their designated crop genera and are invaluable as sources for breeding materials and research, as well as housing some endangered or threatened plant species.

Maintaining vegetatively propagated plants requires a system of field, greenhouse, and screenhouse facilities, depending on the requirements of the particular crop (Jahn and Westwood 1982). The wide diversity of collections and their growth requirements produces a challenge for clonal crop curators. Developing duplicates or backup collections for this germplasm creates an additional challenge (Reed *et al.* 2004a). Unlike seed-propagated crops where seeds can be exchanged and stored easily at a secondary site, clonal crops require more land and labor or alternative storage techniques for insuring security.

The development of cryopreservation techniques for clonal crops began in the late 1970s (Sakai and Nishiyama 1978; Sakai *et al.* 1978; Uemura and Sakai 1980; Katano *et al.* 1983; Sakai 1984; Kartha 1985). These techniques used controlled-rate cooling followed by plunging in liquid nitrogen (LN) and were developed into protocols that were used successfully to freeze and recover growing shoot tips (Grout *et al.* 1978; Sakai and Nishiyama 1978; Sakai *et al.* 1978; Kartha *et al.* 1979; Towill 1981; Sakai 1984; Towill 1984; Harada *et al.* 1985; Sakai 1985; Stushnoff 1985; Withers 1985; Reed and Lagerstedt 1987; Taniguchi *et al.* 1988; Towill 1988; Tyler and Stushnoff 1988). By the 1990s, germplasm cryostorage of clonal propagules had begun (de Boucaud and Brison 1995; Moriguchi 1995; Niino 1995; Reed and Hummer 1995; Stushnoff and Seufferheld 1995; Schafer-Menuhr 1996; Reed and Chang 1997; Dulloo *et al.* 1998; Forsline *et al.* 1998; Hirai *et al.* 1998; Panis *et al.* 1998; Reed *et al.* 1998; Reed *et al.* 2000). It is now possible to plan and implement clonal cryopreserved storage to safeguard many irreplaceable collections (Reed 2001). A range of cryopreservation techniques are used for numerous genera and species throughout the world (Benson 1999; Reed 2008), and procedures for new genera or species continue to evolve. Routine cryopreservation is now possible for many species, and additional species are being added to the success column on a regular basis. In this manuscript, the cryopreserved clonal collections of the NPGS are described.

Plant Tissues for Cryopreservation

Any totipotent tissue may be used for cryopreservation of clonal plants. The most commonly used tissues are shoot tips, and to a lesser extent, somatic embryos and embryonic axes (shoot and root axis of a seed). Embryonic axes derived from seed are used for seed-propagated crops when the whole seed cannot be cryopreserved due to high oil content of the cotyledons, large seed size, or general lack of cold tolerance. Axes have a unique genetic makeup rather than a clonal character; however, they are processed using the same techniques as clonal propagules, so they are included in the clonal cryopreservation category. In clonal cryopreservation procedures, clonal integrity is required, so callus is not used and conservative use of plant growth regulators is maintained to reduce the possibility of somaclonal variation (Nehra *et al.* 1992; Skirvin *et al.* 1994; Sahijram *et al.* 2003; Bairu *et al.* 2006; Bairu *et al.* 2008; Bairu *et al.* 2011). The use of shoot tips and somatic embryos requires tissue culture systems with established micropropagation regimes (Bell and Reed 2002; Normah *et al.* 2002; Sarasan *et al.* 2006; Bamberg *et al.* 2016). Fortunately, some clonally propagated genetic resources are maintained *in vitro* and could be used for cryopreservation (Reed *et al.* 2013).

Tissue cultures, especially of tropical plants, may carry endophytes (Abreu-Tarazi *et al.* 2010; Reinhold-Hurek and Hurek 2011). Endophytes, and any microorganism if present, manifest themselves during post-cryopreservation recovery, hindering plant regrowth; hence, it is a good practice to test cultures before cryoprocessing (Wilson 1996; Van den Houwe and Swennen 2000; Hamill *et al.* 2005; Orlikowska *et al.* 2017). Some plants (*e.g.*, *Saccharum* and *Musa*) exhibit intense phenolic secretion when cut or otherwise injured. Phenolic compounds may inhibit shoot multiplication and plant recovery after liquid nitrogen storage (Kerns and Meyer 1986; Qin *et al.* 1997; Kumari and Verma 2001; Ozyigit *et al.* 2007; Mneney and Ndakidemi 2014). Supplementing micropropagation medium, cryopreservation solutions, or recovery medium with antioxidants often mitigates the problem (Lux-Endrich *et al.* 2000; Lorenzo *et al.* 2001; Huang *et al.* 2003; Khan *et al.* 2007; Uchendu *et al.* 2009; Uchendu *et al.* 2010; Reed 2013; Shimelis 2015). Shoot tips of a few taxa such as *Allium sativum*, *A. logicuspis*, and selected *Citrus* species can use propagules directly from greenhouse or field plants for cryopreservation (Ellis *et al.* 2006; Volk *et al.* 2012). Shoot tips of *A. sativum* and *A. longicuspis* are excised from cloves of field-harvested bulbs, and shoot tips of *Citrus* species are extracted from greenhouse-grown twigs. Propagules of both genera are sterilized in 75% (*v/v*) isopropyl alcohol (10 min) and 2% (*v/v*) sodium hypochlorite before processing. However, post-cryopreservation recovery is done *in vitro* so a workable system of culture is still required.

The pioneering research of Sakai (1960) opened a possibility of cryopreserving dormant winter buds (DB). Successful dormant bud cryopreservation was reported for several fruit-tree genera including *Diospyros* (Matsumoto *et al.* 2001), *Malus* (Sakai and Nishiyama 1978; Forsline *et al.* 1998; Stushnoff and Suefferheld 1995; Seufferheld *et al.* 1999; Jenderek *et al.* 2011), *Juglans* (Jenderek *et al.* 2014), *Prunus* (Towill and Forsline 1999), *Pyrus* (Suzuki *et al.* 1997; Kovalchuk *et al.* 2014), and several forest and ornamental

woody plant genera including *Pinus* (Kuoksa and Hohtola 1991), *Betula* (Ryynänen 1996), *Ulmus* (Harvengt *et al.* 2004), and *Fraxinus* (Volk *et al.* 2009).

At the NPGS, dormant buds were used for cryopreservation of a wide range of *Malus* (apple) accessions, selected *Prunus cerasus* (tart cherry) and *Salix* (willow) species (Towill and Forsline 1999; Towill *et al.* 2004; Towill and Widrlechner 2004; Towill and Bonnart 2005; Volk *et al.* 2008). Cryopreservation of dormant buds requires fewer resources than using shoot tips or any other *in vitro*-derived material. Currently, routine cryostorage of dormant buds is reported for only a few genera of temperate trees and shrubs. This is mainly due to a lack of genus- or species-specific processing protocols, the narrow time window of deep dormancy required for twig harvesting and processing (Jenderek *et al.* 2017), or the absence of reliable post-cryopreservation viability testing techniques that could substitute for grafting.

Advantages and Shortcomings of Material Type

Both meristem shoot tips (MS) and dormant buds (DB) propagules have their advantages and shortcomings. Shoot tips (apices) can be used at any time of year, but their processing requires a tissue culture laboratory and aseptic cultures, microscopy skills, and an established *in vitro* plant recovery system (Fig. 1*a, b*). It takes 3 to 5 yr. before a tree or a shrub that is recovered becomes a plant capable of producing fruit. The use of shoot tips requires micropropagation of about 180 to 200 shoots per accession. It takes from 2 to 7 mo (species dependent) to produce the number and the quality of shoots required for MS excision. With rare exception, the *in vitro* introduction of field- or greenhouse-grown material is done at the primary NPGS clonal repositories (Table 1). Usually, a primary repository provides 5 to 10 aseptic cultures per accession to the Plant and Animal Germplasm Resources Preservation Unit (PAGRPU) in Fort Collins, CO (also a part of the NPGS) for cryopreservation. Upon arrival, all cultures are kept in quarantine (6 wk) and then routinely all monocotyledonous cultures are tested for endophytes (2 to 3-wk procedure).

Cryopreservation of dormant buds is suitable only for species that undergo a dormancy phase and some period of low temperature that in the northern hemisphere takes place during a winter season. The process requires suitably sized budwood (previous-year growth; and thin twigs, with a species-dependent diameter, usually 4 to 8 mm); the budwood is processed in 3.5- or 7-cm twig segments that usually have one or more buds depending on twig morphology (Fig. 1*c–e*). Before plunging into liquid nitrogen vapor, DB must be desiccated to about 25 to 30% moisture content and slow-cooled to −30°C. After rewarming, DB recovery for the majority of tree and shrub species requires grafting onto rootstocks, a process that involves a skilled grafter, proper rootstock selection, and post-grafting plant management. Fruit production on the recovered DB typically occurs in 1 to 2 yr. This process bypasses the normal juvenile phase that would delay fruit production when a tree is recovered from a cryopreserved aseptic shoot tip. Storage of DB requires more space in a LN tank than for MS since the buds (on 3.5- or 7-cm twig segments) are much larger than the 0.8 to 1 mm shoot tip explants. A MVE XLC 1830 cryotank (Chart Industries, Garfield Heights, OH) can store 19,600 2-mL cryovials with MS; that is, 1237 accessions per tank (16 cryovials per accession, 10 propagules per vial). For DB, the individual buds are much larger and require storage in larger tubes; hence, there is room for only 3960 polyolefin tubes (1.9 × 28 cm), with 247 accessions per tank (16 tubes per accession, 10 DB segments per tube) (Fig. 1*f*). However, cryoprocessing of DB is faster and about tenfold less expensive than cryopreservation of MS.

Cryostorage in the National Plant Germplasm System

Applied cryopreservation and storage of cryopreserved clonal propagules is currently performed at the PAGRPU. From 1991 to 2010, the initial accessions were cryopreserved at the National Clonal Germplasm Repository, Corvallis, OR, and shipped for cryostorage at the PAGRPU. The main tissues originally used for clonal cryopreservation were MS, and a few embryonic axes (Table 2). As of June 2016, cryopreserved clonal storage at Fort Collins included 3903 clonal accessions (12.6% of the NPGS clonal holdings) cryopreserved with ≥40% post-cryoviability and stored in liquid nitrogen tanks. These accessions belong to 96 taxa grouped in 20 collections. The cryostored inventories include genetic resources of asexually propagated genotypes such as apple (*Malus*), banana, (*Musa*), blackberry, dewberry and raspberry (*Rubus*), blueberry (*Vaccinium*), currant, gooseberry (*Ribes*), garlic (*Allium*), grasses (*Cynodon*, *Lolium*, and *Zoysia*), hazelnut (*Corylus*), hop (H*umulus*), mint (*Mentha*), pear (*Pyrus*), potato (*Solalnum*) and selected wild relatives, strawberry (*Fragaria*), sugarcane (*Saccharum*), sweet-potato (*Ipomoea*), tart cherries (*Prunus*), and various species of willow (*Salix*). Initial cryopreservation was targeted at designated core collections that were deemed most representative of the genetic diversity of each genus, so most of the cryopreserved accessions are representatives of the core collections (Table 2). Micropropagation protocols and the cryopreservation procedures used are available at the PAGRPU website (https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/plant-and-animal-genetic-resources-preservation/docs/clonal-protocols/).

The PAGRPU laboratory standard for cryopreserved clonal storage is to achieve ≥40% post-cryopreservation viability and

Figure 1. Preparation of clonal materials for cryopreservation: (*a*) shoot excision for meristem cryopreservation; (*b*) placing a cane with cryovials in the liquid nitrogen dewar; (*c*) twigs with dormant buds as received for cryopreservation; (*d*) cutting of dormant-bud twig segment; (*e*) dormant bud segments in polyolefin tubes; (*f*) storage vault with liquid nitrogen tanks at the NPGS site in Fort Collins.

≥60 viable propagules per accession stored in a liquid nitrogen tank (termed 40/60 standard). A total of 4362 accessions (14.0% of NPGS clonal holdings) were cryoprocessed, but the viability of 459 accessions was <40% and/or less than 60 viable propagules were deposited in liquid nitrogen tanks, hence, they do not fit the PAGRPU criteria of an effective back up (Table 2). Out of the 3903, 44.5% of accessions were cryopreserved as MS, 55.5% as DB, and <0.1% as embryonic axes. Even using published and established cryopreservation protocols, not all cryoprocesses were successful, *i.e.*, they did not result in ≥40% post-cryopreservation viability. Variability in viability can result from genotypic variation in response to applied procedures, or suboptimal culture medium, culture age, culturing conditions, pretreatments, cold acclimation, dehydration, or conditions of the growing season for DB. The least effective procedures were those applied for tart cherry (12.9%) and willow accessions (48.2%) possibly due to the lack of control over winter hardening conditions.

Cryostorage Contingencies

Cryoprocessing efficiency depends on the kind of propagule processed, the plant species, and the number of technicians involved in the process, *i.e.*, available resources. Monocots require much more time for shoot excision, making dicot plant species easier and faster to process. The clonal cryopreservation efforts of the NPGS accelerated over the years as techniques became standardized (Fig. 2). Early storage (1990s) was part of the research program to develop clonal cryopreservation methods, and a few accessions were stored as each method was perfected (Reed 1999). Later as storage became more routine, PAGRPU processed large numbers of accessions from *in vitro* collections provided by the clonal repositories and implemented the 40/60 storage standard. To achieve 60 viable propagules after cryopreservation at a 40% viability level, at least 150 propagules should be processed (Volk *et al.* 2016).

Table 1. USDA-ARS, National Plant Germplasm System (NPGS), and related sites that hold clonally propagated materials. The clonal repositories are sited in environments compatible with the crop species held. The total number of NPGS maintained clonal accessions available for distribution is approximately 31,072

Primary NPGS repositories	Number of genera	Number of species	Total accessions
National Arid Land Plant Genetic Resources, Parlier, CA[a]	13	70	603
National Clonal Germplasm Repository, Corvallis, OR	64	664	8754
National Clonal Repository for Citrus and Date, Riverside, CA	9	10	1789
National Clonal Germplasm Repository for Tree Fruit/Nut Crops and Grapes, Davis, CA	21	248	7257
National Germplasm Repository, Brownwood, TX	1	12	2328
North Central Regional Plant Introduction Station, Ames, IA[a]	152	622	2456
Ornamental Plant Germplasm Center, Columbus, OH[b]	54	300	1013
Plant Genetic Resources Conservation, Griffin, GA[a]	45	196	1823
Plant Genetic Resources, Geneva, NY	7	103	4410
Subtropical Horticulture Research Station, Miami, FL	327	802	2790
Tropical Agriculture Research Station, Mayaguez, PR	285	483	1152
Tropical Plant Genetic Resources Management, Hilo, HI	19	58	709
United States Potato Genebank, Sturgeon Bay, WI[a]	1	92	836
Western Regional Plant Introduction Station, Pullman, WA[a]	24	64	542
Woody Landscape Plant Germplasm, Washington, DC	172	701	1493

Repositories holdings might be accessed at http://www.ars-grin.gov/npgs/collections.html, under repository locations

[a] Clonal collections maintained at NPGS repositories that predominantly hold seed collections

[b] Clonal collections maintained outside NPGS

Routinely, the laboratory processes 150 to 160 propagules per accession. Using MS, the average number of accessions cryopreserved by a technician varied from 35 (for monocotyledons) to 80 (for dicotyledonous species) per year, whereas the number of accessions cryopreserved by DB was 200 to 300 accessions per processing season.

For cryopreservation of clonal propagules, crucial equipment includes a functional tissue culture laboratory with laminar flow hoods, growth chambers, autoclaves, and laboratory appliances as well as the necessary materials, cryotanks, liquid nitrogen supply, an inventory management database, and a control (security) system to warn of disturbance or equipment failure. Cryopreserved materials are entered into the GRIN database with a unique identifier, information on the date of storage, the viability of the controls and cryopreserved material, the number of propagules, the type of propagule cryopreserved, and the storage location. Records are kept on cryopreservation technique, the growth medium used, and the origin of tissue cultures or dormant buds. GRIN also stores information on basic curatorial activities at the primary repositories (*e.g.*, regeneration, planting location for perennials, germplasm exchange, distribution, and passport information including GPS data and research data pertaining to the genetic resources, based on publications). GRIN also holds information on descriptors used in germplasm characterization, listings of crops evaluated and the evaluation data.

Value and Uses of Cryopreserved Collections

The loss of genetic resources from field genebanks is due to a variety of causes (Groenendael *et al.* 1996; Heywood and Iriondo 2003). The most common is the failure of plants from diverse locations and genetic backgrounds to thrive in the soil or climatic conditions of the genebank (Stuefer 1994). Diseases or insect attack can also decimate living collections, and these may become more common with climate change and its unknown effects on living organisms (Botkin *et al.* 2007; Nicotra *et al.* 2010; Dullinger *et al.* 2012; Dullinger *et al.* 2013). For clonal crops, the loss of breeding collections after a retirement or change in research focus is a common occurrence due to the resources required to maintain living plant collections (Jarret and Florkowski 1990; Pence 2010). Seed banking is considered the most cost-effective preservation method (Pence 2011), but it is not appropriate for clonal crops. Information on costs of *ex situ* preservation of clonal genetic resources is difficult to establish or find in literature and it varies from country to country (Pence 2011). Dulloo *et al.* (2009) reported that preservation of coffee genetic resources *via* cryopreservation was more cost efficient than preservation in the field.

Adding these collections to clonal repositories and providing a backup in liquid nitrogen is an economical alternative for preserving important genetic resources. Although these cryopreserved collections may rarely be used, they provide viable alternatives to duplicate field genebanks and also allow a reduction in the number of security duplicate accessions needed

Table 2. Genera, propagule type, cryopreservation technique, and number of clonal accessions cryostored at the USDA-ARS National Plant Germplasm System, Plant and Animal Genetic Resources Preservation Unit, Fort Collins, CO

Genus	Propagule type[z]	Cryopreservation technique[y]	Unique accessions cryopreserved to a 40/60 standard	Accessions in clonal collection	Accessions cryostored (%)	Cryopreserved accessions meeting the 40/60 standard (%)
Allium (garlic)	ms	v	100	312	43.3	74.1
Corylus	ea, db, ms	d, ed, dsc	5	798	0.6	83.3
Cynodon	ms	ed	33	175	18.9	61.1
Fragaria	ms	sc, dv	280	1382	20.3	98.2
Humulus	ms	ed, sc	90	387	23.3	84.1
Ipomoea	ms	ev	166	776	21.4	100
Lolium	ms	ed	15	Breeding collection		
Malus	db	dsc	2155	4280	50.4	94.1
Mentha	ms	v, dv, ed	43	393	10.9	82.7
Musa	ms	dv	22	160	13.8	88.0
Prunus (tart cherry)	db	dsc	12	130	9.2	12.9
Pycnanthemum	ms	ed	32	61	53	100
Pyrus	ms, db	sc, dv, dsc	219	1839	11.9	93.6
Ribes	ms, db	sc, dv, dsc	79	716	11.0	91.9
Rubus	ms	sc, dv	187	706	26.5	89.5
Saccharum	ms	dv	40	408	9.8	66.7
Salix	db	dsc	25	60	41.7	48.1
Solanum	ms	dv	61	836	7.3	100
Solanum PVP	ms	dv	332	332	dd	94.6
Vaccinium	ms	dv	42	828	5.1	100
Zoysia	ms	ed	5	5	100	100

[z] Propagule type: *ms* shoot tip, *ea* embryonic axis, *db* dormant buds

[y] Cryopreservation technique: *d* desiccation, *dsc* desiccation and slow cooling, *dv* droplet vitrification, *ed* encapsulation dehydration, *ev* encapsulation vitrification, *sc* slow cooling, *v* standard vitrification

in a field collection. Clonal crops constitute a small fraction (*ca.* 4.5%) of the total genetic resources maintained by the USDA-ARS, National Plant Germplasm System, but the clonal plant group includes staple food (banana, potato, and sweetpotato) and industrial crops (sugarcane), fruits and nuts (pear, cherry, plum, peach, apple, citrus, hazelnut, walnut, and pecan), berries (blueberry, raspberry, cranberry, current, blackberry, and strawberry), ornamentals (maple, willow, birch, camellia, oak, and rose), and other specialty crops (hops and mint). Active breeding programs that use these collections exist for most of these crops, and all of them contribute greatly to the quality of our life and health.

Challenges for the Future

Although published cryopreservation procedures are not always applicable for all accessions, slight changes can often

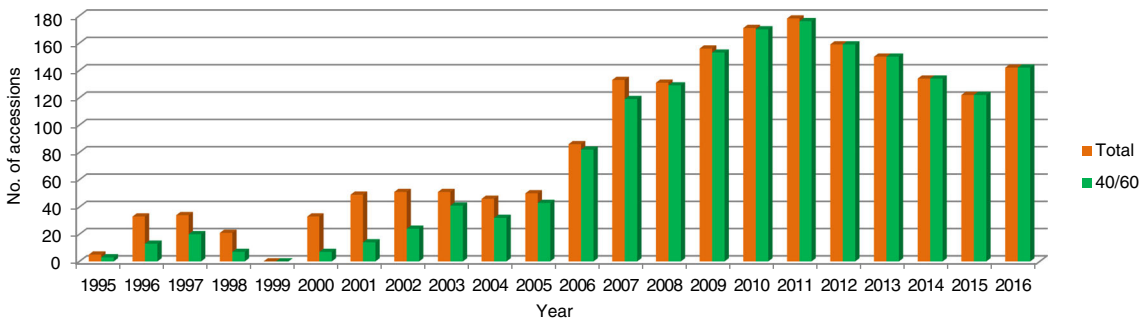

Figure 2. Cryostorage of clonal propagules in the USDA-ARS, National Plant Germplasm System since 1995 including the number of cryopreserved clonal accessions processed yearly and the number that meet the genebank standard of 40% viability and 60 viable propagules (40/60) instituted in 2004.

make the protocol work for most accessions in a genus (Reed *et al.* 2003). A standard evaluation of the suitability of a protocol for a group of accessions is the first step toward storing a collection, followed by suitable training of the staff (Turner *et al.* 2001; Reed *et al.* 2004a; Reed 2008). Consideration for the natural growth conditions of a species such as desiccation tolerance or cold tolerance or lack thereof can indicate which type of protocol is most likely to be successful. Drought-tolerant grasses (*Zoysia*, *Lolium*, *Cynodon*) were highly adaptable to cryopreservation by encapsulation-dehydration (Chang *et al.* 2000; Reed *et al.* 2006). Shoot tips of plants that are desiccation sensitive require more gradual dehydration steps and other protocols are more suitable (Reed 2001). Tissue culture protocols aimed at improving shoot growth are often the key step to success. Changes in plant growth regulators such as removing auxin from the recovery medium can reduce callus production and increase shoot regrowth (Chang and Reed 1999). Poor growth *in vitro* often results in poor recovery from cryopreservation. Small changes or omissions from a protocol can often render it ineffective, so staff training and adherence to protocols should be emphasized. A study of three laboratories implementing two cryopreservation protocols showed that specific step-by-step instructions, and careful adherence to those steps resulted in high recovery of growing shoot tips after cryopreservation (Reed *et al.* 2004b). Developing efficient shoot micropropagation procedures, refining cryoprocedure details, and optimizing plant recovery will increase the viability and quality of cryostored genetic resources. Applying reliable cryopreservation protocols to species that have not been studied will enlarge the scope of plant species that can be successfully cryopreserved. Establishing factors that will increase cryoprocessing efficiency will also promote this type of plant preservation and contribute to lowering costs of material introduction into cryostorage (Keller *et al.* 2008).

While cryopreserving genetic resources threatened with epidemic disease outbreak has a priority, *e.g.*, Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (Ploetz 2006) or citrus greening caused by *Candidatus Liberibacter asiaticus* (Graca 1991; Folimonova *et al.* 2009), continuous efforts are needed to increase the number of cryostored accessions representing the diversity of a collection. The remaining wild diversity of breeding and selection material for clonally derived plant crops is diminishing, making all plant collections more valuable and possibly irreplaceable. Loss of plant germplasm translates to a decline in our food diversity and in the portfolio of options for developing crop cultivars that are suitable for cultivation today and in the future. While a reasonable awareness of the necessity to preserve genetic resources exists in our society, inclusion of crop species not represented in NPGS and economic clonal crop long-term backups are adding to the challenges facing this group of crops.

The NPGS cryopreserved clonal collections are growing annually, and additional genera and species are added to the queue for storage as techniques develop and additional genera are tested and adapted for storage. These clonal backups provide valuable security for those crops that cannot be stored as seed but are vital to our economy, our diet, and our way of life.

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