HortScience 60(11):1886–1891. 2025. https://doi.org/10.21273/HORTSCI18758-25

Documentation of Cryopreserved Dormant Buds and Shoot Tips in the US National Plant Germplasm System

Gayle M. Volk, Adam Henk, Barbara Ambruzs, and Remi Bonnart
US Department of Agriculture–Agricultural Research Service, National Laboratory for Genetic Resources Preservation, 1111 S. Mason Street, Fort Collins, CO 80521, USA

Katheryn Chen
Department of Soil and Crop Sciences, Colorado State University, 307 University Ave, Fort Collins, CO 80523-1170, USA

Stacey Estrada
US Department of Agriculture–Agricultural Research Service, North Central Region Plant Introduction Station, 1305 State Avenue, Ames, IA 50014, USA

Bradford D. Hall, Alison LaTona, Allison Level, Cullen McGovern, James Q. Robinson, Ashley Shepherd, Dianne Skogerboe, Elise Staats, Maria Jenderek, and Daren Harmel
US Department of Agriculture–Agricultural Research Service, National Laboratory for Genetic Resources Preservation, 1111 S. Mason Street, Fort Collins, CO 80521, USA

Keywords. clonal, cryopreservation, data review, GRIN-Global, liquid nitrogen, National Laboratory for Genetic Resources Preservation

Abstract. **Genebanks around the world have implemented cryopreservation programs to conserve clonally propagated crop collections as dormant buds and shoot tips. In 1988, the US National Plant Germplasm System (NPGS) cryopreservation program placed its initial dormant bud samples into long-term storage at the National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, CO, USA. Shoot tip cryopreservation at NLGRP became routine in the early 2000s, with an early emphasis on potato cryopreservation. Accurate data documentation methods ensure critical information is available for decades after materials are placed into cryostorage. Therefore, we provide key data fields, methods for sample documentation, and newly standardized data review processes based on NLGRP procedures. In addition, taxonomic information is provided for the 5457 unique NPGS accessions with cryopreserved NLGRP inventories, as well as some longevity data.**

The US National Plant Germplasm System (NPGS) maintains more than 620,000 plant accessions representing more than 200 crops across 20 locations. Most of the NPGS collections are kept as orthodox seeds and undergo periodic field regeneration; however, more than 40,000 accessions are clonally propagated plants perpetually growing in field, greenhouse/screenhouse, or in vitro conditions (Jenderek and Reed 2017). These clonally propagated crop collections, primarily fruit, nuts, ornamentals, and some vegetables, require expensive, resource-intensive maintenance and are particularly vulnerable to pests, diseases, and abiotic stresses. It is critical to safeguard these crops because NPGS collections provide the genetic variation and important traits needed to overcome threats to agricultural production, and to increase the quality and productivity of nutritious foods and nursery crops (Bretting 2018; Byrne et al. 2018).

The US Department of Agriculture (USDA)–Agricultural Research Service National Laboratory for Genetic Resources Preservation (NLGRP) in Fort Collins, CO, USA, serves as the NPGS backup facility and is therefore responsible for maintaining duplicates of these vast collections to ensure their security. Orthodox seeds are primarily stored at freezer (–18 °C) conditions, whereas shoot tips and dormant buds are kept in liquid nitrogen (LN) to preserve the specific genotypes of the clonally propagated collections. In the 1980s, the NPGS established seven new repositories for clonally propagated crops at Corvallis, OR, USA; Davis, CA, USA; Riverside, CA, USA; Hilo, HI, USA; College Station, TX, USA; Miami, FL, USA; and Mayaguez, PR, USA (Postman et al. 2006). This required the development of new procedures to cryopreserve nonseed propagules for long-term backup at the NLGRP. From 1989 to 2015, the National Clonal Germplasm Repository in Corvallis, OR, USA, also managed an active clonal tissue culture and cryopreservation program that focused primarily on temperate small fruit, pears, and hops. Corvallis later transferred these cryopreserved materials to the NLGRP for long-term storage (Jenderek and Reed 2017).

NLGRP clonal cryopreservation programs initially focused on cryopreserving apple dormant buds using methods developed by the USDA and collaborators (Forsline et al. 1998; Towill et al. 2004; Tyler and Stushnoff 1988). The NLGRP has since developed and successfully implemented cryopreservation methods for a variety of crops, including potato (Jenderek et al. 2023), garlic (Ellis et al. 2006), citrus (Volk et al. 2015, 2019), grape (Bettoni et al. 2019), and kiwifruit (Volk et al. 2022). In addition, methods developed by other programs have been adopted, including mint (Senula et al. 2007), mountain mint (Jenderek et al. 2013), strawberry (Pinker et al. 2009), and banana/plantain (Skogerboe and Jenderek 2023). Detailed methods for some NLGRP cryopreservation processes have been made available in e-book format (Volk 2020).

NLGRP cryopreservation data are maintained in the NPGS information management system: the Germplasm Resources Information Network (GRIN-Global). Specific procedures for the documentation of clonal cryopreservation data in GRIN-Global were not initially established. This resulted in inconsistent use of data fields and reporting methods for more than 30 years. It is critical to address the inconsistent documentation because information about genebank holdings must be transferred between generations (Weise et al. 2020) and communicated to crop managers at the active NPGS repository sites. In 2019, NLGRP scientists and staff identified key data fields and documentation systems to ensure consistent, standardized storage of necessary data in GRIN-Global. Then, from 2022–24, the NLGRP systematically reviewed and updated data for accurate and consistent documentation of clonally propagated collections.

Accessions and Inventories

The NPGS identifies plant genetic resources in terms of "accession" and "inventory." An accession describes a single genetic resource collected at one time and from one location, and one that is uniquely identifiable through provenance, improvement status, pedigree, taxonomy, and/or cultural context.

Received for publication 26 May 2025. Accepted for publication 23 Jun 2025.
Published online 18 Sep 2025.
The US Department of Agriculture (USDA) is an equal-opportunity provider, employer, and lender. Mention of tradenames or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.
D.H. is the corresponding author. E-mail: daren.harmel@usda.gov.

In GRIN-Global, an inventory describes each physical sample of an accession. Characteristics associated with an inventory include physical plant or plant part form (plant, seed, meristem, etc.), quantity, location, and condition.

A single accession may have multiple inventories associated with it, and each inventory is a unique representative of the accession. For clonally propagated crops, an accession typically describes a single genotype. When cryopreserved, one or more NLGRP inventories are associated with an accession. For historical reasons, an NSSL prefix identifies NLGRP inventories due to the original name of the NLGRP, the National Seed Storage Laboratory (NSSL).

We describe the methods developed to document cryopreserved NLGRP inventories of shoot tips and dormant buds, as well as the data cleanup project that resulted in an accurate representation of NLGRP clonal holdings. We provide an up-to-date listing of clonal propagules cryopreserved at the NLGRP, as well as some long-term viability results.

Materials and Methods

Receiving, processing, and documenting cryopreserved inventories. NPGS clonal plant collections are maintained at active sites in orchards, vineyards, greenhouses, screenhouses, and as in vitro plants. To initiate a cryopreservation activity at the NLGRP for a crop in which procedures are available, curatorial teams at the NLGRP and the active site coordinate conservation targets and timelines. NPGS staff document cryopreservation requests sent to the NLGRP in the GRIN-Global Curator Tool as an order with associated order items. Upon receipt, NLGRP staff verify all the materials on the packing list were received. The NLGRP data management group then uses the order records to create NSSL identifiers to track the material received at the NLGRP. Technicians then process the material, cryopreserve it, test its viability, and transfer it to long-term storage in LN tanks. Data associated with each step are provided to NLGRP data management in a spreadsheet with a specific format, referred to as an ecryocard (Table 1). Automated processes ensure the data are uploaded correctly into GRIN-Global.

Storage criteria. Currently, the NLGRP aims to cryopreserve 170 propagules for each accession, resulting in 17 containers of 10 dormant buds or shoot tips. Two of these containers are used for viability assessments; the remaining 15 containers are placed into long-term storage. Ideally, a clonal accession (genotype) is backed up successfully at the NLGRP when the 40/60 standard is met (Volk et al. 2017b). To meet this standard, the inventories belonging to the accession have a viability of 40% or greater as determined by the specified viability method. In addition, the standard calls for a predicted minimum of 60 viable propagules across all cryopreserved inventories (usually no more than two) of an accession. Sometimes, achieving 40% viability for an inventory is impossible, so producing 60 viable propagules after multiple cryopreservation events must suffice until improved procedures are developed and implemented. In some cases, inventories that do not meet these standards are placed into long-term storage, as any regenerable propagules may prevent total loss of valuable plant genetics.

GRIN-global data documentation and review. GRIN-Global is a relational database that divides data storage into separate categories of information. Each category consists of a "parent" table that provides the broadest overview and separate "child" tables that capture many-to-one relationships back to the parent table. For example, "Inventory" is a parent category that stores information about the location and state (i.e., a plant in the field, in vitro, or propagules that are cryopreserved) of the physical material. Individual events that happen to an inventory throughout its life are recorded in a child "Inventory action" table because an action table can document

Table 1. GRIN-Global data fields for documenting inventories that are cryopreserved as dormant buds or shoot tips at the National Laboratory for Genetic Resources Preservation (NLGRP).

Dataview and column	Relationship to the NLGRP inventory
Order	
Created By	Cooperator at the active site
Intended Use	"Backup" or "Research"
Inventory	Initial parent "source" inventory
Distribution Form	The form of the germplasm sent to the NGLRP for processing
Inventory	
Inventory	Unique inventory number starting with "NSSL"
Accession	NPGS accession
Inventory Maintenance Policy	Describes long-term storage conditions and stored form type
Location Section 1	LN tank number
Location Section 2	Section in LN tank
Location Section 3	Cryobox number
Location Section 4	Used for nonstandard storage
Inventory Status	"Backup germplasm" for preserved NPGS inventories; "Sample in Processing" for samples being processed
Quantity On Hand	Number of containers in storage (e.g., vials)
Quantity On Hand Units	The containers used for storage
Standard Distribution Form	The propagule that will be distributed
Parent Inventory	The source plant material from which the cryopreserved inventory was derived, which may be an intermediate in vitro culture
Backup Inventory	The active site inventory sent to the NLGRP
Pathogen Status	Microbial activity observed during cryopreservation
Preservation Method	The cryopreservation method
Inventory Viability	
Inventory Viability ID	An autogenerated record ID number representing the viability test
Inventory Viability Rule	The method for determining viability
Inventory	Same as Inventory; associates viability test with inventory record
Tested Date	Date viability evaluated
Percent Viable	Percent Viable propagules according to the criteria in the associated Viability Method; often, this refers to the regrowth of viable plants
Sample Count	Total propagules tested for viability
Replication Count	Total number of replications in the viability test
Inventory Viability Data	
Inventory Viability	Same as Inventory Viability ID; links raw data records to the summarized viability record
Counting Cooperator	The cooperator who did the viability test
Replication Number	The identifier for this specific replication
Count Number	The instance (data collection event) counted (1 is initial, 2 is final)
Normal Count	Viable propagules counted
Replication Count	The total number of propagules tested in this replication
NSSL Site Inventory	
Inventory	Same as NSSL number
Moisture X	The moisture calculated in instance "X"
Moisture X type	How the moisture was calculated
Moisture X Date	Date the moisture was calculated
Estimated Viable in LN2	The estimated number of viable propagules stored at time of cryopreservation
Inventory Action (Action Name):	
Date Tissue Culture Introduction	Date the in vitro culture was initiated at the active site or the NLGRP
Harvested	Date budwood sent to the NLGRP was harvested at the active site
Received	Date material was received at the NLGRP
Last Culture	Date of last subculture before cryopreservation
Liquid Nitrogen Exposure	Date of exposure to LN
Stored	Date the inventory was moved to the LN vault
Sample in Processing	Date processing at the NLGRP started

ID = identification; LN = liquid nitrogen; LN2 = liquid nitrogen; NPGS = National Plant Germplasm System; NSSL = National Seed Storage Laboratory.

various processing steps as well as multiple occurrences of the same event.

All data available in GRIN-Global for cryopreserved clonal inventories belonging to the NPGS and stored at the NLGRP were reviewed and corrected from 2022–24. It was critical to document which specific inventories and their associated data fields were reviewed, and to keep records of the initial data stored in those fields. The data review was conducted systematically for each of the crop collections (usually designated by the same genus) in GRIN-Global. Spreadsheet workbooks were developed to evaluate each dataset. Records were reviewed and corrected for consistent use of fields and units. Incomplete records were completed when possible, using historical documents and/or information from the original cryoprocessing technician. Workbooks contained the original records retrieved from GRIN-Global. The corrected data were then entered into GRIN-Global along with a list of the reviewed dataviews and fields, review criteria, and any intermediate spreadsheets used during the review process. These workbooks and additional information, such as scanned paper documents and associated electronic fields, were archived in online folders at the conclusion of the project.

The GRIN-Global Curator Tool was used to access and update relevant data during the review process; original data were downloaded to a workbook, standardized, and then uploaded back into GRIN-Global. Each reviewed inventory was classified into broad categories that include "Backup germplasm," "Sample in processing," "Research," "Inventory does not exist," "Accession has property rights," or "Unknown status." "Backup germplasm" inventories, at a minimum, include quantity, recorded physical storage location, and viability. For inclusion in the reports, the associated viability must be greater than 0%. Internal-use reports provide viability information to inform curation teams about the quality of the inventory.

*"Order" and **"Order Request Item" dataviews.*** Fields in the "Order" dataview and "Order Request Item" dataview (order-specific itemized list of inventories in an order) are used when inventories are created at the NLGRP. If the **Order Type** and **Intended Use Note** fields in the "Order" dataview indicate that the material sent to the NLGRP is intended for NPGS backup purposes, the corresponding NLGRP inventories are assigned the "Sample in processing" status. Other status types describe material intended for some other purpose, such as "Research" or "Accession has property rights." The individual responsible for completing the order is indicated in the **Created By** field and is referenced as the Cooperator associated with the NLGRP inventory's **"Received"** action. The **Inventory** and **Distribution Form** fields in the "Order Request Item" dataview identify the specific source plant and germplasm form provided to the NLGRP. The corresponding NLGRP inventory references this as the **Backup Inventory** and **Parent Inventory**.

"Inventory" dataview. Inventory records capture information about the condition, location, and relationship to other inventory records. Key data fields are **Quantity On Hand**, **Quantity On Hand Units**, **Inventory Maintenance Policy**, **Inventory Type**, **Inventory Status**, **Location** (**Section 1**, **Section 2**, **Section 3**, or **Section 4**), **Parent Inventory**, **Backup Inventory**, **Pathogen Status**, **Preservation Method**, **Accession**, and **Inventory Identifier** (the NSSL number). Additional information may also be captured in optional fields, including a free-text **Note** field.

The required **Inventory Maintenance Policy** field assigned to the inventory provides key information about the propagule type and storage regime. For example, CRYO-LIQUID-MERISTEM indicates that meristems (excised shoot tips) are stored in the liquid phase of LN. The NLGRP primarily uses several options for the **Inventory Type** field: IV [in vitro: tissue culture plants in StarPac bags (AgriStart, Inc., Conroe, TX, USA), test tubes, or magenta boxes stored in a growth chamber]; DB (dormant buds: twig segments containing one or more dormant buds in tubes in LN), and MS (meristems: excised shoot tips in vials stored in LN). **Distribution Critical Amount** indicates the minimum number of containers on hand before an inventory is no longer available for regular distribution. **Replenishment Critical Amount** is the critical cut-off to indicate the need for a replacement inventory. Both fields are usually set at 2.

Quantity on Hand is the number of the **Quantity on Hand Units** (containers), in storage at the NLGRP. These codes describe the type of container housing the propagules and also serve to propagate the **Unit of Distribution** field. Units associated with cryopreserved inventory include ct (count: individual propagules), vl (vial: 1.2-mL cryovial), tb (tubes: sealed tube, usually polyolefin used for dormant buds), and 50mL_vl (50-mL polypropylene tube used for some samples).

The physical storage location of cryopreserved dormant buds and shoot tips at the NLGRP is described using four fields. **Location Section 1** identifies the LN tank, **Location Section 2** identifies the sector within the tank, **Location Section 3** indicates the cryobox containing the cryopreserved inventory, and **Location Section 4** is used when an additional location description is needed, such as if cryovials are stored within 10 × 10 cryo storage boxes.

Fields in the NLGRP inventory record relate the cryopreserved material to other, existing inventory records associated with a given accession. The **Backup Inventory** field always identifies the original source germplasm (using the active site inventory identifier), associated with an NSSL inventory derived from it. This indicates the field inventory being backed up at the NLGRP. **Parent Inventory** indicates specific inventory (active site inventory or NLGRP inventory) from which this inventory directly descended. For cryopreserved shoot tips, it may reflect an intermediate NLGRP inventory instead of the original source inventory from the primary repository. For dormant buds, however, **Backup Inventory** and **Parent Inventory** are both the active site inventory identifier. **Pathogen Status** provides a location to document whether any sort of contamination was observed during the preservation and viability assessment procedures. This information is valuable for future inventory regeneration efforts, particularly if minor contamination was observed that did not inhibit plant regrowth. **Preservation Method** indicates the method used to preserve the propagules in storage. A separate GRIN-Global "Methods" table records some information about specific methods and, via a standardized file-naming convention, links to a document maintained in a separate file system with a detailed description of the preservation process.

"NSSL Site Inventory" dataview. The "NSSL Site Inventory" dataview provides fields specific to NLGRP activities, including **Moisture**, **Moisture Type**, **Estimated Viable in LN2** (liquid nitrogen), **Shoots_Per_Vial**, and **Estimated_Shoots**. Captured data include adjusted moisture content measurements for dormant buds as **Moisture X**, method for calculation as **Moisture X Type**, and data collection date. The **Estimated Viable in LN2** field records the estimated total number of viable propagules placed in long-term storage as calculated by the processing technician and calculated by multiplying the percent viable (usually based on regrowth), the value in the **Shoots_Per_Vial** field, and the data in the **Quantity on Hand** field. These data reflect the initial quantity placed into storage; the value does not change when units are removed from cryostorage. The **Shoots_Per_Vial** field records the number of propagules in each container for the inventory. The **Estimated_Shoots** field is a calculated field determined by multiplying the data from the following fields: **Shoots_Per_Vial**, **Percent Viable**, and **Quantity on Hand**.

"Inventory Viability" and "Inventory Viability Data" dataviews. It is important to differentiate data captured in the "Viability" and "Viability data" tables. The child "Viability data" table captures raw scored values associated with a viability test; the "Viability" table provides the summary overview of the viability test methodology, sample size, and overall percentages.

The "Inventory Viability" dataview includes key fields that document sample viability testing including **Inventory Viability Rule**, **Percent Viable**, and **Replication Count**. The **Inventory Viability Rule** field records the method used to test the viability of the propagules in storage. This must be an entry in the "Viability rule" table that links to a document with detailed descriptions about how to recover the propagules of this inventory from LN and how to perform the viability/regrowth test procedures. The **Percent Viable** field is the percentage of the sampled propagules that met the viability criteria expressed in the **Inventory Viability Rule** field. The **Replication Count** field indicates the number of replications performed during the viability test (e.g., a viability test of 20 propagules performed as 2 replications of 10 propagules each).

The "Viability data" table is a child table of "Viability" used to store itemized data for each testing event. Each row in the table describes an observation for the viability test indicated in the **Inventory Viability** field. Data captured in the table include an identifier for the observation (**Count Number**), the observation date (**Count Date**), the number of viable propagules observed (**Normal Count**) in the number of propagules tested (**Replication Count**) for the specific replication (**Replication number**), and the technician who collected the data (**Counting Cooperator)**.

"Inventory Action" dataview. The "Inventory action" database table is a child table of "Inventory." It describes activities performed on inventories throughout the life of the material. For cryopreserved materials, each inventory must have an action with the name **"Exposed to LN"** and its completed date, the date on which the inventory was cryopreserved. At the completion of an inventory's data review process, the action **"Associated information review"** was added to document the date the data were corrected (completed date) in the GRIN-Global database.

Accession inventory attachments. GRIN-Global includes an Attachment Wizard to simplify and streamline uploading and linking computer files to many different types of records within GRIN-Global using standardized codes. These files may include images, documents, spreadsheets or other file types containing observation information, viability data, cryopreservation or viability testing methods, and so on.

Reporting. NLGRP data in GRIN-Global can be retrieved by querying the database with the desired fields. Alternatively, summary information is available to NPGS curators through internal-use web-based reports for "NLGRP Clonal Accessions" and "Clonal Summary" available through the GRIN-Global public website. The NLGRP Clonal Accessions report displays information (including viability and quantity on hand) about accessions with NLGRP inventories and can show all records or only accessions that are currently cryopreserved (viability greater than 0%, and at least one container in LN storage). The "NLGRP Clonal Totals Report" selection provides the number of accessions cryopreserved at the NLGRP (greater than 0% viability and at least one container in LN), described by genus and propagule type. Although these criteria are much less stringent than the 40/60 standard, they provide a consistent method to retrieve information from GRIN-Global for reporting purposes.

"Long-term storage of citrus shoot tips." An experiment was initiated in 2011 to determine long-term viability of citrus shoot tips in cryostorage using *Citrus aurantium* 'Seville' sour orange, *Citrus limon* 'Eureka' lemon, and *Citrus paradisi* 'Reed Marsh' grapefruit (Volk et al. 2015, 2017a). Three inventories containing 10 shoot tips of each accession were warmed and micrografted at time points between 2011 and 2024 to determine regrowth levels. Significant changes over time were calculated using regression analyses.

Results and Discussion

NLGRP inventories. The long-standing clonal cryopreservation program at the NLGRP has resulted in the successful storage of inventories representing 26 NPGS crops. In some cases, viability levels or quantities do not meet current standards because they were processed at a time when different standard levels were in use, inadequate quantities of material were available, or because the current methods did not achieve the desired viability/regrowth levels. Because of the number of accessions within these large collections, curatorial decisions must be made as to whether accessions will be reprocessed or whether the cryopreserved quantity and quality are adequate, freeing up resources to preserve additional vulnerable accessions at the NLGRP. Ongoing research programs seek to identify modifications or improved methods that will improve cryopreservation results. The lack of methods and resources to implement available methods significantly affects the ability of the NLGRP to cryopreserve many of the clonally propagated crop collections within the NPGS.

After the NLGRP clonal data review, it was determined that the NLGRP has a total of 5457 NPGS unique accessions from clonal collections maintained as vegetative propagules in LN, with 2856 accessions preserved as dormant buds and 2656 accessions preserved as shoot tips. Overall, ∼14% of the NPGS clonal collections are represented at the NLGRP. Of these, 638 of the accessions cryopreserved as dormant buds meet the 40/60 standard and 2394 of the accessions cryopreserved as shoot tips meet the 40/60 standard. Materials remain in storage that were cryopreserved as early as 1988 (Table 2 and Fig. 1). Long-term efforts to cryopreserve dormant buds and shoot tips at the NLGRP have resulted in one of the largest collections of clonally propagated plant materials in the world, with other large collections held by Bioversity International (Nagel et al. 2024); the National Institute of Agricultural Sciences, South Korea (Kim et al. 2012); the National Institute of Agrobiological Sciences Genebank, Japan (Fukui et al. 2011); the International Potato Center, Peru (Vollmer et al. 2022); and the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany (Nagel et al. 2024; Wang et al. 2014).

Long-term viability of cryopreserved inventories. The field of cryobiology is relatively new, and the longevity of plant germplasm cryopreserved as dormant buds and shoot tips is not well documented. We sampled three citrus shoot tip accessions cryopreserved at the NLGRP for more than 10 years and demonstrate no significant changes in viability after 140 months of storage in the vapor phase

Table 2. National Plant Germplasm System (NPGS) accessions at the National Laboratory for Genetic Resources Preservation (NLGRP) cryopreserved as dormant buds (DB) and shoot tips (MS) with more than one container in liquid nitrogen storage and more than 0% viability, as well as columns showing the number of stored accessions that meet the 40/60 standard as dormant buds and shoot tips.[i]

Genus	NPGS accessions at NLGRP	NPGS accessions at NLGRP as DB	DB > 60 and at least one inventory > 40% viability	NPGS accessions at NLGRP as MS	MS > 60 and at least one inventory > 40% viability
Actinidia	4	0	0	4	3
Allium	100	0	0	100	98
Ampelopsis	1	0	0	1	1
Ananas	5	0	0	5	4
Citrus	508	0	0	508	450
Corylus	1	0	0	1	0
Cydonia	1	0	0	1	1
Cynodon	25	0	0	25	9
Fragaria	285	0	0	285	269
Humulus	91	0	0	91	80
Ipomoea	175	0	0	175	146
Juglans	9	9	4	0	0
Lolium	9	0	0	9	0
Malus	2403	2403	408	2	2
Mentha	78	0	0	78	72
Musa	25	0	0	25	23
Prunus	113	113	52	0	0
Pycnanthemum	32	0	0	32	32
Pyrus	293	105	40	216	172
Ribes	230	180	90	75	75
Rubus	201	0	0	201	179
Saccharum	29	0	0	29	28
Salix	46	46	44	0	0
Solanum-non-PVP	307	0	0	307	279
Solanum-PVP	401	0	0	401	391
Vaccinium	51	0	0	51	50
Vitis	34	0	0	34	30
Total	5457	2856	638	2656	2394

[i] *Solanum* (potato) accessions in the NPGS active collection are reported separately from those that, at the time of this writing, are held under an active Plant Variety Protection (PVP) certificate (queried 10 May 2025). After 20 years, the PVP expires and the accession is released for incorporation into the NPGS's active *Solanum* collection.

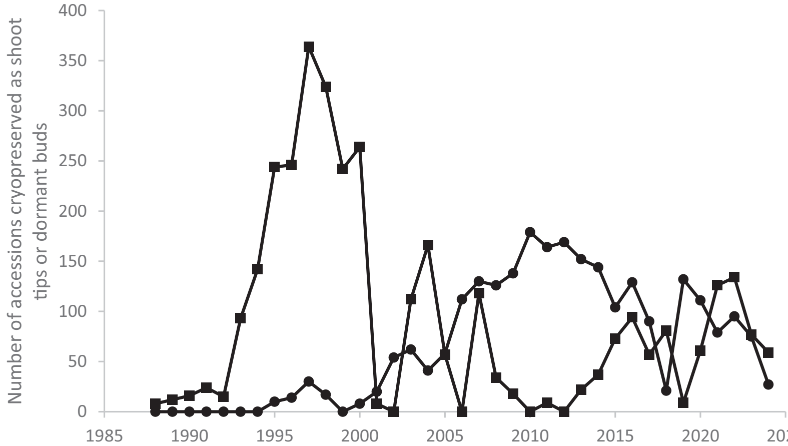

Fig. 1. Number of accessions cryopreserved as dormant buds (squares) and shoot tips (circles) in the National Plant Germplasm System annually from 1988 to 2024.

of LN (LNV; Fig. 2). *F* significance values for the regressions were 0.139 for *C. aurantium* 'Seville' sour orange, 0.482 for *C. limon* 'Eureka' lemon, and 0.927 for *C. paradisi* 'Reed Marsh' grapefruit.

Anecdotal evidence reveals that NPGS *Malus* inventories cryopreserved as dormant buds as early as 1988 and retrieved 30 years later were propagated successfully onto rootstocks to replenish field trees that have been lost in the *Malus* field collection in Geneva, NY, USA (Gutierrez B, personal communication). In addition, Volk et al. (2008) compared the viability of *Malus* dormant buds maintained at the NLGRP from the Canadian Clonal Genebank of Plant Gene Resources of Canada and found that a subset that was tested for regrowth after 10 years exhibited lower, but insignificant, regrowth compared with the initial LNV-exposed regrowth levels at the time of preservation. In addition, rice cells stored in LNV at the NLGRP in 1981 were warmed and grown successfully in 2020, demonstrating survival after 39 years of cryostorage (Samuels et al. 2021). Vollmer et al. (2022) provide long-term viability monitoring data for cryopreserved *Solanum* (potato) after 2, 4, and 8 years, with no significant differences in viability after the defined interval. In contrast, Jenderek et al. (2023) reported an overall decline of 25% in regrowth after 10 years of cryostorage for 10 accessions of *Solanum* crop wild relatives cryopreserved as shoot tips. Recently Jenderek et al. (2025) compared the regrowth of 25 *Rubus* accessions that were cryopreserved as shoot tips (droplet vitrification) and stored for 15 to 20 years. Regrowth levels dropped by 8.7%, on average, compared with their initial viability. These ranges in responses after extended cryopreservation durations suggest that declines in viability over time are possible, and that additional long-term research is needed to understand more fully the longevity of cryopreserved shoot tips and dormant buds in genebank settings. This type of research may be difficult because experiments performed across multiple generations of genebank staff may have variation in regrowth assessment technique.

Conclusion

Cryopreserved clonal propagules may remain in storage for decades without needing to be replenished. Thanks to these long storage times, valuable collections of diverse, clonally propagated crops and their wild relatives can be backed up in a quiescent state, safe from various biotic and abiotic stresses they might otherwise experience. Long-term storage necessitates clear data communication across generations of genebank staff and across multiple NPGS genebank sites. Standardization of NLGRP clonal data reporting resulted in accurate documentation of cryopreservation data for dormant buds and shoot tips that can be easily sorted or queried. Including automated processing and data checks to GRIN-Global data entry now provides a consistent upload process with substantially fewer errors than manual data edits to each inventory record. It is recommended that annual data checks be performed to ensure the uploaded data maintain high quality standards. In addition, this standardized procedure provides staff an opportunity to review progress on inventories that are in progress and to update the status as needed. In the future, clonal cryopreservation processing and dataflows should be further streamlined by incorporating one- or two-dimensional barcodes, radio-frequency identification tags, or similar technologies so that each step of the process may be uploaded easily and immediately to GRIN-Global as it is completed.

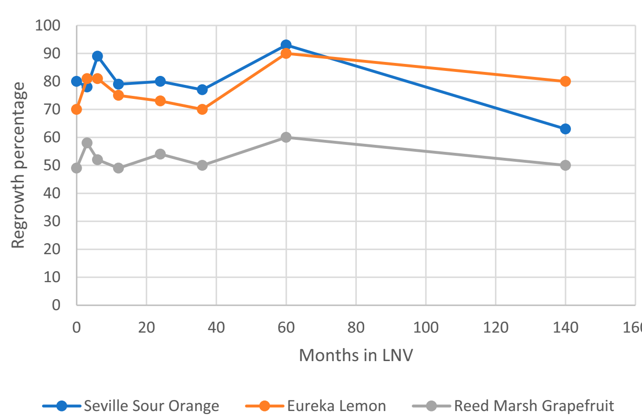

Fig. 2. Viability/regrowth levels of three *Citrus* accessions cryopreserved as shoot tips and stored in the vapor phase of liquid nitrogen (LNV) for 0 to 140 months.

References Cited

Bettoni JC, Kretzschmar AA, Bonnart R, Shepherd A, Volk GM. 2019. Cryopreservation of 12 *Vitis* species using apical shoot tips derived from plants grown in vitro. HortScience. 54(6): 976–981. https://doi.org/10.21273/HORTSCI13958-19.

Bretting PK. 2018. 2017 Frank Meyer medal for plant genetic resources lecture: Stewards of our agricultural future. Crop Sci. 58(6): 2233–2240. https://doi.org/10.2135/cropsci2018.05.0334.

Byrne PF, Volk GM, Gardner C, Gore MA, Simon PW, Smith S. 2018. Sustaining the future of plant breeding: The critical role of the USDA-ARS National Plant Germplasm System. Crop Sci. 58(2):451–468. https://doi.org/10.2135/cropsci2017.05.0303.

Ellis D, Skogerboe D, Andre C, Hellier B, Volk G. 2006. Implementation of garlic cryopreservation techniques in the national plant germplasm system. Cryo Lett. 27(2):99–106.

Forsline PL, Towill LE, Waddell JW, Stushnoff C, Lamboy WF, McFerson JR. 1998. Recovery and longevity of cryopreserved dormant apple buds. J Am Soc Hortic Sci. 123(3):365–370. https://doi.org/10.21273/JASHS.123.3.365.

Fukui K, Shirata K, Niino T, Kashif IM. 2011. Cryopreservation of mulberry winter buds in Japan. Acta Hortic. 908:483–488. https://doi.org/10.17660/ActaHortic.2011.908.62.

Jenderek MM, Ambruzs BD, Tanner JD, Bamberg JB. 2023. High regrowth of potato crop wild relative genotypes after cryogenic storage. Cryobiology. 111:84–88.

Jenderek MM, Ambruzs BD, Yeater KM, Reed BM. 2025. Evaluating shoot-tip regrowth of 25 *Rubus* L. species and hybrids after 15 to 20 years of cryopreserved storage. Cryobiology. 118:105159.

Jenderek MM, Holman GE, Denoma J, Reed BM. 2013. Medium- and long-term storage of the *Pycnanthemum* (Mountain mint) germplasm collection. Cryo Lett. 34(5):490–496.

Jenderek MM, Reed BM. 2017. Cryopreserved storage of clonal germplasm in the USDA National Plant Germplasm System. In Vitro Cell Dev Biol Plant. 53(4):299–308. https://doi.org/10.1007/s11627-017-9828-3.

Kim HH, Popova E, Shin DJ, Yi JY, Kim CH, Lee JS, Yoon MK, Engelmann F. 2012. Cryobanking of Korean *Allium* germplasm collections: Results from a 10 year experience. Cryo Lett. 33(1):45–57.

Nagel M, Pence V, Ballesteros D, Lambardi M, Popova E, Panis B. 2024. Plant cryopreservation: Principles, applications, and challenges of banking plant diversity at ultralow temperatures. Annu Rev Plant Biol. 75(1):797–824.

Pinker I, Halmagyi A, Olbricht K. 2009. Effects of sucrose preculture on cryopreservation by droplet-vitrification of strawberry cultivars and morphological stability of cryopreserved plants. Cryo Lett. 30(3):202–211.

Postman J, Hummer K, Stover E, Krueger R, Forsline P, Grauke LJ, Zee F, Ayala-Silva T, Irish B. 2006. Fruit and nut genebanks in the US National Plant Germplasm System. HortScience. 41(5): 1188–1194. https://doi.org/10.21273/HORTSCI.41.5.1188.

Samuels FMD, Stich DG, Bonnart R, Volk GM, Levinger NE. 2021. Non-uniform distribution of cryoprotecting agents in rice culture cells measured by CARS microscopy. Plants (Basel). 10(3):589.

Senula A, Keller ERJ, Sanduijav T, Yohannes T. 2007. Cryopreservation of cold-acclimated mint (*Mentha* spp.) shoot tips using a simple vitrification protocol. Cryo Lett. 28(1):1–12.

Skogerboe DM, Jenderek MM. 2023. Improvement in micropropagation and cryopreservation of *Musa*. Acta Hortic. 1359:209–214. https://doi.org/10.17660/ActaHortic.2023.1359.27.

Towill LE, Forsline PL, Walters C, Waddell JW, Laufmann J. 2004. Cryopreservation of *Malus* germplasm using a winter vegetative bud method: Results from 1915 accessions. Cryo Lett. 25: 323–334.

Tyler NJ, Stushnoff C. 1988. The effects of prefreezing and controlled dehydration on cryopreservation of dormant vegetative apple buds. Can J Plant Sci. 68(4):1163–1167. https://doi.org/10.4141/cjps88-144.

Volk GM. 2020. Training in plant genetic resources: Cryopreservation of clonal propagules. https://colostate.pressbooks.pub/clonalcryopreservation/. [accessed 23 Apr 2025].

Volk GM, Bonnart R, Shepherd A, Krueger RR, Lee R. 2015. Cryopreservation of *Citrus* for long-term conservation. Acta Hortic. 1065:187–191. https://doi.org/10.17660/ActaHortic.2015.1065.19.

Volk GM, Bonnart R, Shepherd A, Yin Z, Lee R, Polek ML, Krueger R. 2017a. Citrus cryopreservation: Viability of diverse taxa and histological observations. Plant Cell Tiss Organ Cult. 128(2):327–334. https://doi.org/10.1007/s11240-016-1112-4.

Volk GM, Chen K, Bonnart RM. 2022. Plant cryopreservation: Implementation and outreach. Acta Hortic. 1339:93–100. https://doi.org/10.17660/ActaHortic.2022.1339.13.

Volk GM, Henk AD, Jenderek MM, Richards CM. 2017b. Probabilistic viability calculations for cryopreserving vegetatively propagated collections in genebanks. Genet Resour Crop Evol. 64(7):1613–1622. https://doi.org/10.1007/s10722-016-0460-6.

Volk GM, Jenderek MM, Walters C, Bonnart R, Shepherd A, Skogerboe D, Hall BD, Moreland B, Krueger R, Polek ML. 2019. Implementation of *Citrus* shoot tip cryopreservation in the USDA-ARS National Plant Germplasm System. Acta Hortic. 1234:329–334. https://doi.org/10.17660/ActaHortic.2019.1234.43.

Volk GM, Waddell J, Bonnart R, Towill L, Ellis D, Luffman M. 2008. High viability of dormant *Malus* buds after 10 years of storage in liquid nitrogen vapour. Cryo Lett. 29(2):89–94.

Vollmer R, Villagaray R, Castro M, Cárdenas J, Pineda S, Espirilla J, Anglin N, Ellis D, Azevedo VCR. 2022. The world's largest potato cryobank at the International Potato Center (CIP): Status quo, protocol improvement through large-scale experiments and long-term viability monitoring. Front Plant Sci. 13:1059817.

Wang B, Wang R-R, Cui Z-H, Bi W-L, Li J-W, Li B-Q, Ozudogru EA, Volk GM, Wang Q-C. 2014. Potential applications of cryogenic technologies to plant genetic improvement and pathogen eradication. Biotechnol Adv. 32(3):583–595.

Weise S, Lohwasser U, Oppermann M. 2020. Document or lose it: On the importance of information management for genetic resources conservation in genebanks. Plants (Basel). 9(8):1050.