Apple CGC Minutes
October 20, 2022; 1:00-4:00 EDT
Teleconference hosted by Plant Genetic Resources Unit (PGRU)
Geneva, NY

Attendees: Gayle Volk (chair), Herb Aldwinckle, Peter Bretting, Matt Clark, Larissa Carvalho Costa, Kate Evans, Gennaro Fazio, Chris Gottschalk, Ben Gutierrez, Peter Herzeelle, Nick Howard, Oscar Hurtado-Gonzales, Awais Khan, Sarah Kostick, Jason Londo, Jim Luby, Jim McFerson, Cameron Peace, Greg Peck, Lauri Reinhold, Chris Richards, Terence Robinson, Jessica Waite, Kenong Xu

Minutes provided by: Gayle Volk

National Program Report (Bretting, see attached for PDF of presentation)
Discussion:

- The 2018 Farm Bill requested an NPGS Plan to describe backlogs for characterization and maintenance. This was completed in 2021 and is in the review process.
- Evaluation is the determination of agricultural and horticultural traits used by breeders that are of importance to farmers (often referred to as phenotyping)
- Characterization is the measurement of highlight heritable, often genetic traits that are used to determine trueness to type and genetic relationships (often referred to as genotyping)
- Prebreeding is the first step in genetic enhancement. Moving crop wild relatives to a stage where they could be more readily used in a cultivar breeding programs. Not generally a high priority in the NPGS due to limited resources available.

National Germplasm Resources Laboratory (NGRL) (Kinard) See attached

National Genetic Resources Advisory Council Report (McFerson)
This reactivated 14 member committee reports on all National Genetic Resources programs (plant, animal, microbe) to the Secretary of Agriculture. They reviewed the 2018 Farm Bill report that addressed needs and shortcomings in the NPGS. Submit NPGS success stories!

Plant Germplasm Quarantine Program Report (Hurtado-Gonzales)
A powerpoint was presented describing the activities of the Plant Quarantine program. New processing pipelines have been proposed and approved to streamline the quarantine process using a combination of sequencing technologies and RT-qPCR to detect pathogens throughout the testing and clean-up process. Ongoing efforts seek to compare incoming germplasm with existing materials in collections using fingerprinting/KASP technologies to determine if the materials are already available in the US (perhaps under a different name). Therapy clean-up processes are the slowest part of the quarantine procedure.

Curator Report (Gutierrez, see attached for written report)
Highlights:

- New Orchard Manager: Peter Herzeelle
- Dawn Dellfave is retiring 12/31/2022
- Public open house on 9/17/2022 had over 100 attendees

- A new non-research request tool in GRIN-Global has reduced the number of legitimate order requests by more than 50%, thus drastically reducing the number of orders filled each winter
- Field efforts: Previous concerns about waterlogged soils are being addressed by the addition of new drainage systems. A new deer fence was constructed.
- Fire blight was a significant concern in FY 2020 (see publication by Dougherty et al. 2021. 11, 144, https://doi.org/10.3390/agronomy11010144) and the issue has not been completely resolved. Although fireblight infections were lower in FY 2021, FY 2022 was another devastating year with old infections flaring up. There is still streptomycin resistant fireblight in the NPGS Apple Collection. As a result, distribution of dormant budwood in FY2023 will again be limited. Current control methods include including Kasumin, Blossom Protect, alternating applications of copper and Apogee every 7-14 days. In 2021, chemical applications resulted in some phytotoxicity, but were corrected in 2022. Russeting and fruit cracking was the main effect of copper applications in 2022.
- In 2020, 218 accessions were grafted onto EMLA7 rootstock, and 169 accessions survived (46% success), in 2021, 268 accessions were grafted onto EMLA7 rootstock, and 144 accessions survived (54% success), in 2022, 520 accessions were grafted into EMLA7 rootstock and 484 accessions survived (93% success). Grafting on G.890 had lower levels of survival.
- Rootstock trials will determine rootstock compatibility for wild *Malus* species, with the hope to improve fireblight tolerance of the orchards in the future.
- Most of the W3 orchard was removed using tree selection/grafting plans that were developed a few years ago.
- The Impact Statement is a work-in-progress. A draft document was circulated for comment, and there will be email follow up discussions. There's a need for adding Success Stories to the document, and community participation in this activity is encouraged. Here is the current version: https://www.ars.usda.gov/northeast-area/geneva-ny/plant-genetic-resources-unit-pgru/apple-collection/apple-impact/
- Budwood exposure to liquid nitrogen appears to decrease the fireblight colony forming units of grafted budwood. Is this enough to reduce the transfer of fireblight during the repropagation process?
- NLGRP cryopreserved dormant budwood recovery requests have been put on hold until current repropagation efforts are completed.
- The Gala x M. sieversii G1 orchard will be removed next.
- A high-density, replicated planting is proposed for the current G1 orchard location.

Discussion topics:

- How long does it take to see fireblight in grafted trees? It could take up to a year—this should be considered when grafting and evaluating the viability of the cryopreserved apple accessions. It is important to consider the presence of fireblight when backing up accessions at NLGRP.
- The Apple CGC discussed the need for fingerprinting new accessions prior to acceptance and to develop a process to decide which new accessions to accept to optimize field space. Genotyping platforms through WSU are available for fingerprinting efforts. Oscar Hurtado-Gonzales has added a genotyping step into the PGPQ processing pipeline to screen materials prior to clean-up.
- Are stakeholders (especially nurseries) willing to accept the risk of receiving streptomycin resistant fireblight that could be in distributed apple accessions? The level of concern likely varies with recipient.

- Oscar H.G. has found that some nurseries distribute virus infected rootstocks, which affects his results after the quarantine cleanup process. Ongoing efforts to have a close self-sufficient program that will generate its own clean rootstock material. There is a need to educate stakeholders on using virus free rootstocks since PGQP's release material (mostly budwood) could become contaminated in stakeholder's hands.
- Fireblight is likely becoming more prevalent due to the warm, wet springs.
- In Upstate NY, streptomycin resistant fireblight has been present for years. Growers routinely rogue trees as needed.
- It is important to prune heavily and remove all cankers to remove the fireblight from the infected trees. It returns if a mild approach is taken.
- Is seedling rootstock compatible with all the species that could be recovered from liquid nitrogen? EMLA 7 is compatible. Consider using EMLA 7 for viability assessment after cryopreservation.
- Oscar H.G. is working with the Geneva curation team to perform large-scale virus testing in the collection.
- Virus loads may also affect grafting success.
- Request for the Apple CGC to receive up-to-date collection inventory information (number of accessions, seedlings, losses, etc)

GRIN-U and Security back-up (Volk, see attachment)

- Prior to 2021: 2052 apple accessions were backed up as dormant buds at NLGRP. In 2021, 83 accessions were cryopreserved at NLGRP and grafted in Geneva. Of these, 36 are now fully backed-up at NLGRP. 40 accessions met the 40% viability standard, but not enough material was sent for processing—additional budwood could be processed at some point. In 2022, 102 apple accessions were cryopreserved at NLGRP and grafted in Geneva. 16 of those are fully backed up. 35 met the 40% viability standard, but not enough material was sent for processing.
- Current cryopreservation standards are to have 40% viability and at least 60 predicted viable shoot tips in cryostorage. G. Volk proposed that we aim for these standards, but accept a lower level (6 tubes of 10 buds each, 40% viability OR 8 tubes of 10 buds each, 30% viability) because often we do not receive enough budwood to meet our standards.
- GRIN-U.org was launched in July 2021. It provides online resources for training, including ebook chapters on apple shoot tip and dormant bud cryopreservation and a webinar that was provided by Ben Gutierrez in 2021. We would be happy to collaborate on developing more content. See attached PDF for more information.

New Core Subcommittee (Ben Gutierrez) The New Core Subcommittee is identifying potential individuals to be included in a high-density planting. This same planting may also be grown at other locations, including Ithaca, NY.

Land Use Efficiency Subcommitee (Ben Gutierrez). No report.

Proposed New Collection Acquisition and Retention Subcommittee (Gayle Volk). Develop an acquisition policy and retention policy. Identify criteria for accepting new accessions and identify potential candidates that should be added to the NPGS collection. Members: Gayle Volk, Ben Gutierrez, Cameron Peace, Jim Luby. Send out email invite to invite others for first meeting.

SNP array results for the NPGS Apple collection (Volk, attached) The 20K SNP array has been used to genotype unique cultivar (and progenitor species) the NPGS collection and was recently published (Volk et al. 2022 10.3389/fpls.2022.1015658). It revealed hybrid relationships between wild “species” accessions and domesticated cultivars. Of 383 accessions of *M. sieversii*, 151 were pure *M. sieversii*, 215 were hybrid/admixed, and 17 were pure *M. domestica*). Of 36 accessions of *M. orientalis*, 26 were pure *M. orientalis*, 9 were hybrid/admixed, and 1 was pure *M. domestica*). Of 44 accessions of *M. sylvestris*, 35 were pure *M. sylvestris*, 6 were hybrid/admixed, and 3 were pure *M. domestica*. We need to find a way to record this information in a very apparent format in GRIN-Global. Accessions that are not the described species are being updated as hybrids or as the correct species in GRIN, with annotations to describe the previous taxonomic identity.

Fruit RegisTREE & apple genotyping (Peace) Cameron Peace’s lab has made SNP genotyping available for identity and pedigree determination. Species-specific alleles are also being identified. He’s also leading a heritage apple group that is developing tools to document fruit trees in the landscape and in private and non-profit collections.

2021 Evaluation Grant Results (Gottschalk) The Apple CGC Evaluation Grant was awarded to Chris Gottschalk to perform genotyping and resequencing of *M. angustifolia* concurrently with his genome sequencing and assembly of a reference quality genome for *M. angustifolia* PI 613880 from the NPGS Apple Collection. Seedlots that were received have low germination.

Meeting adjourned at 4 pm EDT.

The National Plant Germplasm System: 2022 Status, Prospects, and Challenges

Peter Bretting

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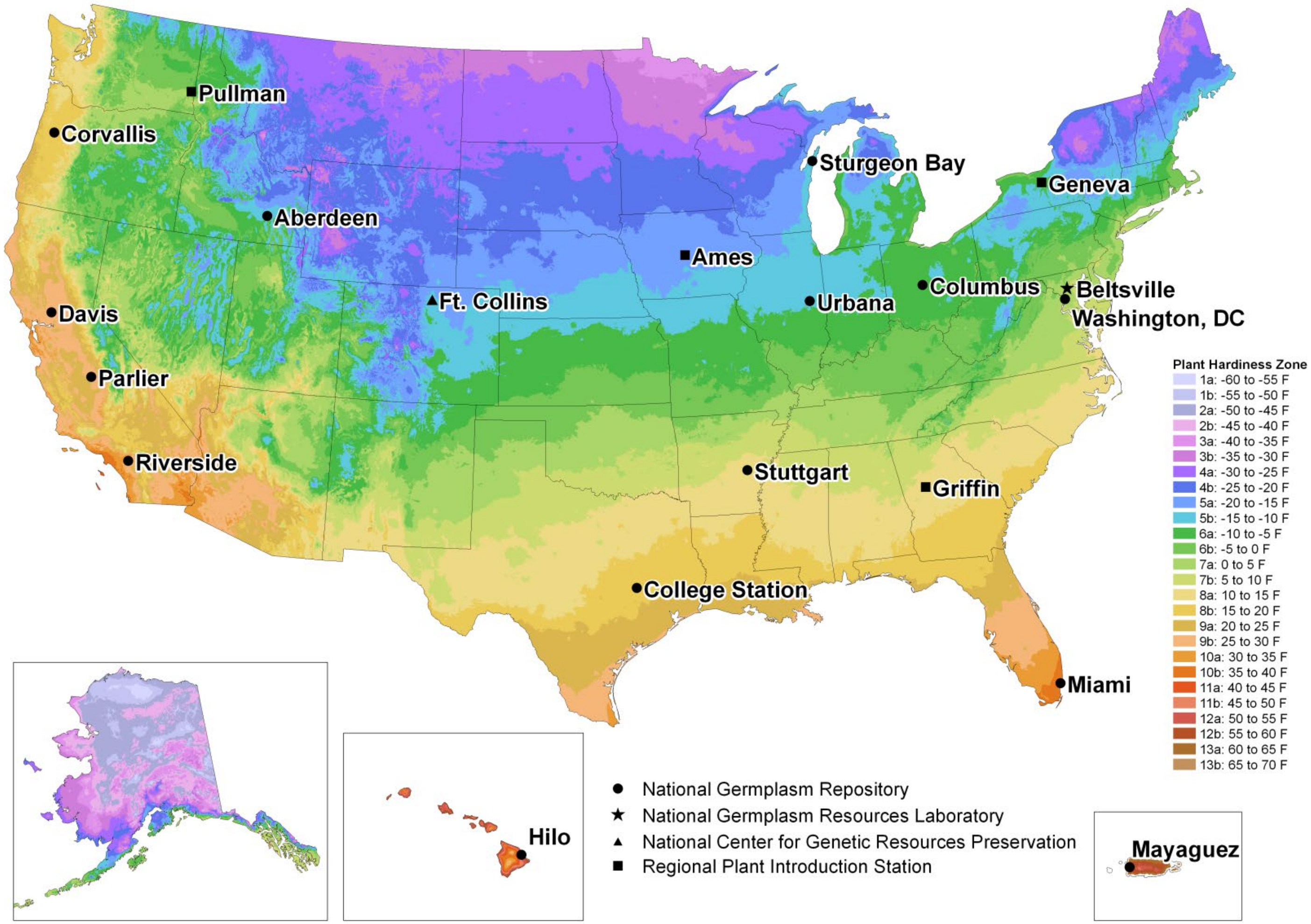
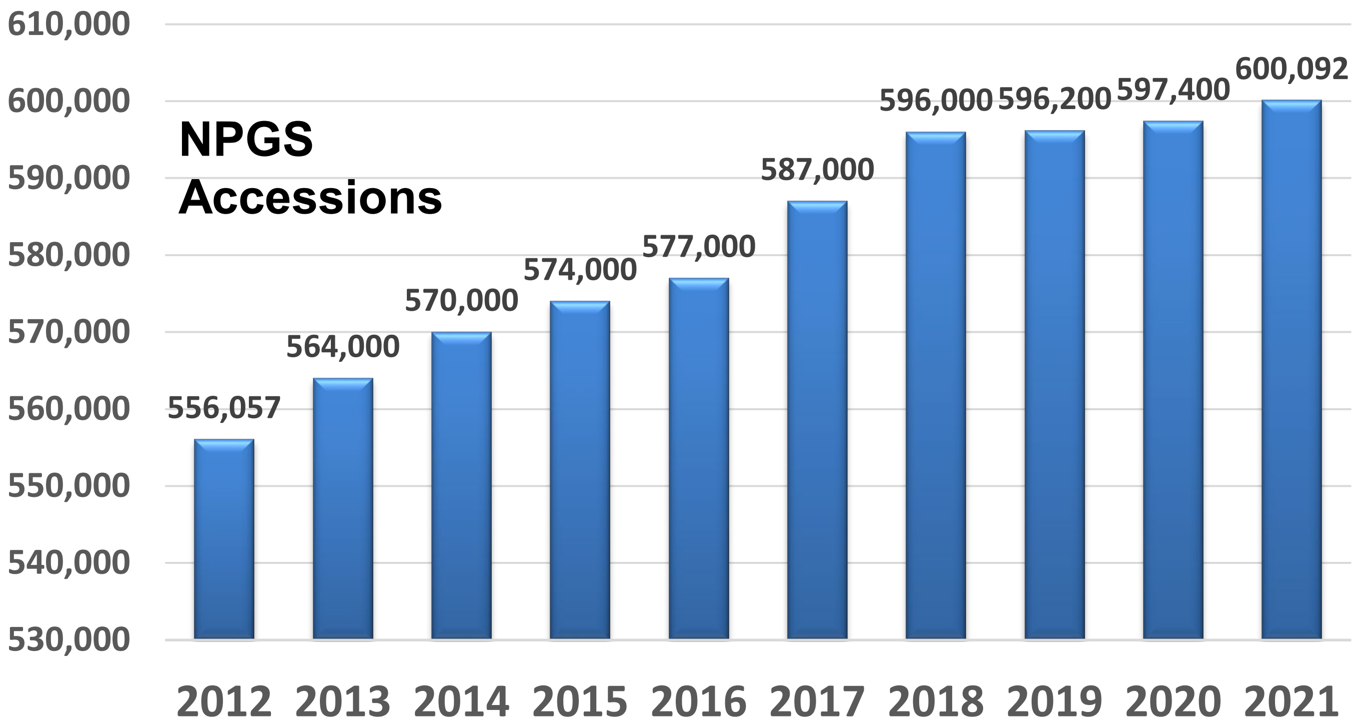
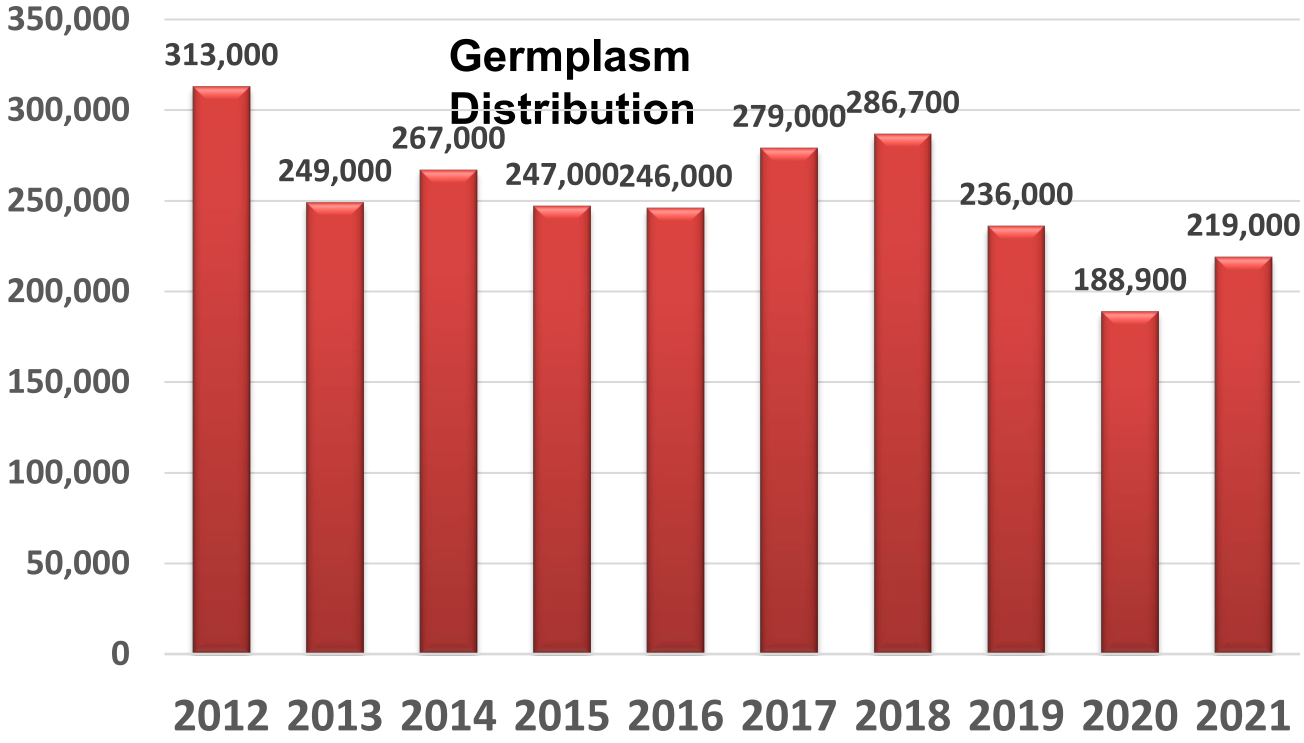
USDA National Plant Germplasm System (NPGS)



Number of NPGS Accessions 2012-2021



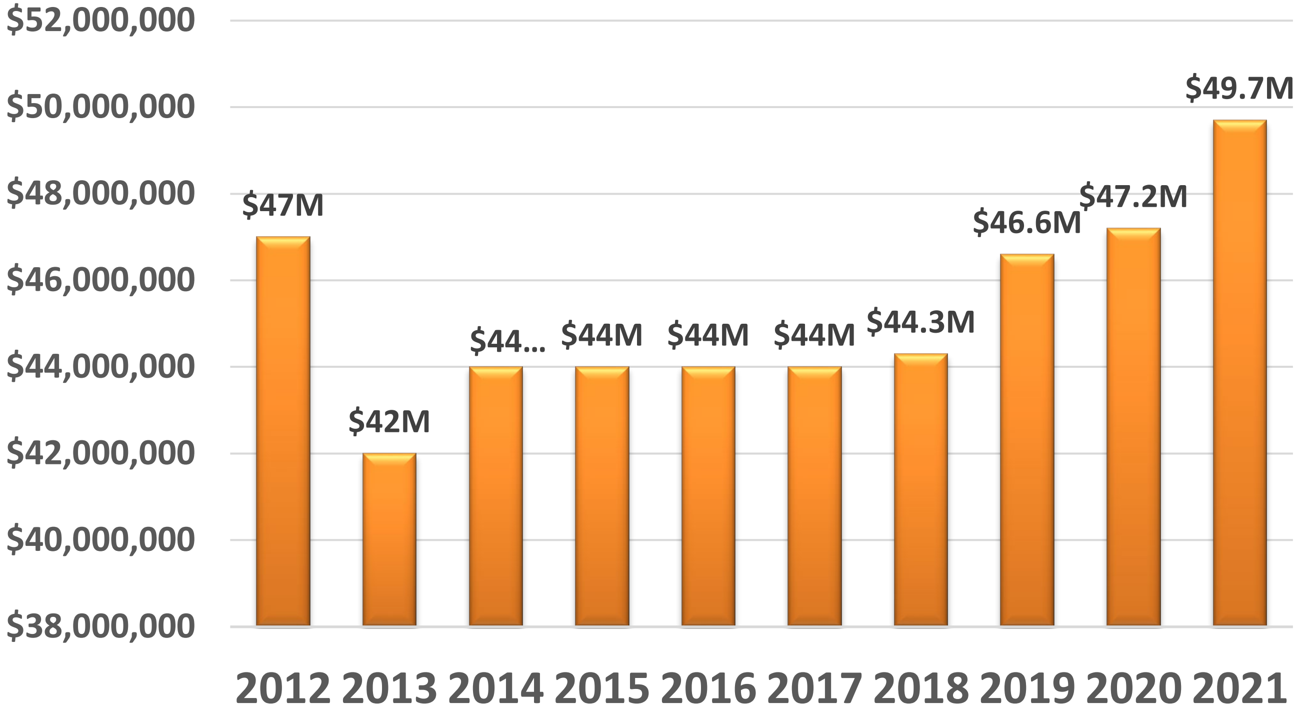
DEMAND FOR NPGS GERMPLASM
2012-2021

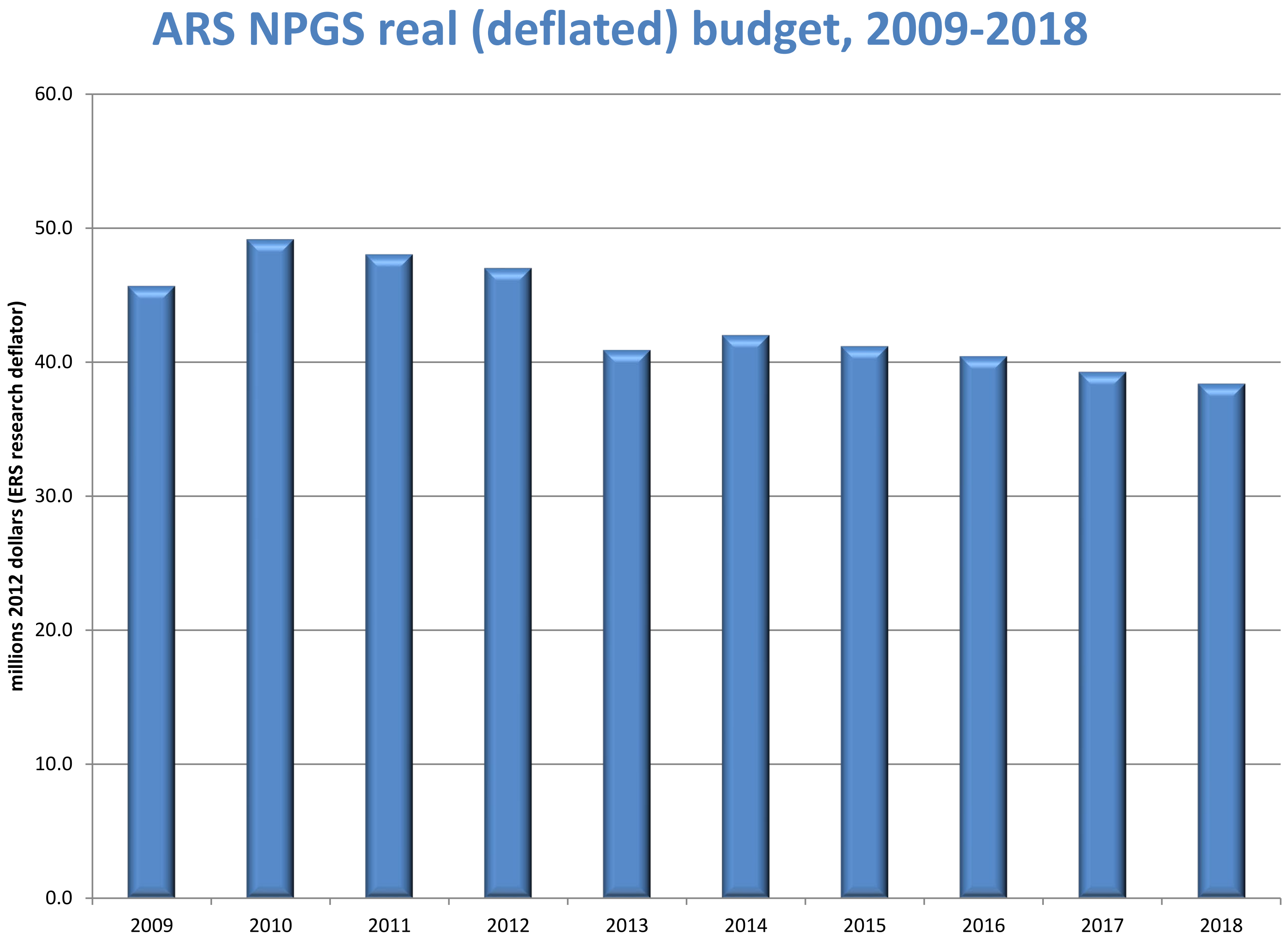


Effects of CoVID-19 as of October 2022

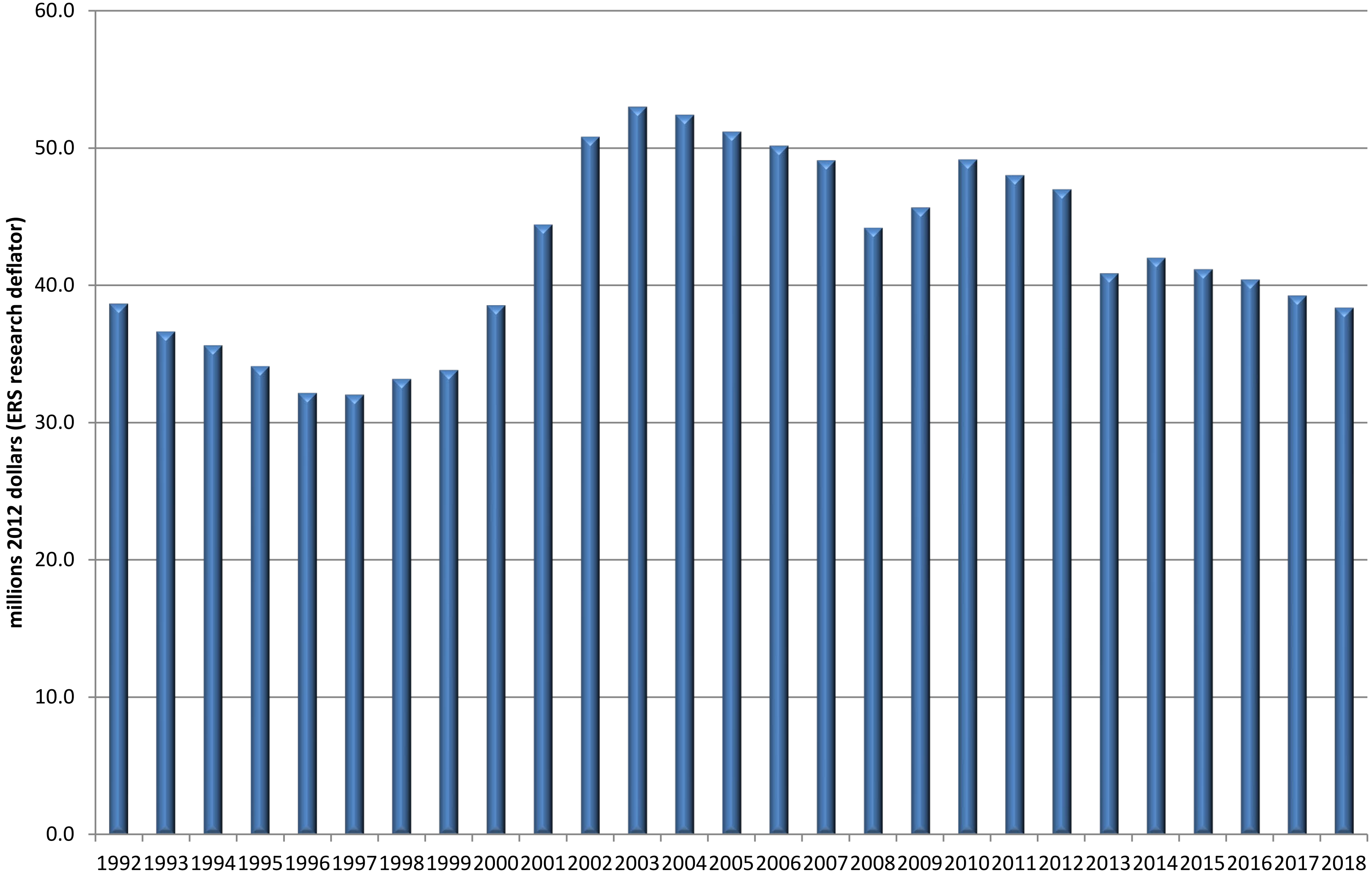
- **All NPGS genebanks are shipping germplasm (PGR).**
- **# of samples distributed fell by ca. 20% in 2020. The distribution rate increased substantially from the 2020 level in 2021 but is still a bit below the long-term average.**
- **Genebanks are fully operational. Some genebanks are finding it difficult to hire temporary, often student, labor, because of the low unemployment rate and demographic changes.**
- **GRIN-Global has functioned normally throughout the pandemic.**

ARS National Plant Germplasm System Budget 2012-2021




ARS NPGS real (deflated) budget, 2009-2018
millions 2012 dollars (ERS research deflator)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018

ARS NPGS real (deflated) budget, 1992-2018



Some key challenges for the NPGS

- **Expanding the NPGS operational capacity and infrastructure to reduce PGR management backlogs and meet increased demand for PGR and associated information.**
- **Increased operational costs (labor, inputs, overall inflation). See** https://www.ers.usda.gov/amber-waves/2022/june/investment-in-u-s-public-agricultural-research-and-development-has-fallen-by-a-third-over-past-two-decades-lags-major-trade-competitors/?cpid=email#
- **NPGS personnel transitions—hiring, training, etc.**
- **Developing and applying cryopreservation and/or in vitro conservation methods for clonal and some seed PGR.**
- **BMPs and procedures for managing accessions (and breeding stocks) with an increasing diversity of GE traits in more crops, the occurrence of adventitious presence (AP), and the products of gene editing.**
- **Acquiring and conserving additional PGR, especially of crop wild relatives.**

PGR Management Priorities: Foundations for Crop Innovation

- Acquisition
- Maintenance
- Regeneration
- Documentation and Data Management
- Distribution
- Characterization
- Evaluation
- Enhancement
- Research in support of the preceding priorities

NPGS Personnel Transitions

- **Farewell and best wishes to Joanne Labate, Vegetable Curator (ARS-Geneva); Lorie Bernhardt, Seed Distributions (ARS-Stuttgart); Esther Peregrine, Assistant Curator (ARS-Urbana); Renée White, IT Specialist (ARS-Ft. Collins), and Kim Hummer, RL (ARS-Corvallis).**
- **Welcome and best wishes to Jeff Gustin, Maize Genetic Stock Curator and Benjamin Bartlett Soybean Assistant Curator (ARS-Urbana); Erin Galarneau, Grape Curator (ARS-Geneva); Madhugiri Nageswara-Rao, Tropical Ornamental Curator (ARS-Miami) and Gul Shad Ali, Tropical Crops Curator (ARS-Miami); Lauri Reinhold, Curator (ARS-Corvallis); Anne Frances, Botanist and Matthew Riggs, IT Specialist (ARS-Beltsville); Warren Chatwin, Pecan Curator (ARS-College Station); Jonathan Moser, Seed Distributions (ARS-Stuttgart); Harlan Svoboda, Herbarium Curator (ARS-USNA); Robert Krueger, RL (ARS-Riverside); and Marilyn Warburton, RL, Sarah Dohle, Curator, Paul Galewski, Curator, and Bailey Hallwachs, SOS Coordinator (ARS-Pullman); and Claire Heinitz, RL (ARS-Davis and Parlier).**
- **We are recruiting staff at Corvallis, OR; Parlier, CA; and Geneva, NY.**

PGR Management Training Initiative

- **Numerous NPGS PGR managers have retired recently; no formal, comprehensive program existed for training new PGR managers.**
- **G. Volk (ARS-Ft. Collins) and P. Byrne (CSU-Ft. C.) lead a project, supported by ARS and a NIFA grant, to design and develop a training program for PGR management to be delivered primarily through distance-learning.**
- **A now three module, 3 credit hour Colorado State online course Plant Genetic Resources: Genomes, Genebanks, and Growers to be taught in Aug.-Sept. 2022-- the first time for the three-part course. http://pgrcourse.colostate.edu/**
- **Numerous PGR training/educational materials are freely accessible from GRIN-University at https://grin-u.org/**
- **Infographic posters for PGR, genebanks and conservation, and PGR and food security in 6 languages; download at http://genebanktraining.colostate.edu/trainingmaterials.html**

FY 20-21 ARS NPGS Budgetary Increases

- **Small grains PGR ($190,000): Aberdeen, ID.**
- ***Vaccinium* PGR ($150,000): Corvallis, OR.**
- **Hemp PGR ($1.35 million): Geneva, NY.**
- **Pecan PGR ($400,000): College Station, TX.**

USDA-ARS
National Germplasm Resources Laboratory
Beltsville, Maryland
2022 Report to PGOC, RTACs, and CGCs

The National Germplasm Resources Laboratory (NGRL) supports the acquisition, introduction, documentation, evaluation, and distribution of germplasm by the National Plant Germplasm System (NPGS) and other components of the U.S. National Genetic Resources Program (NGRP). The Laboratory is comprised of the Plant Exchange Office (PEO), the Database Management Unit (DBMU), and the Plant Disease Research Unit (PDRU).

Dr. Dimitre Mollov transferred to the ARS Horticultural Crops Research Unit in Corvallis, OR, in June 2021. NGRL hopes to fill this vacant Plant Pathologist position in 2022.

Dr. Anne Frances joined NGRL as a Botanist in August 2021. Anne comes to ARS and NGRL after serving as the Lead Botanist for NatureServe, a conservation science-based NGO, for ten years. Anne is a scientist in the Plant Exchange Office project.

Plant Exchange Office

Plant Exploration and Exchange Program:

- The PEO supports the collection of germplasm for the NPGS through the management of the Plant Exploration and Exchange Program. Guidelines for developing plant exploration and exchange proposals will be distributed to CGC chairs in February 2022. Proposals must be endorsed by the appropriate CGC or other crop experts to be considered for funding.
- Most explorations approved for funding in FY 2020 and FY 2021 were postponed due to the pandemic. It is unclear at this writing (January 2022) whether the postponed explorations and any new ones approved for FY 2022 will be able to proceed this year. Due to funding constraints imposed by proposals already approved, it may not be possible to approve new exploration or exchange proposals for funding in FY 2023. Please consult with PEO before developing proposals for FY 2023.
- Two explorations were conducted in FY 2021. One international exploration was conducted in the country of Georgia for *Salix* by in-country scientists. One domestic exploration was conducted in Illinois for *Aronia* species, deciduous shrubs used as ornamental landscape plants and as an edible fruit crop. All postponed explorations will be rescheduled when pandemic-related travel restrictions are lifted and conditions are considered safe.
- All foreign explorations supported by PEO must comply with the principles in the Convention on Biological Diversity covering access and benefit sharing related to genetic resources. Prior informed consent to collect genetic resources is obtained from the host country before the exploration. The PEO is involved in most requests to foreign

governments for permission to collect and negotiates the terms of agreements when necessary.

Collaboration on Crop Wild Relatives in the U.S.:

The NGRL is collaborating with NatureServe, the US Botanic Garden, and other partners on the conservation of *Vitis* species native to North America, which are crop wild relatives and used as rootstock for the cultivated grapevine (*Vitis vinifera*). Conservation status assessments are being completed and an invitational workshop is planned for fall of 2022.

GRIN Taxonomy for Plants:

- GRIN Taxonomy, available through GRIN-Global (https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch), provides online current and accurate scientific names and other taxonomic data for the NPGS and other worldwide users. This standard set of plant names is essential for effective management of ARS plant germplasm collections, which now represent ca. 16,300 taxa. A broad range of economically important plants is supported by GRIN nomenclature, including food, spice, timber, fiber, drug, forage, soil-building or erosion-control, genetic resource, poisonous, weedy, and ornamental plants. The search page (excluding the World Economic Plants search) was rewritten in 2021 to allow a broader range of searches and provide the option to export most search results.
- GRIN Taxonomy includes scientific names for 27,931 genera (14,715 accepted) and 1,422 infra-genera (1,355 accepted) and 125,758 species or infra-species (69,125 accepted), with over 67,798 common names, geographical distributions for 61,212 taxa, 510,559 literature references, and 32,468 economic importance records. These numbers increase regularly.
- Since 2008, a project to provide thorough coverage of wild relatives of all major and minor crops in GRIN Taxonomy has been underway. We have completed our initial work on 386 major and minor crops from 174 genera, and CWR from 4,295 taxa have been mapped to these crops and others under progress. An interface to query these data is available (https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearchcwr.aspx). We invite feedback from NPGS curators and CGC members for those CWR classifications already developed.

Facilitation of Germplasm Exchange:

The PEO helps expedite the distribution of germplasm from the NPGS to foreign scientists and international genebanks through a long-standing collaboration with USDA-APHIS at Building 580, BARC-East in Beltsville. The pandemic caused a backlog in shipment of orders in 2020. Although the backlog was significantly reduced in 2021, international shipments remain challenging. Only one APHIS inspector is currently available to inspect NPGS outgoing shipments, and logistical delays related to global shipping are continuing.

In 2021, 735 public orders containing a total of 48,196 samples of NPGS accessions were shipped from Beltsville to individuals in 72 countries for research and education. This is more orders than have been shipped from Beltsville in any previous year. In addition, PEO facilitated the agricultural inspection of arriving germplasm shipments containing accessions from numerous foreign countries for researchers and curators at NPGS sites.

Crop Germplasm Committees:

- Many committees continue to meet regularly and are active, although the second year of the pandemic continued to create challenges, especially for committees that typically meet in person. Committees are urged to meet at least annually, and especially to update their Crop Vulnerability Statements. Several CGCs recently completed new versions. The NPGS has been fortunate to fill numerous vacant positions in the last 1–1.5 years, and we hope more will be filled in 2022. These new staff would especially benefit from active and supportive CGCs.
- A virtual meeting/web conference for CGC Chairs is scheduled for March 3, 2022. The 2021 CGC Chairs meeting was held February 21, 2021, and the presentations are archived on the CGC page at https://www.ars-grin.gov/CGC.
- NGRL has a Zoom conferencing account that is available to the CGCs to use for hosting virtual meetings.
- Please send updates to the individual crop committees of the CGC page on GRIN (https://www.ars-grin.gov/CGC) to Gary Kinard.

Database Management Unit

GRIN and GRIN-Global:

- At the time of this report, the GRIN-Global plant database included the following:

  600,495 active accessions representing 16,308 species and 2,568 genera
  3,506,531 inventory records
  2,147,592 seed germination/viability testing records
  9,126,834 characteristic/evaluation records
  1,404,683 attachment files, primarily digital images

These numbers increase regularly, some almost daily.

- Incremental improvements were made in the GRIN-Global applications throughout 2021. One of the major enhancements was in GRIN Taxonomy, which received a major redesign. Search pages, especially for nodulation data, received a new interface, a browse feature was added with family, genus, species, and common name search options, and the capability to perform species-level searches on geographical distribution was improved. In June 2021, a major enhancement was made to allow for public display and ordering, if the curator implements it, of multiple inventories or propagule forms of single accession. This is particularly relevant for clonal collections that may curate both asexual (whole plant with cuttings, stolons, etc. distributed) and sexual (seeds, pollen, fruit) forms of a single accession This also assists with requesting cuttings from a specific gender of dioecious accessions where both male and female plants are curated. Another feature added in 2021 was implementation of a tool to filter automatically incoming orders that have characteristics potentially indicative of illegitimate requests, which we call Non-Research Requests (NRR). This NRR Tool allows staff to manage efficiently and consistently such requests NPGS-wide, including using system-generated emails to communicate decisions about submitted orders.

- Current information about the project, including user documentation and release notes from each version of the software, can be found on the project website at https://www.grin-global.org/.

Plant Disease Research Unit

The PDRU conducts research on pathogens that infect clonally propagated prohibited genus (i.e., quarantine) plant germplasm, including their etiology, detection, and elimination by therapeutic procedures. This project provides direct support to the APHIS Plant Germplasm Quarantine Program and helps facilitate the safe introduction, conservation, and international exchange of valuable plant germplasm. PDRU also collaborates on virus related problems with NPGS germplasm repositories, state departments of agriculture, and university scientists. Additional updates will be provided for those committees whose crops are within the scope this project's research.

Key NGRL Contacts

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Plant Disease Research Unit
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PLANT GENETIC RESOURCES UNIT REPORT TO THE APPLE CGC

October 20, 2022

Ben Gutierrez, Tori Meakem, Peter Herzeelle, Dawn Dellefave, and Gan-Yuan Zhong

OPERATIONS

The PGRU Clonal team operates with Gan-Yuan Zhong (lead scientist), Erin Galarneau (*Vitis* curator), Ben Gutierrez (Apple/Tart Cherry curator), Dawn Dellefave (program support/database manager), Tori Meakem (Molecular Biologist), Peter Herzeelle (farm manager), and five permanent field technicians through USDA-ARS PGRU and NE9 funding. PGRU hosted three interns in 2022 who worked on sieboldin in apple and grape and tart cherry characterization.

FIELD PROJECTS

In 2021, close to 5000ft of drainage tile was installed/repaired on the Wellington (Vegetable) and McCarthy (Clonal) farms and the grass waterway was extended from the apple collection. There are still significant drainage and erosion issues on the McCarthy farm. The field did some extensive regrading throughout the farm in response to soil erosion. To prevent further erosion, we are developing a new 6-9 month cover crop plan for maintaining herbicide strips. To reduce deer damage and improve security of the farms, PGRU installed 10,560 lineal feet of 8' deer fencing; this has substantially improved our nursery production. Plans for the new pesticide facility are moving forward. A temporary pesticide platform and mixing area are being constructed now.

FIRE BLIGHT

Fire blight continues to be a significant challenge for the Apple Collection. Strain testing in 2022 confirmed the same streptomycin-resistant strain of *E. amylovora* since 2020. In collaboration with fire blight specialists at Cornell University, we modified our management program for apple to account for streptomycin-resistance, including Kasumin, Blossom Protect, alternating applications of copper and Apogee every 7-14 days. In 2021, chemical applications resulted in some phytotoxicity, but were corrected in 2022. Russeting and fruit cracking was the main effect of copper applications in 2022.

REGENERATION

Extensive accession regeneration is ongoing since 2020. Table below summarize the 2022 and 2023 nurseries.

Year	Rootstock	No. Trees Grafted	No. Trees Survived	Grafting Success Rate	No. Accessions Grafted	No. Accessions Survived	Accession Success Rate
2023		-	-	-	489	-	-
2022	EMLA7	1,949	1,332	68%	520	484	93%
	G.890	213	72	34%	68	35	51%

2021	EMLA7	780	261	33%	268	144	54%
2020	EMLA7	654	364	56%	218	169	46%
	G.890	81	74	91%	27	27	100%
2019	EMLA7	247	135	55%	70	49	70%
	G.890	29	16	55%	15	10	66%
2017	EMLA7	14	11	79%	14	11	79%
	G.210	184	80	43%	59	38	33%
2016	EMLA7	140	39	28%	60	24	40%

STATUS OF W3 BLOCK

The W3 was partially removed (91%) in 2021. The remaining (140) individuals were regenerated in 2022 and the mother trees will be removed as these are established.

PROPOSAL for G1

The G1 planting was established 15+ years ago as 7 *M. sieversii* accessions crossed with Royal Gala. Though they have been useful for several genetic studies, they have become difficult and expensive to maintain. Fire blight pressure is high in this block as well, causing some concerns as a source of inoculum for the USDA Apple Collection and Cornell orchards. As interest has decreased over the past several years, we're proposing to remove this block in 2022, and preserve the space for the new high-density core collection.

PROPOSAL HIGHLIGHTS FOR NEW CRIS PROJECT PLAN

Apple Rootstock and High-Density Apple Planting: The largest threat to the apple collection is fire blight, a bacterial disease which spreads quickly through an orchard and can kill susceptible trees within a season (Dougherty et al. 2021). Apple rootstocks can mediate scion qualities (Marini and Fazio 2018; Singh et al. 2019), which could improve PGRU maintenance practices through enhanced resistance to fire blight and other biotic and abiotic stresses, improve grafting success rate and nursery viability, reduced root suckering, and reduced tree size to accommodate higher density plantings.

Currently, the apple collection is maintained on 6 × 3.6 m spacings on M.7 rootstocks across 30 acres. M.7 is a semi-dwarf (50-60% size of a standard seedling) and is moderately resistant to fire blight but produces many root suckers, which requires annual maintenance through chemical control and pruning. Modern apple rootstocks have increased disease resistance to fire blight and other diseases and have reduced-to-no root suckering. PGRU has used G.890 and G.210 rootstocks sporadically in past years with mixed success but has not sufficiently evaluated their performance in the collection.

To evaluate new rootstocks, we will propagate a diverse panel of 100 PGRU *Malus* accessions onto four Geneva series rootstocks: G.890, G.210, G.30, and CG.6006, with M7 as a control (see Table 1 below). Each is a semi-dwarf rootstock that produces a tree 10-20% smaller than M.7 and has improved disease resistance, with reduced suckering relative to M.7 and a low tendency for biennial bearing compared to M.7. *M. domestica* and wild *Malus* accessions will be selected for evaluation based on genetic diversity using genetic data, ploidy variation, and phenotypic diversity. Scions will be indexed for viruses (**sub-objective 1.B**) through the USDA-APHIS Pome Quarantine Program as latent apple viruses can infect susceptible rootstocks impacting the perception of compatibility (Fazio et al. 2015). Virus-free scions and rootstocks will be used where possible to reduce the impact of graft success due to latent viruses (Fazio et al. 2015). Accessions will be grafted onto the rootstocks, with 4-5 replicate trees per accession following established PGRU nursery standards.

Table 1. Rootstocks to be evaluated for their suitability for apple germplasm preservation

Rootstock	Size	Disease Resistance	References
M.7 Control	Semi-dwarf (50-60%)	Moderate fire blight resistance	(Singh et al. 2019)
B.10	Semi-dwarf (40-50%)		No Reference
G.969	Semi-dwarf (40-50%)	Strong resistance to fire blight Phytophthora root rot	(Reig et al. 2018)
G.484	Semi-dwarf (40-50%)	Strong resistance to fire blight and Phytophthora root rot	No Reference
G.66	Semi-dwarf	Strong fire blight resistance	(Autio et al. 2017; Reig et al. 2018)

Nursery trees will be planted in a randomized block and maintained through best practices include topping, irrigation, fertilization, and pest control. After two years, nursery trees will be dug and re-planted as a randomized high-density planting for further evaluation, emphasizing training systems to promote vegetative growth and ease of maintenance (Lordan et al. 2018). Two annual inventories will determine graft success rates and tree survival rates throughout the nursery and early high-density stages. Growth rate, trunk width, height, graft compatibility, and survival rates will be measured each fall. Accession, species, rootstock, growth, and year factors will be analyzed using generalized linear mixed models.

Apple Cryopreservation and Fire Blight (hypothesis driven): Fire blight, a disease caused by the bacterium Erwinia amylovora, is a major threat to the UDSA apple collection (Dougherty et al. 2021), requiring extensive interventions and regeneration of severely infected trees. Fire blight can be present in asymptomatic tissues (Tancos et al. 2017) and carried over during propagation which later proliferates in regenerating tissues, impacting PGRU propagation, distribution, and potentially cryopreserved germplasm. We hypothesize that cryopreservation techniques will significantly reduce fire blight inoculum in apple propagules. Currently, it is uncertain whether E. amylovora can survive cryopreservation, although other microbes have been reported to survive cryotemperatures (Bajerski et al. 2020). To determine the impact of cryotreatments on fire blight-infected apple scions, dormant buds will be inoculated with fire blight (Bell and Van Der Zwet 1987) and processed following NLGRP protocols (Volk et al. 2020). Cryotreatments will occur early to mid-January in Geneva, NY, following at least three successive days at or below 0℃. Scions will be cut into 2 cm nodal sections and:

1. Desiccated to 25-30% moisture content at -5℃.

2. Frozen to -30℃ at the rate of 1℃/hour.
3. Exposed to liquid nitrogen vapor for at least 1 hour.

Sample subsets will be removed during each progressive step and stored at 5℃ prior to E. amylovora culturing, including lab inoculated and non-inoculated controls. Each treatment will include five biological replicate samples, each with 10 to 20 buds. Five buds from each treatment replication will be grafted to determine bud viability. Depending on results, second-year samples will expand to cover more genetic diversity, including apple cultivars and wild progenitors amenable to cryopreservation. ANOVA will be used to determine statistical variation between treatments. Linear mixed models will be used to determine the effects of species and genotype diversity assay in the second year. If successful, methods for dormant bud cryopreservation could develop into a SOP for regeneration of priority and diseased accessions or international germplasm distribution. In a pilot study, we observed a 96% reduction of fire blight colony forming units (CFUs) in 'Gala' samples exposed to all three cryopreservation stages, compared to untreated, inoculated samples. Additional sampling is needed to determine significance.

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Cryopreservation of Apple Dormant Buds

Prior to 2021: 2052 apple accessions were backed up as dormant buds at NLGRP, with a minimum of 19 predicted viable buds per accn.

2021: 83 accessions were cryopreserved at NLGRP and grafted in Geneva

- 36 fully backed-up at NLGRP
- 40 met the 40% viability standard, but not enough material was sent for processing

2022: 102 accessions were cryopreserved at NLGRP and grafted in Geneva

- 16 fully backed-up at NLGRP
- 35 met the 40% viability standard, but not enough material was sent for processing

Cryopreservation standards:

- 40% viability
- 60 predicted viable bud segments
- Process 16+ tubes of 10 buds each, use 1-2 for grafting viability, store the rest

Online Learning:
Plant Genetic Resources Management and Use

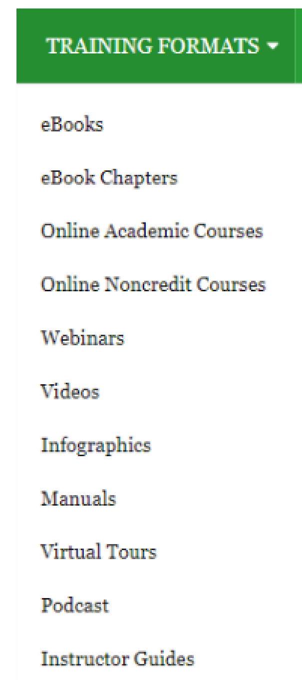
- GRIN.U.Education YouTube site
- Training materials are freely available through GRIN-U.org

Three 1 credit (5 week) online courses offered through Colorado State University

https://pgrcourse.colostate.edu/

GRIN-U.org

- Launched July 2021
- 162 searchable items to date



Quarterly email sent to GRIN-U.org subscribers with updates

Check Out Our New Posts!

Cowpea Diversity 2: Producer and Consumer Traits

WATCH VIDEO Colorado State University Center for Science Communication Cowpeas (Vigna unguiculata) provide numerous benefits

Create content for education and training

Plant genetic resources conservation and use

Freely available online

Mostly college-level

Used by instructors, technical staff, students, managers, etc.

Types of content

- eBooks
- Infographics
- Videos
- Webinars
- Virtual Tours
- Courses

Topics

- Genebanking fundamentals
- Collection maintenance
- Phenotyping and genotyping
- Crop wild relatives
- Plant breeding
- Crops and cultures
- Virtual tours
- Documentation and GRIN-Global
- Success stories

Public Ebooks on GRIN-U.org

Crop Wild Relatives and their Use in Plant Breeding

Gayle Volk and Patrick Byrne

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Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules

Gayle Volk

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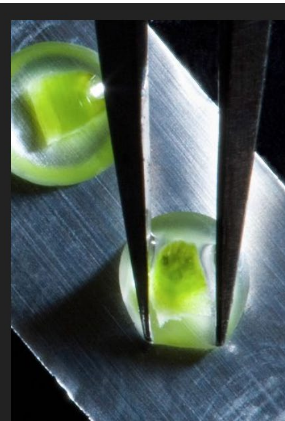
READ BOOK



Field Tour of the USDA National Clonal Germplasm Repository for Tree Fruit, Nut Crops, and Grapes in Davis, California

Gayle M. Volk and John E. Preece

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READ BOOK

Crop Diversity: A Virtual Crop Science Field Tour

Fundamentals of Plant Genebanking

Gayle Volk

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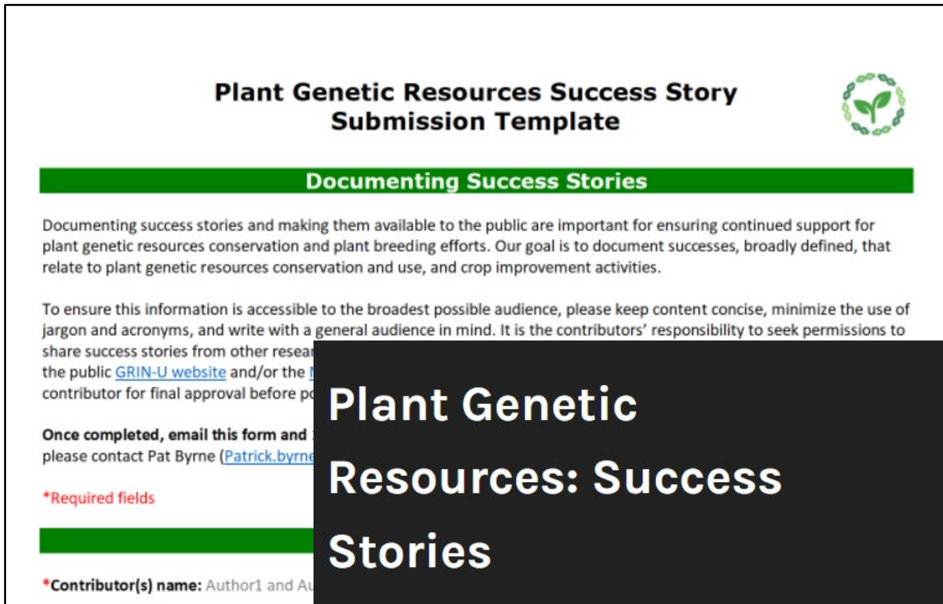
- Applications of Plant Pathology

Seeking Success Story Submissions


Plant Genetic Resources Success Story Submission Template

Documenting Success Stories

Documenting success stories and making them available to the public are important for ensuring continued support for plant genetic resources conservation and plant breeding efforts. Our goal is to document successes, broadly defined, that relate to plant genetic resources conservation and use, and crop improvement activities.

To ensure this information is accessible to the broadest possible audience, please keep content concise, minimize the use of jargon and acronyms, and write with a general audience in mind. It is the contributors' responsibility to seek permissions to share success stories from other resea
the public GRIN-U website and/or the
contributor for final approval before p

Once completed, email this form and
please contact Pat Byrne (Patrick.byrne

*Required fields

***Contributor(s) name:** Author1 and Au


Development of Rice Variety IR36
International Rice Research Institute (IRRI), Philippines

In the 1980's rice variety IR36 was the most widely used variety in rice lands throughout Asia due to its multiple resistance to diseases and pests that were causing significant yield loss. The development of IR36 was led by the International Rice Research Institute (IRRI), which has a long history of rice breeding, including the breakthrough success with IR8, a key variety of the green revolution. To improve rice beyond the success of IR8, IRRI expanded its germplasm collection with the goal of developing rice varieties with multiple disease and pest resistance.

IR36 improved rice variety grown in IRRI field plot

"IR36" by IRRI images is licensed under CC BY-NC-SA 2.0

PROJECT GOALS
- Create improved rice variety resistant to main diseases and pests
- high disease and pest pressure

production
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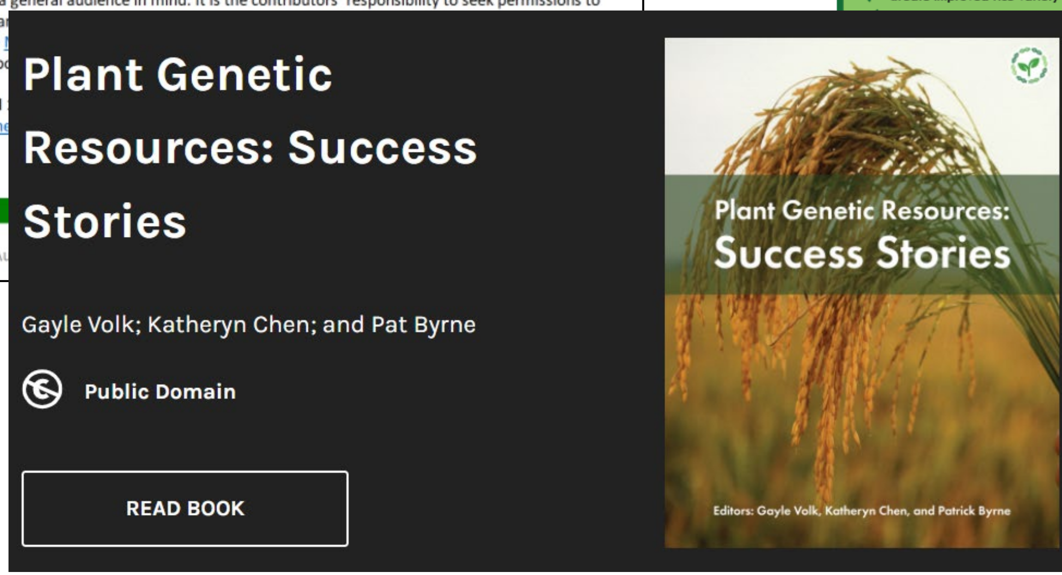
Solutions Developed
The development of disease resistant rice varieties helped stabilize rice production and protected farmers from economic losses. While IRRI gradually released various disease resistant varieties, IR36 had the most success as it possessed the most comprehensive resistance. Thanks to IR36, farmers harvested an average of $1 billion worth of extra rice annually between 1980 and 1984. Five years after its release, a review team stated that the development of IR36 alone justifies the original investment in IRRI 21 years prior.

n Gray, A.A. Mahama, Candice Gardner, K. Chen (editor)
r success stories, visit GRIN-U.org



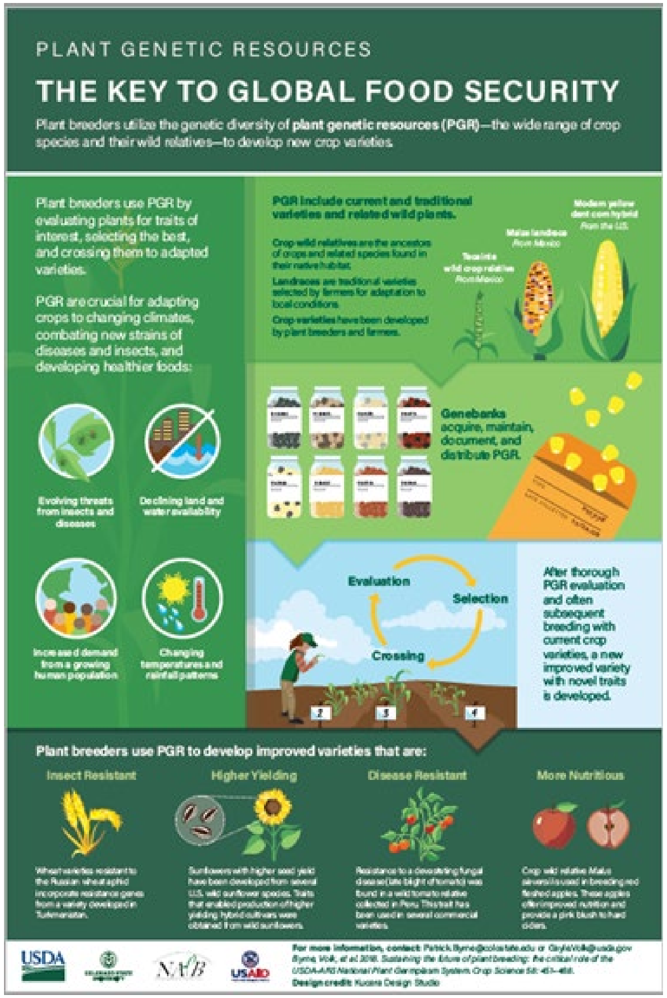
Coming soon!

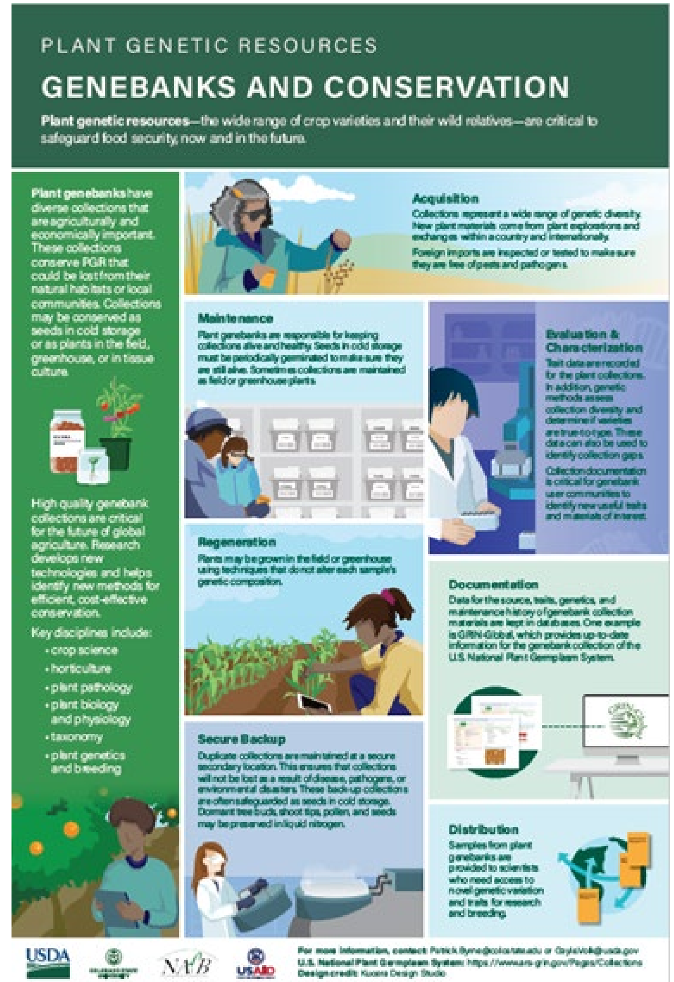
Climate-Ready Plant Collections: Conserving, Using and Building Capacity

Gayle Volk; Tara Moreau; and Pat Byrne

READ BOOK

Infographics available on GRIN-U (print as posters or handouts)





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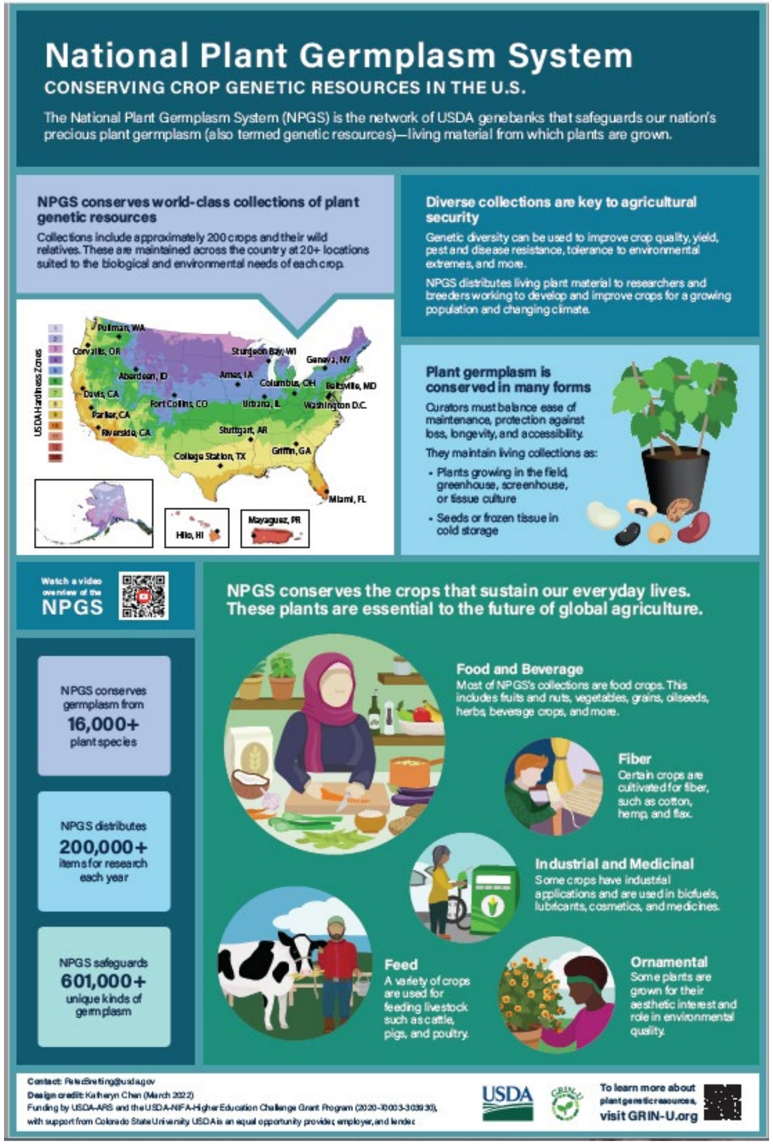

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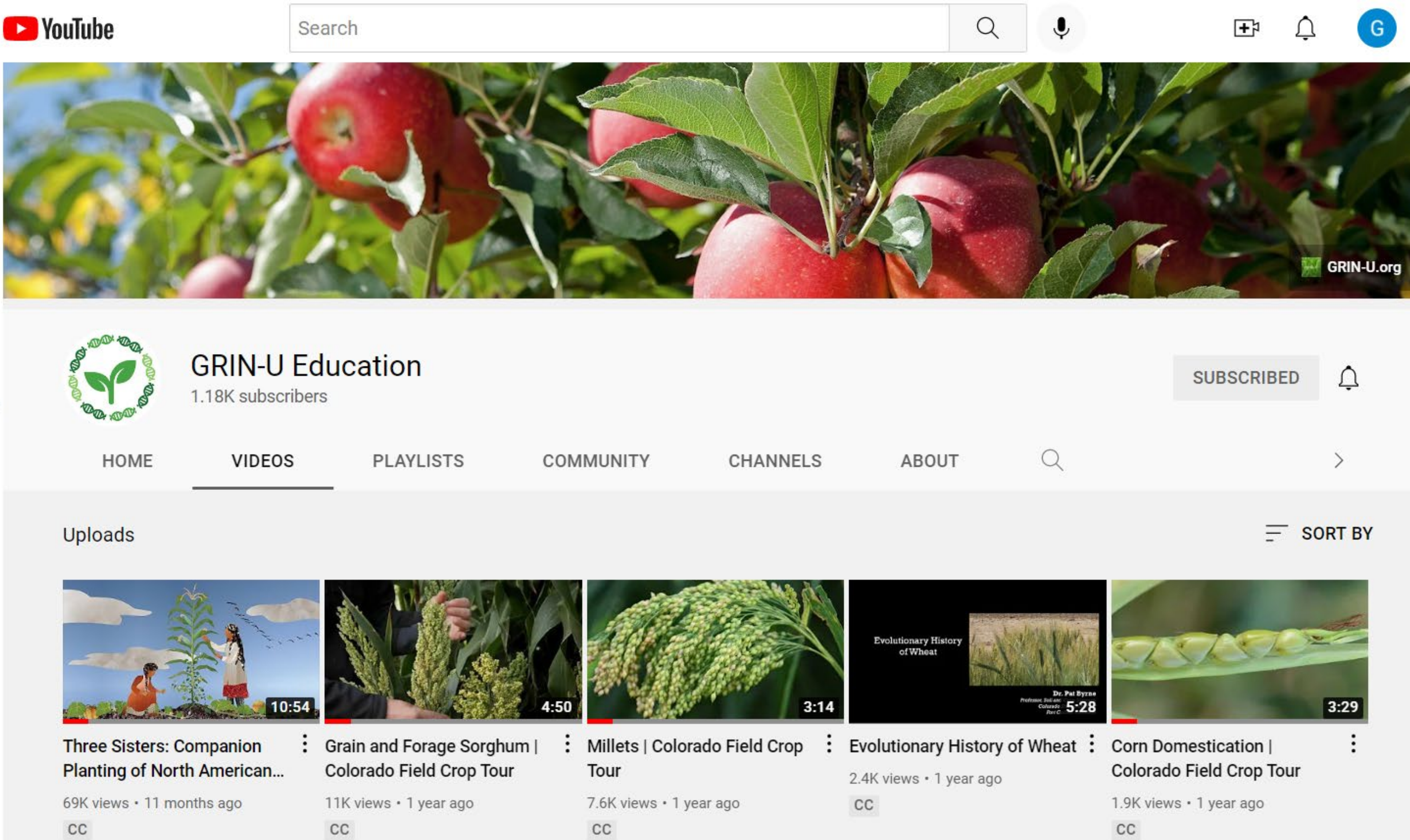
English, French, Spanish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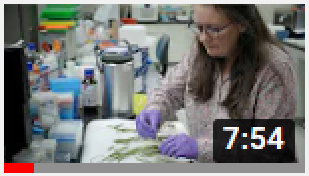
3 (of 6 planned) National Laboratory for Genetic Resources Preservation Virtual Tour Videos

1



Conserving Plant Diversity | NLGRP Virtual Tour

GRIN-U Education

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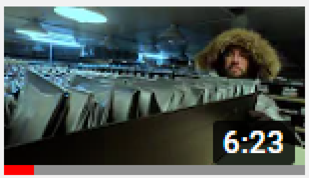

Storing Precious Seed | NLGRP Virtual Tour

GRIN-U Education

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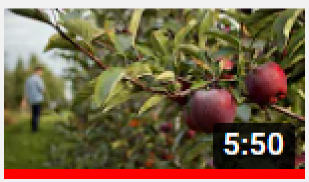


Apple Cryopreservation | NLGRP Virtual Tour

GRIN-U Education

frontiers | Frontiers in Plant Science

DNA profiling with the 20K apple SNP array reveals *Malus domestica* hybridization and admixture in *M. sieversii*, *M. orientalis*, and *M. sylvestris* genebank accessions

Gayle M. Volk[1*], Cameron P. Peace[2], Adam D. Henk[1] and Nicholas P. Howard[3]

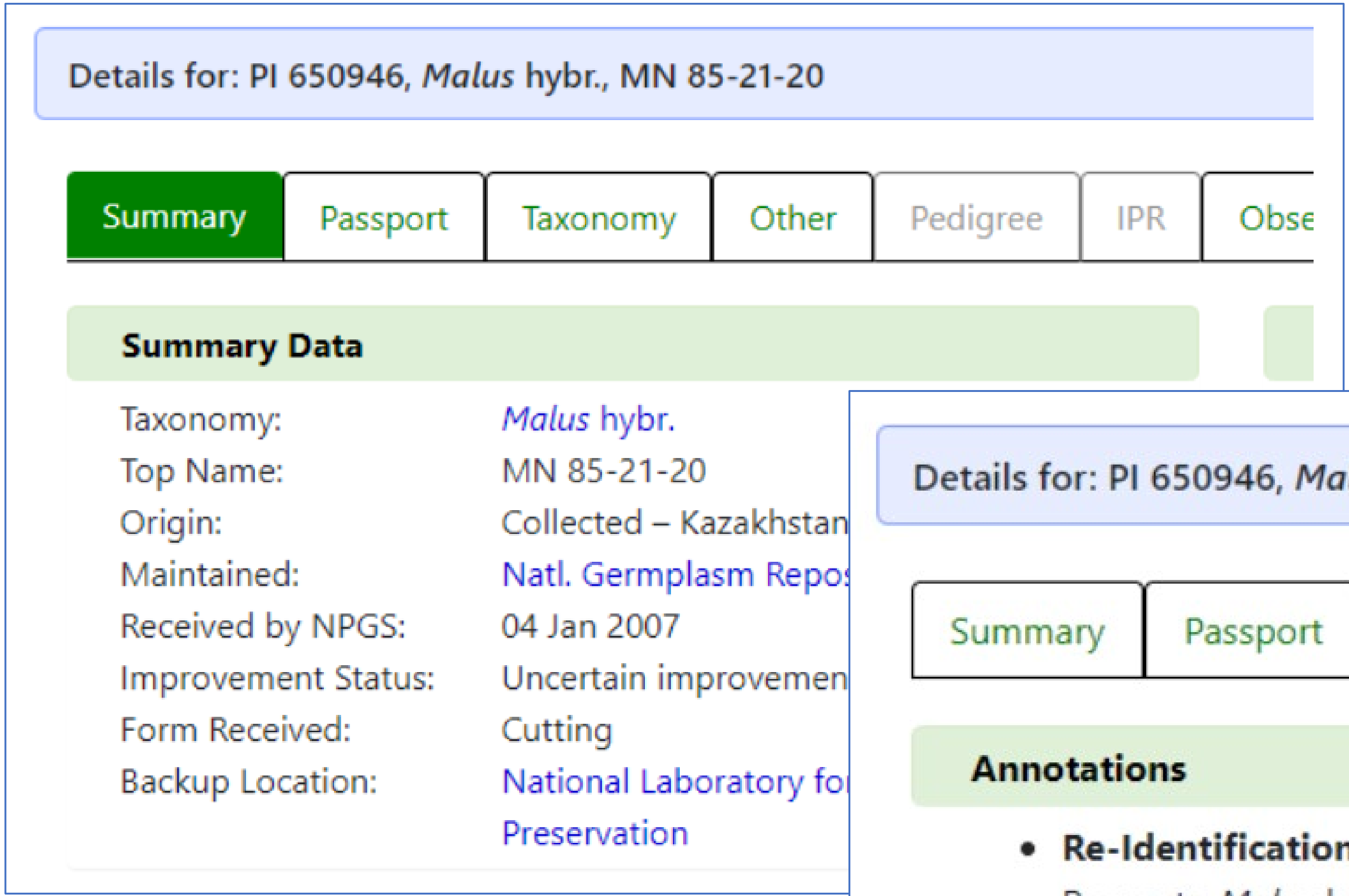
DOI 10.3389/fpls.2022.1015658

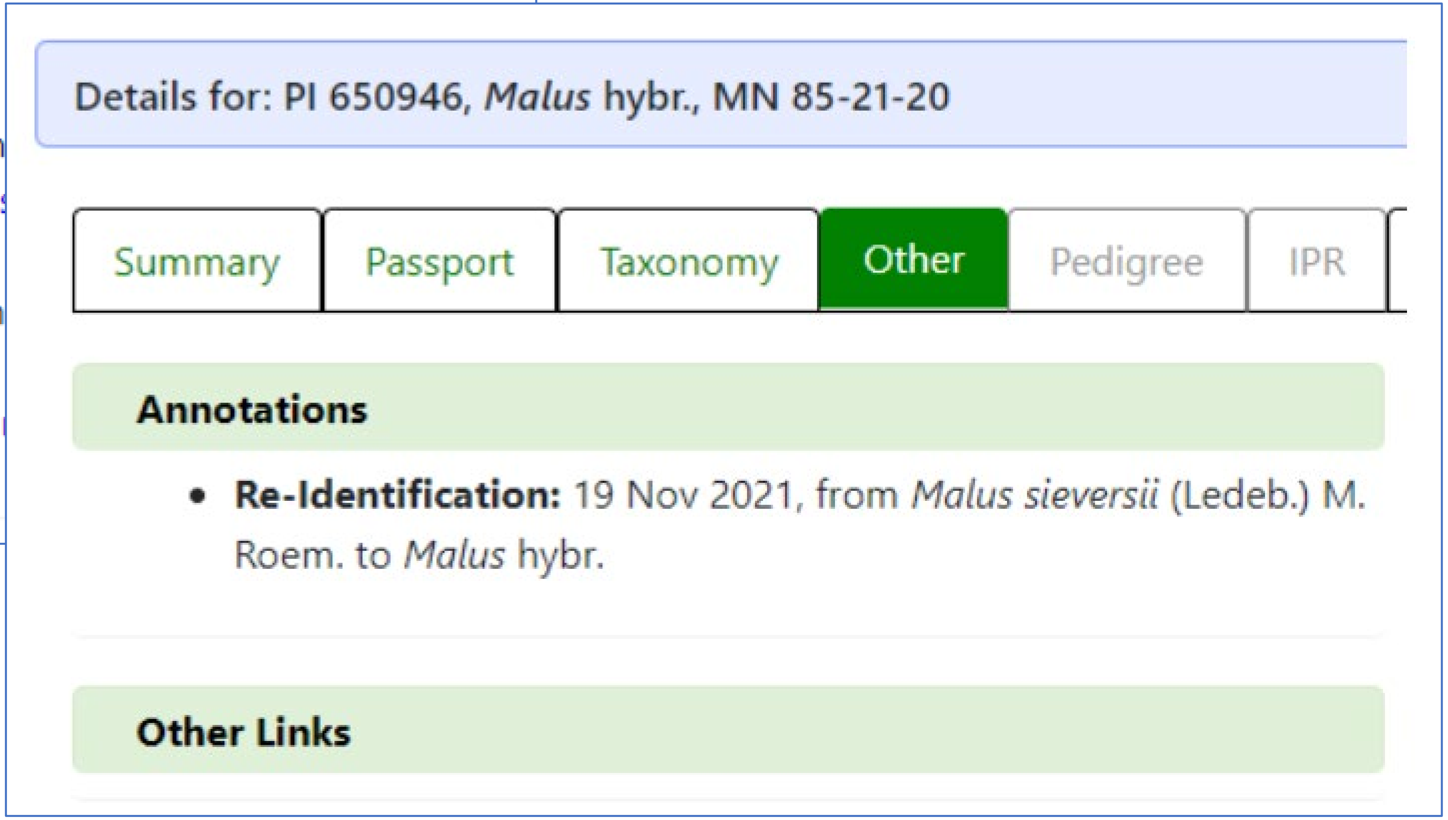
M. sieversii (383 accn)
- 151 pure *M. sieversii*
- 215 hybrid/admixed
- 17 pure *M. domestica*

M. orientalis (36 accn)
- 26 pure *M. orientalis*
- 9 hybrid/admixed
- 1 pure *M. domestica*

M. sylvestris (44 accn)
- 35 pure *M. sylvestris*
- 6 hybrid/mixed
- 3 pure *M. domestica*

Switch from *M. sieversii* to *Malus* hybrid: Change GRIN-Global designation





Verified that a wild is true-to-species:
Verified that a cultivar is true-to-type:

Add feature to GRIN (public):

- Taxonomy Status: Confirmed/Validated (and how?), to the Summary Data on the landing page?

or

- Create a descriptor “Verified”– would have to know where to find it.

Details for: PI 657752, *Malus sieversii* (Ledeb.) M. Roem., DM 5

Summary | Passport | Taxonomy | Other | Pedigree | IPR | Observation

Summary Data

Taxonomy:	*Malus sieversii* (Ledeb.) M. Roem.
Top Name:	DM 5
Origin:	Collected – Kyrgyzstan
Maintained:	Natl. Germplasm Repository - Geneva
Received by NPGS:	05 Feb 2009
Form Received:	Cutting

****

Availability

Form	Quantity	Note	Inventory	Cart
Scion	2 count	NOTE: The deadlines for requesting germplasm from our site are July 15 for summer bud wood and November	PI 657752 .01 PL	!