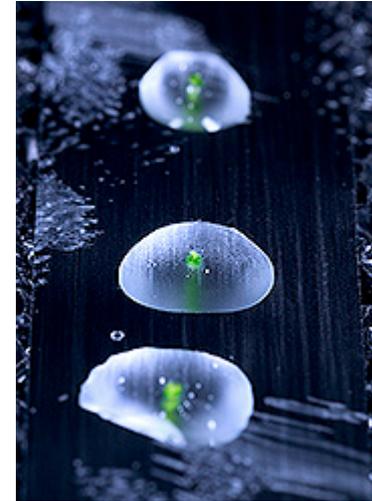


The Critical Role of International Collaborations to Improve Conservation and Utilization of Crop Collections

Clare Coyne, USDA-ARS Pullman, WA
Gayle Volk, USDA-ARS Fort Collins, CO



Vegetatively Propagated Collections

Propagated by grafting or cuttings, rather than from seeds

Genebank maintenance in fields, greenhouses/screenhouses, or in tissue culture

High economic value, nutritious, add variety to diets, and provide job opportunities for local and/or international consumption



Vegetatively Propagated Collections

Offer high levels of novel diversity that is often not available in other genebank collections

Materials are difficult (or impossible) to transfer across international (and some state) borders, often making accessions irreplaceable

May have limited numbers of associated research/breeding programs

Highly susceptible to abiotic and biotic threats

Inadequately secured at secondary locations

Fewer available technical resources (propagation, in vitro methods, etc)



USDA National Plant Germplasm System

597,773 accessions representing 2552 genera

Seed propagated: 557,263 (92.8%)

Clonally propagated: 40,510 (7.2%)

- Fruits & nuts: 69 crops with 26,935 accn
- Vegetables: 5 crops with 2439 accn
- Grasses: 9 crops with 871 accn
- Other (cacao, hops, rubber): 6 crops with 722 accn
- Ornamentals: 74 genera with 9543 accn



GRIN-Global Public Website: <https://npgsweb.ars-grin.gov/gringlobal/search>

USDA- Plant Genetic Resources Unit in Geneva, NY

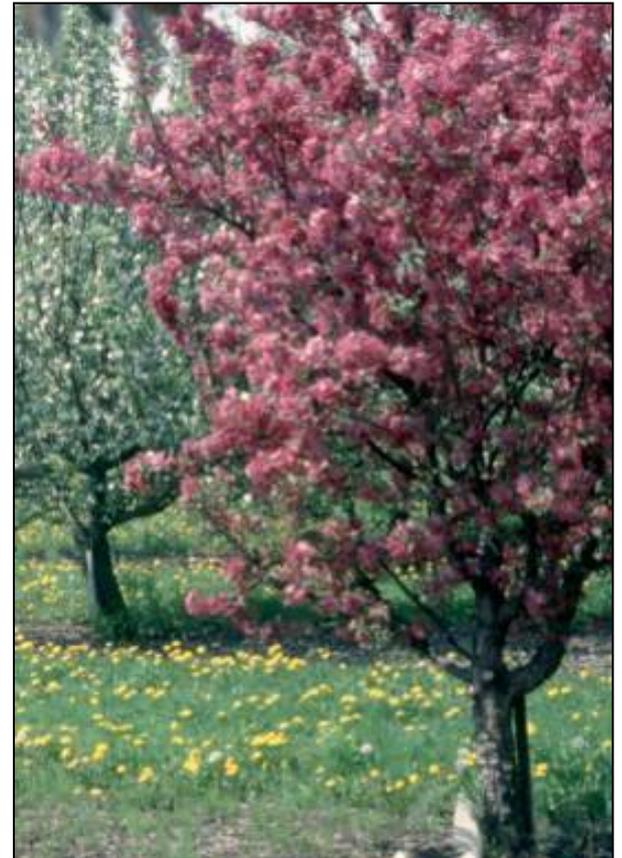
2569 grafted trees (modern, historic, cider, crab, wild species)

3209 wild seedlings and crosses

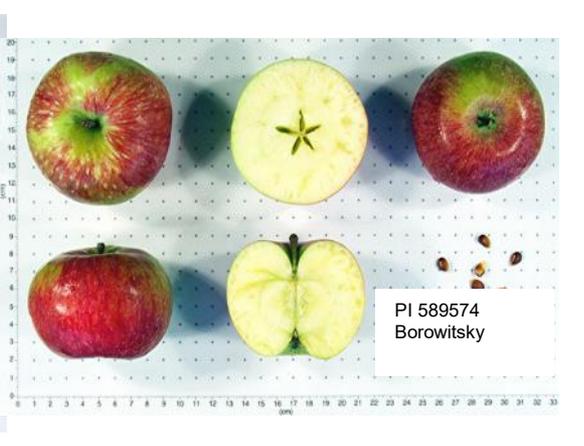
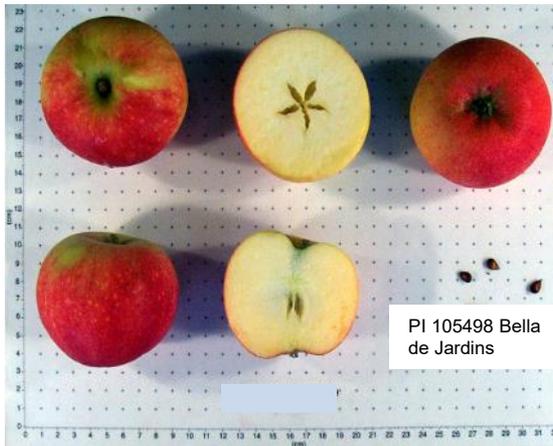
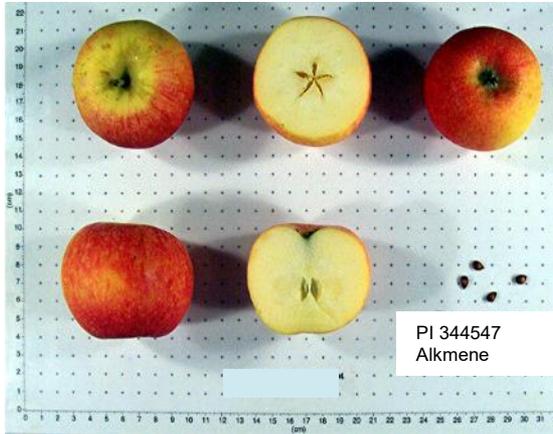
1533 seed accessions

35 *Malus* species, 15 hybrid species

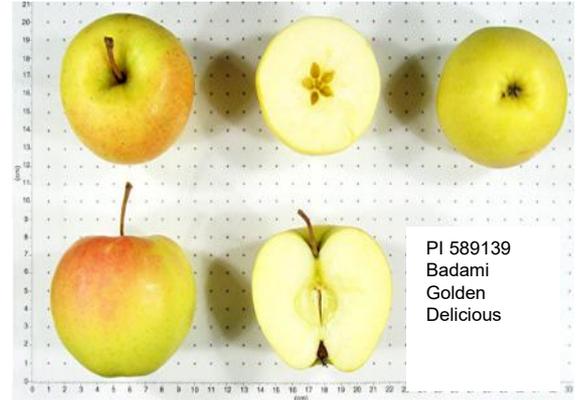
- Genotyped diploid cultivars using 9 SSRs



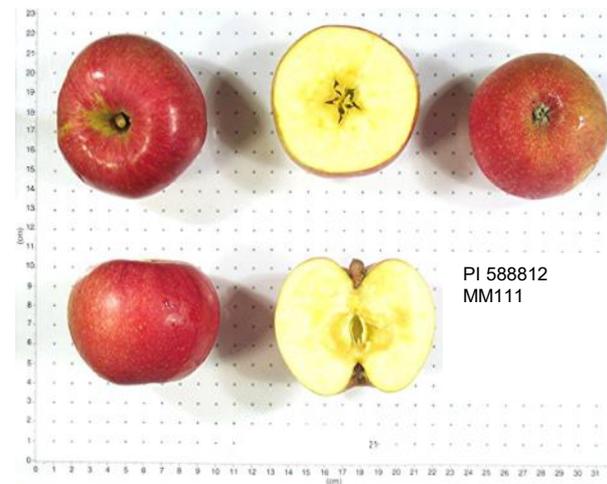
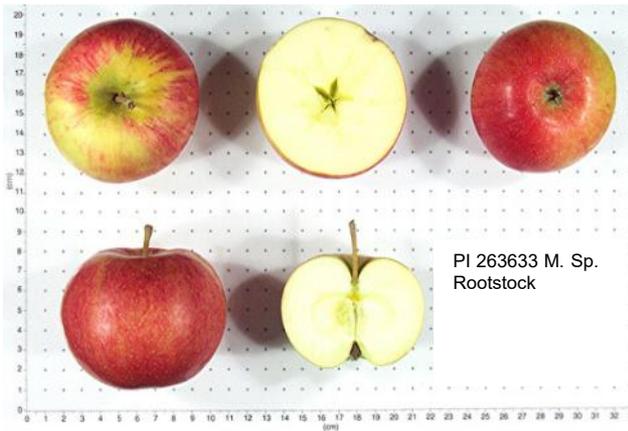
Identified 238 sets of synonyms



Identified 23 sport families, represented by 104 individuals



Identified Rootstock escapes: Scions match rootstocks



HORTSCIENCE 44(3):589–594. 2009.

Identification of Historic Apple Trees in the Southwestern United States and Implications for Conservation

Kanin J. Routson¹

*Arid Lands Resource Sciences, University of Arizona, 1955 East Sixth Street,
P.O. Box 210184, Tucson, AZ 85719*

Ann A. Reilley, Adam D. Henk, and Gayle M. Volk

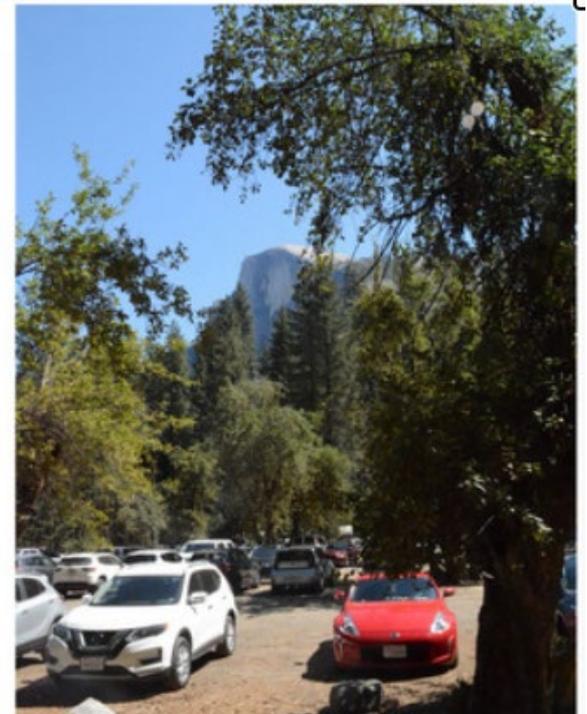
*National Center for Genetic Resources Preservation, U.S. Department of
Agriculture, Fort Collins, CO 80521*



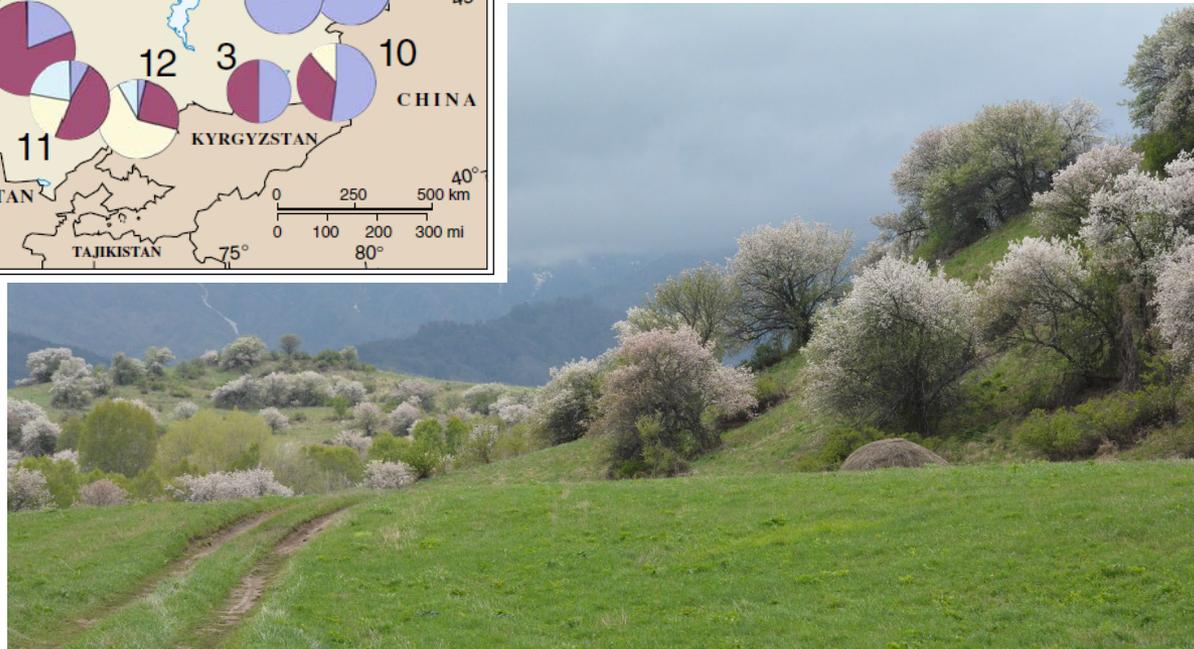
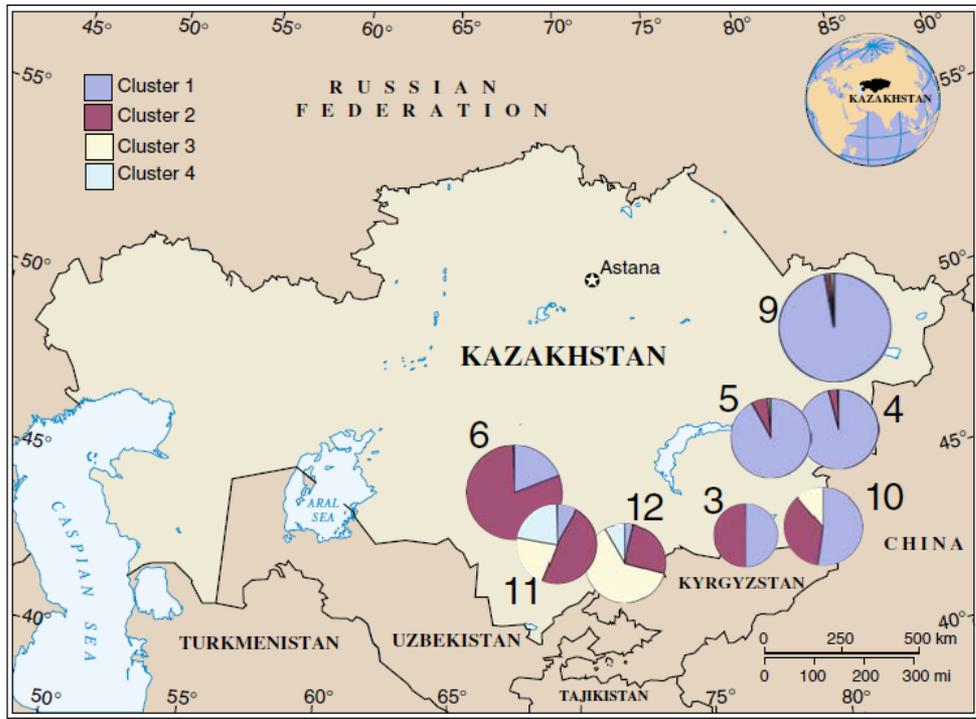
Capitol Reef National Park

Genetic data inform Yosemite National Park's apple orchard management guidelines

Gayle M. Volk¹  | Jonathan Magby² | Adam Henk¹ | Steven Miller² | Rachel Mazur³



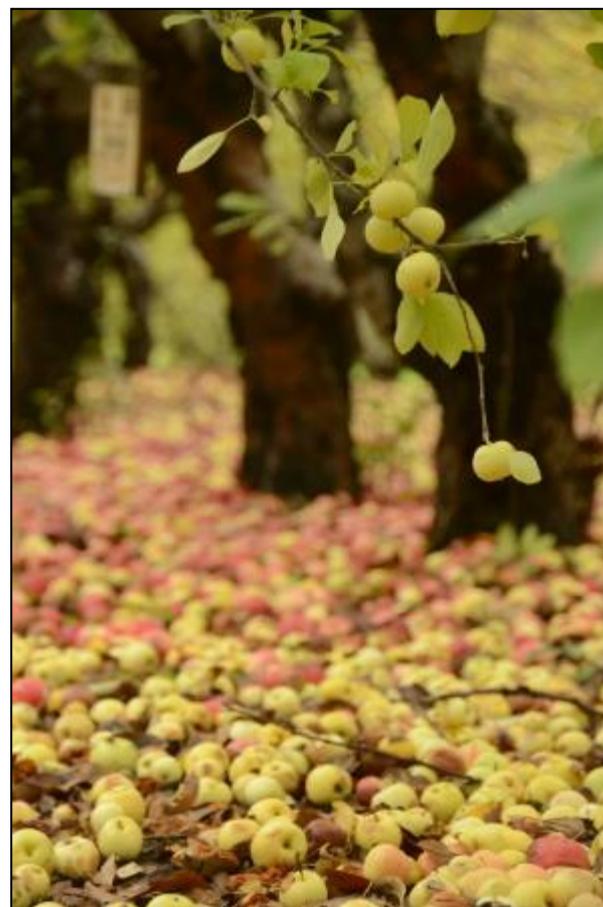
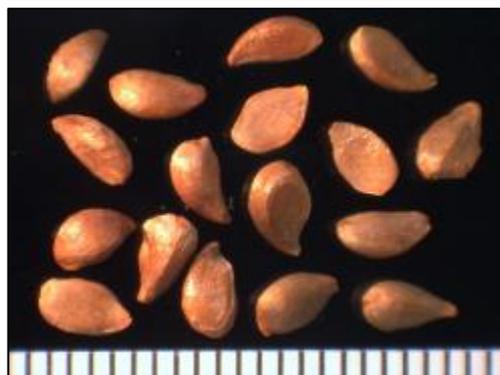
Diversity and conservation of progenitor species *Malus sieversii* in Central Asia



RESEARCH ARTICLE

The vulnerability of US apple (*Malus*) genetic resources

Gayle M. Volk · C. Thomas Chao · Jay Norelli ·
Susan K. Brown · Gennaro Fazio · Cameron Peace ·
Jim McFerson · Gan-Yuan Zhong · Peter Bretting



Apple Plant Explorations

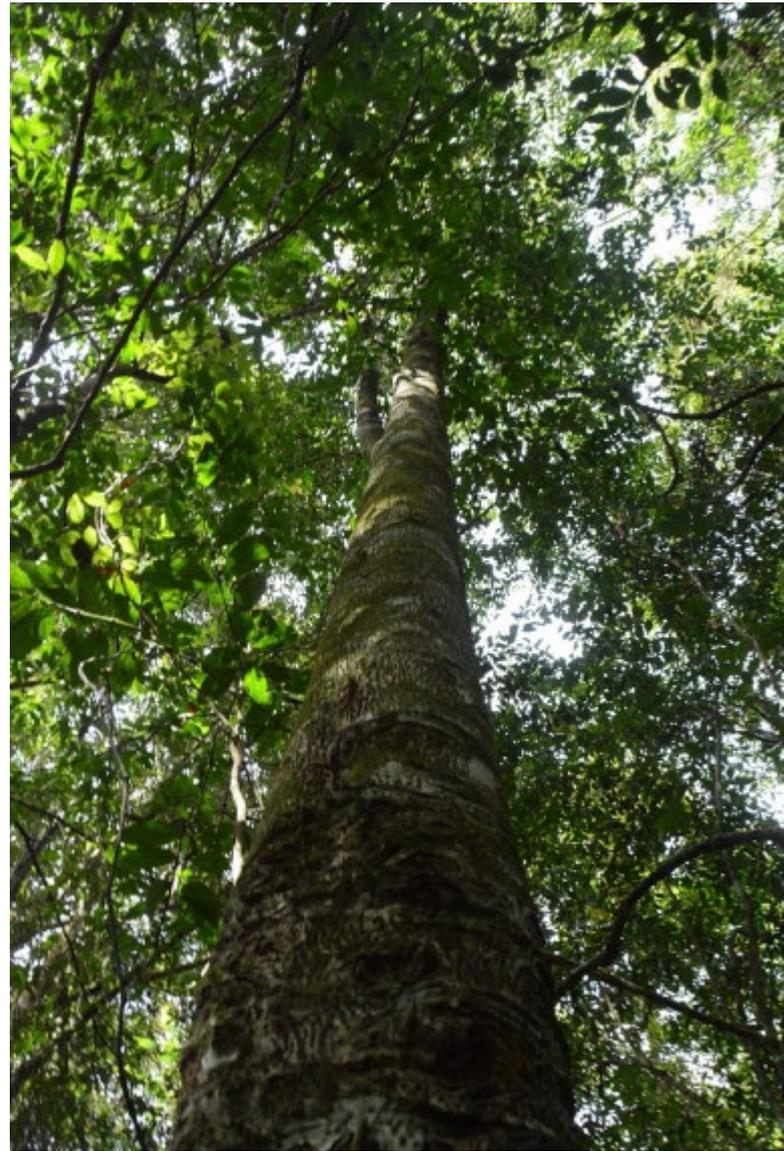
2017, *Malus sylvestris*
Romania and Austria

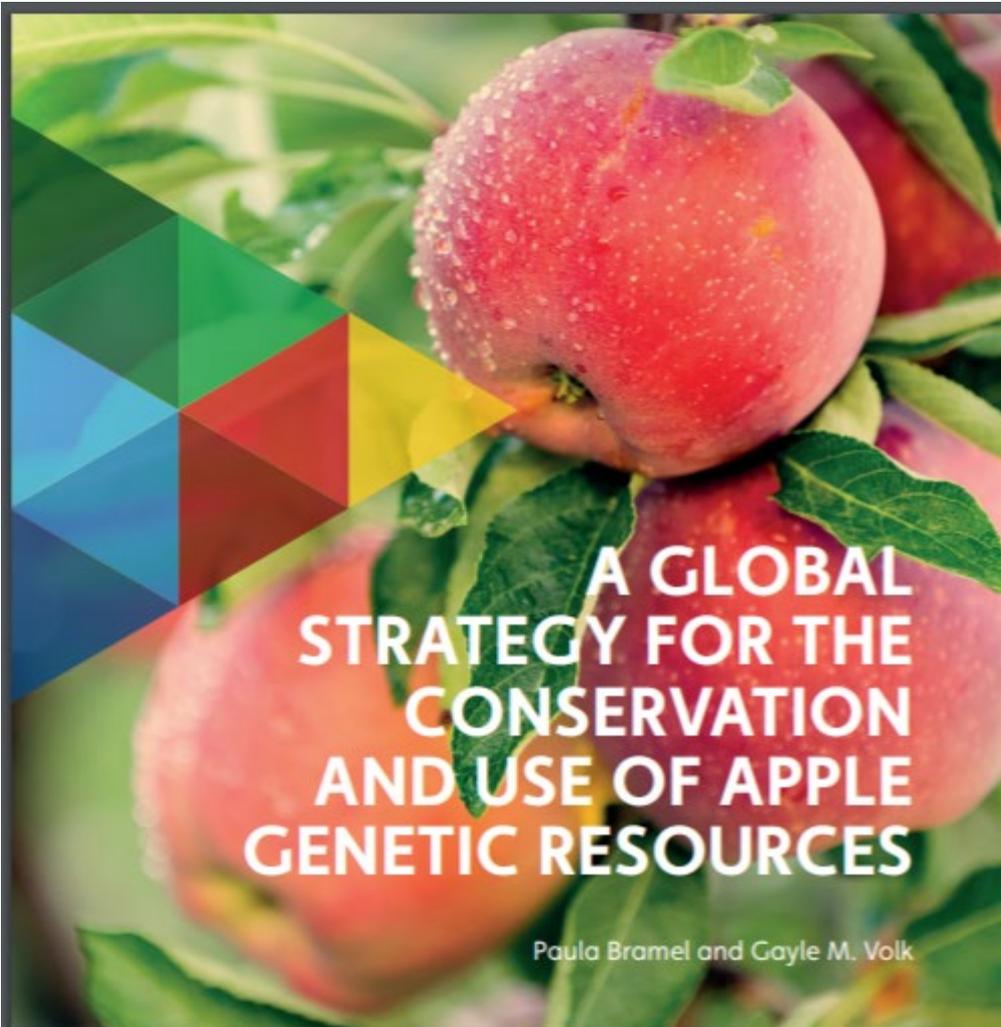


A. Cornille, A. Roman, T. Ursu, T. Kirisits

Apple Plant Explorations

2018 *Malus doumeri*,
Vietnam





A GLOBAL STRATEGY FOR THE CONSERVATION AND USE OF APPLE GENETIC RESOURCES

Paula Bramel and Gayle M. Volk



<https://cdn.croptrust.org/wp/wp-content/uploads/2019/11/Apple-Conservation-Strategy.pdf>

Survey: 35 Apple Collection Inventories

65	Albania	40	Italy-Valor
437	Azerbaijan	1350	Japan
1545	Belarus	38	Kazakhstan
1773	Belgium	698	Latvia
82	Bosnia and Herzegovina	937	Lithuania
388	Brazil	400	New Zealand
287	Canada	429	Poland
1011	China	3821	Russia
65	Czech Republic	119	Slovenia
20	Egypt	520	South Africa
2191	France	191	Spain
1343	Germany	550	Sweden
215	Greece	1300	Switzerland
1210	Hungary	173	The Netherlands
204	India	330	Turkey
220	Ireland	2247	United Kingdom
130	Israel	5291	USDA
256	Italy-Udine		

Total: 29,876 *ex situ* accessions,
mostly *M. x domestica*

Apple Collection Conservation—On a Global Scale

- Worldwide apple production is dominated by 10-20 cultivars
- Crop vulnerability to diseases, pathogens, and environmental threats
- Apple crop wild relatives possess some desirable traits, but access to materials is a challenge
- Quarantine restrictions
- Clonal collections are expensive



Global Conservation Plan for Apple

- Documented international network of collections with agreed-upon standard procedures
- Conserve cultivars as clones in multiple collections/field sites
- Information database to document passport, availability, fingerprint, phenotype, image information
- Possible seed conservation to conserve wild species diversity
- A combination of international and local cultivars with unique traits/characteristics
- Replicate local cultivars in multiple collections for safety duplication



Opportunities with the 20K Apple SNP array

Collection comparisons (>25 international collections participating)

Pedigree information

Linked to markers for breeding

Synonyms

Trueness-to-species (particularly for close crop wild relatives)

Cultivar identities

- Genotyped 1400+ USDA-NPGS apple accessions using the SNP array

Securing Clonal Collections is Expensive, but Critical

- Duplicated plantings/locations
- Seeds for CWR species representatives
- Reduced temperature storage in tissue culture
- Cryopreservation (long-term storage in liquid nitrogen) for cultivars



National Laboratory for Genetic Resources Preservation Fort Collins, CO

Secured at NLGRP:

82% of ~600,000 NPGS seed accessions

15% of the 30,000 NPGS clonal fruit,
nut, and vegetable accessions



LN & LNV (-150 to -196°C) for clonal
shoot tips & dormant buds, as well as
embryonic axes, some seeds, and pollen



-18°C Freezers, seed storage

NLGRP Clonal Cryopreservation Research and Implementation



Dormant buds
from cold-
hardy
perennials:
Apple, sour
cherry, willow



Shoot tips excised
from field or
greenhouse-grown
plants: Citrus, garlic



Shoot tips excised
from tissue
cultured plants:
Grape,
strawberry,
potato, etc.

Cryopreserved clonal crops in NPGS

Shoot tips

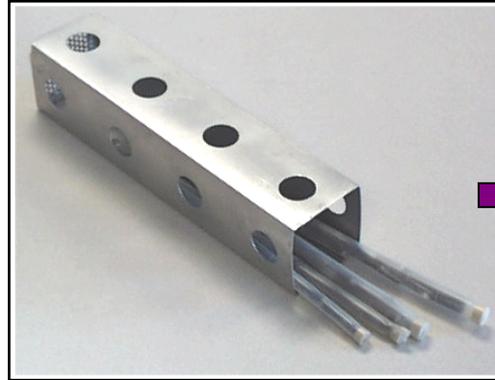
Garlic	102
Strawberry	98
Hops	88
Sweet potato	151
Mint	42
Banana	22
Pear	221
Currants	96
Raspberry	202
Potato	430
Blueberry	41
Sugarcane	28
Citrus	400

Dormant buds

Apple	2052
Sour cherry	32
Willow	19

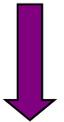
4024 accessions cryopreserved

Dormant bud cryopreservation

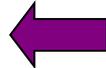


Harvest budwood, desiccate to 25-30% moisture,
Package in polyolefin tubes

Slow cool to -30
LN₂ plunge and store



Assess viability



Thaw, Rehydrate



LN storage

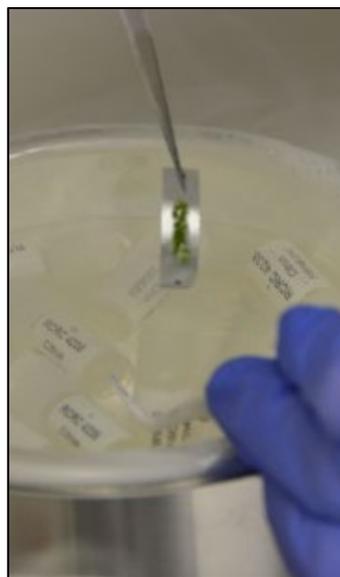
Apple Dormant Bud Cryopreservation

2052 NPGS apple accessions representing 49 taxa
Cryopreserved in Fort Collins 1988-2014



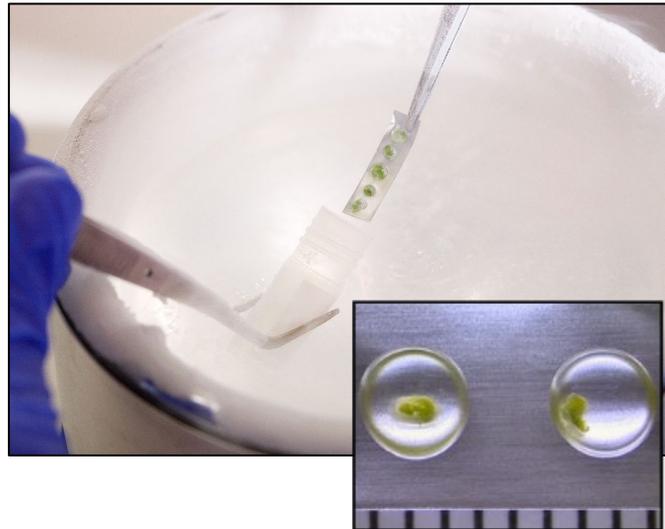
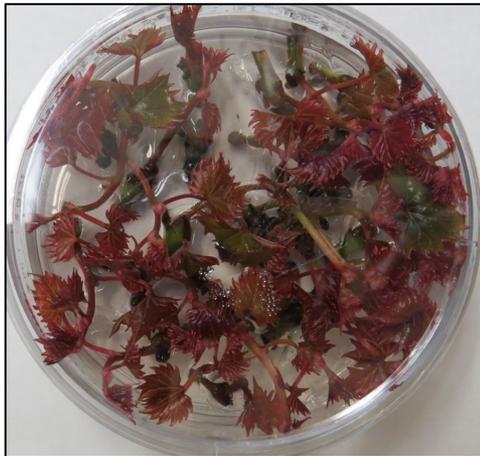
Citrus shoot tip cryopreservation

- Technology developed by NLGRP & Riverside in 2012
- Shoot tips (1 mm) excised from screenhouse-grown trees
- Surface-sterilized, treated with cryoprotectants, cryopreserved
- Recovered by micrografting
- 400 Citrus accessions cryopreserved at NLGRP



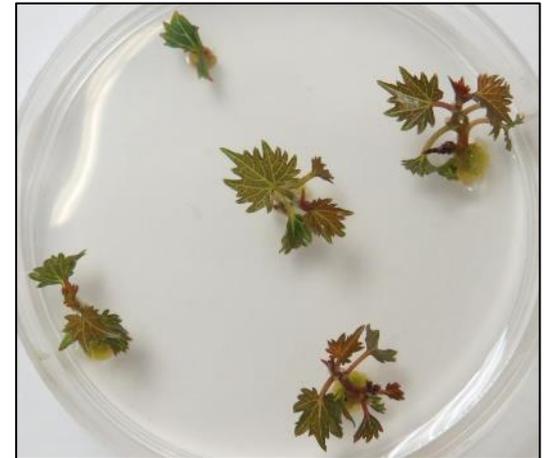
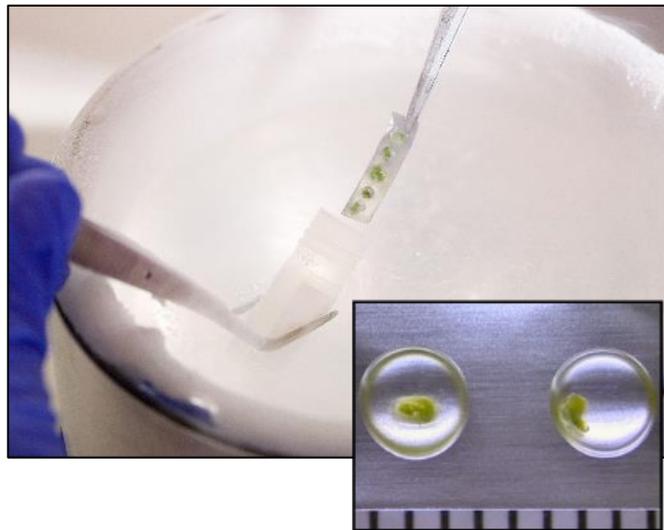
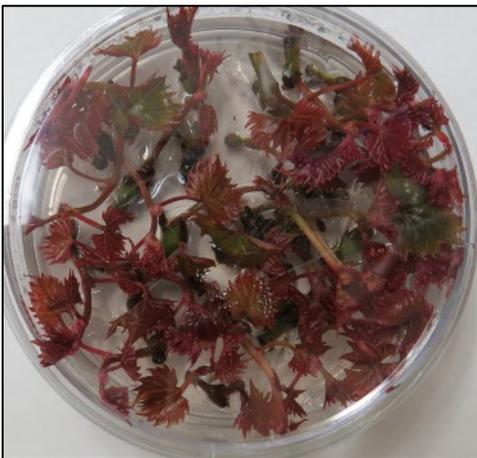
Vitis shoot tip cryopreservation

- Cultivars introduced into tissue culture
- Shoot tips (1 mm) excised from in vitro nodal sections
- Treated with cryoprotectants, cryopreserved
- Recovered by plating onto medium
- 20 Vitis accessions cryopreserved at NLGRP



Challenges for Cryopreserving Clonal Collections

- Facilities
- Specialized skills/training
- Labor intensive
 - 60-75 accessions/year/tech for shoot tips
 - 200+ accessions/year/tech for dormant buds
- Sufficient plant material (quantity, quality/endophytes)
- Tissue culture & cryopreservation methods available & tested



Training Program

Plant Genetic Resources Management and Use

- Funded by a NIFA-Higher Education Challenge Grant with Colorado State, Iowa State and USDA
- Training materials will be freely available through new site associated with GRIN-Global: ebooks, videos, images, virtual tours, PDFs
- Online courses will be offered through Colorado State Univ

PLANT GENETIC RESOURCES

GENEBANKS AND CONSERVATION

Plant genetic resources—the wide range of crop varieties and their wild relatives—are critical to safeguard food security now and in the future.

Plant genebanks have diverse collections that are agriculturally and economically important. These collections conserve PGR that could be lost from their natural habitats or local communities. Collections may be conserved as seeds in cold storage, or as plants in the field, greenhouse, or in tissue culture.

High quality genebank collections are critical for the future of global agriculture. Research develops new technologies and helps identify new methods for efficient, cost-effective conservation.

Key disciplines include:

- crop science
- horticulture
- plant pathology and physiology
- taxonomy
- plant genetics and breeding

Acquisition
Collections represent a wide range of genetic diversity. New plant materials come from plant explorations and exchanges within a country and internationally. Foreign imports are inspected or tested to make sure they are free of pests and pathogens.

Maintenance
Plant genebanks are responsible for keeping collections alive and healthy. Seeds in cold storage must be periodically germinated to make sure they are still alive. Sometimes collections are maintained as field or greenhouse plants.

Regeneration
Plants may be grown in the field or greenhouse using techniques that do not alter each sample's genetic composition.

Secure Backup
Duplicate collections are maintained at a secure secondary location. This ensures that collections will not be lost as a result of disease, pathogens, or environmental disasters. These back-up collections are often safeguarded as seeds in cold storage. Durable tree banks, shoot tips, pollen, and seeds may be preserved in liquid nitrogen.

Evaluation & Characterization
Trait data are recorded for all plant collections. In addition, genetic methods assess collection diversity and determine if varieties are true-to-type. These data can also be used to identify collection gaps. Collection documentation is critical for genebank user communities to identify new useful traits and materials of interest.

Documentation
Data for the source, traits, genetics and maintenance history of genebank collection materials are kept in databases. One example is GRIN-Global, which provides up-to-date information for the genebank collection of the U.S. National Plant Germplasm System.

Distribution
Samples from plant genebanks are provided to scientists who need access to novel genetic variation and traits for research and breeding.

For more information, contact: Patrick Byrne@colostate.edu or Gayle Volk@usda.gov
Byrne, Volk, et al. 2018. Sustaining the future of plant breeding: the critical role of the USDA-ARS National Plant Germplasm System. Crop Science 58: 461-468.
Design credit: Kucera Design Studio

PLANT GENETIC RESOURCES

THE KEY TO GLOBAL FOOD SECURITY

Plant breeders utilize the genetic diversity of **plant genetic resources (PGR)**—the wide range of crop species and their wild relatives—to develop new crop varieties.

Plant breeders use PGR by evaluating plants for traits of interest, selecting the best, and crossing them to adapted varieties.

PGR include current and traditional varieties and related wild plants.

Crop wild relatives are the ancestors of crop and related species. They live in their native habitat.

Landraces are traditional varieties selected by farmers for adaptation to local conditions.

Crop varieties have been developed by plant breeders and farmers.

PGR are crucial for adapting crops to changing climates, combating new strains of diseases and insects, and developing healthier foods.

Genebanks acquire, maintain, document, and distribute PGR.

Evolutionary threats from insects and diseases

Declining land and water availability

Increased demand from a growing human population

Changing temperatures and rainfall patterns

After thorough PGR evaluation and subsequent breeding with current crop varieties, a new improved variety with novel traits is developed.

Plant breeders use PGR to develop improved varieties that are:

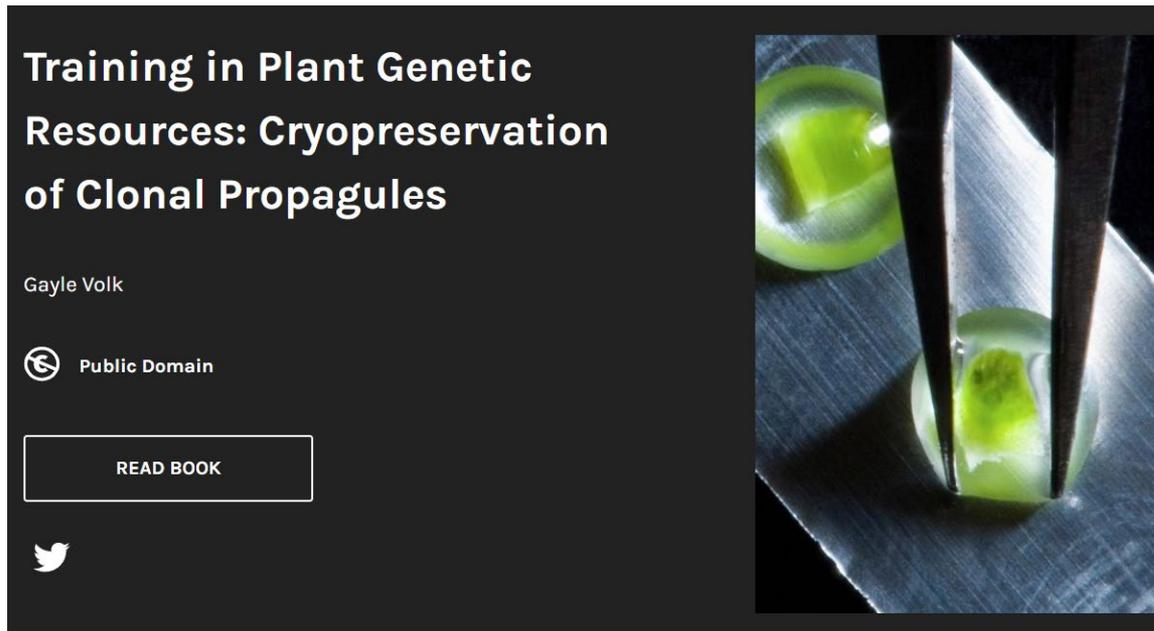
- Insect Resistant**: Wheat varieties resistant to the Russian wheat aphid incorporate resistance genes from a variety developed in Turkmenistan.
- Higher Yielding**: Sunflowers with higher seed yield have been developed from several U.S. wild sunflower species. Traits that enabled production of higher yielding cultivars were obtained from wild sunflowers.
- Disease Resistant**: Resistance to a devastating fungal disease like wheat stem rust was found in a wild tomato relative collected in Peru. This trait has been used in several commercial varieties.
- More Nutritious**: Crop wild relative Minko is used in breeding soft-fleshed apples. These apples offer improved nutrition and provides a pH boost to hard ciders.

For more information, contact: Patrick Byrne@colostate.edu or GayleVolk@usda.gov
Byrne, Volk, et al. 2018. Sustaining the future of plant breeding: the critical role of the USDA-ARS National Plant Germplasm System. Crop Science 58: 451-468.
Design credit: Kucera Design Studio

Temporary Website: <http://genebanktraining.colostate.edu/trainingmaterials.html>

P. Byrne, C. Gardner, M. Munoz-Amatriain, J. Zarestky, W. Suza, G. Kinard, D. Namuth-Covert, G. Morris, K. Jewell

Additional Resources for Clonal Cryopreservation



Bettoni JC, Bonnart R, Volk GM. 2020. Challenges in implementing plant shoot tip cryopreservation technologies. *Plant Cell, Tissue and Organ Culture*.
<https://doi.org/10.1007/s11240-020-01846-x>

Tanner JD, Chen KY, Bonnart RM, Minas IS, Volk GM. 2020. Considerations for large-scale implementation of dormant budwood cryopreservation. *Plant Cell, Tissue and Organ Culture*.
<https://doi.org/10.1007/s11240-020-01884-5>

Temporary Website: <http://genebanktraining.colostate.edu/trainingmaterials.html>

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

 Public Domain

READ BOOK



Crop Wild Relatives and their Use in Plant Breeding

Gayle Volk and Patrick Byrne

 Public Domain

READ BOOK



Temporary Website: <http://genebanktraining.colostate.edu/trainingmaterials.html>

Coming Down the Learning Resources Pipeline...

More ebooks:

Applications of Plant Pathology in Genebank Collections

Fundamentals of Plant Genebanking

Phenotyping

Genomics for Genebanks

More virtual tours:

Crop diversity: A Virtual Crop Science Field Tour

National Laboratory for Genetic Resources Preservation

More infographics:

The Role of Botanic Gardens in Plant Conservation

An official website:



Temporary Website: <http://genebanktraining.colostate.edu/trainingmaterials.html>

Conclusions

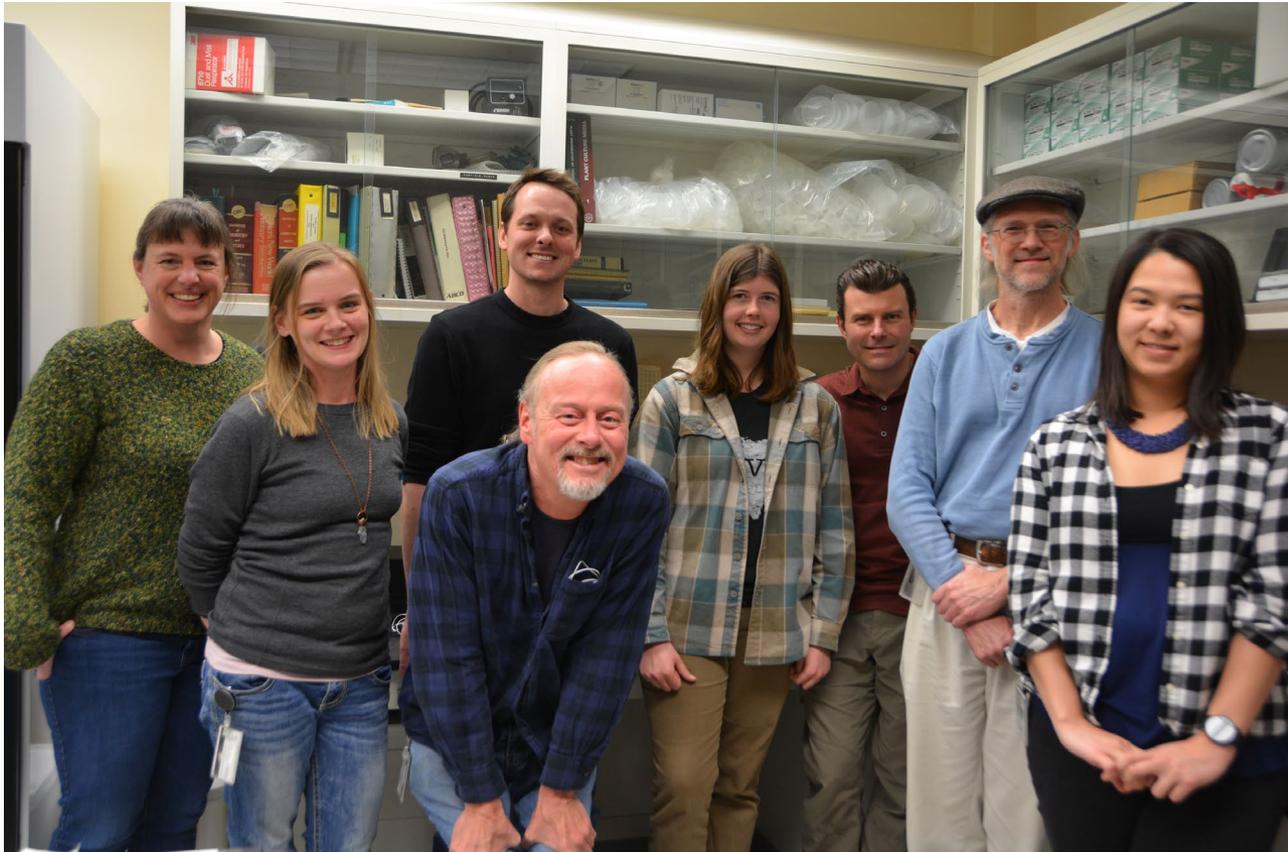
NPGS has many vegetatively propagated collections throughout the genebank system

Expensive to maintain and very vulnerable

International collaborations are needed to build and characterize the collections

Creative, novel back-up strategies are needed to preserve these genetic resources now and into the future

Freely available training content will educate the current and future genebanking community



Ashley Shepherd
Jean Carlos Bettoni
Bradford Hall
Emma Balunek
Remi Bonnart
Adam Henk
Katheryn Chen
Gayle Volk

March 2020