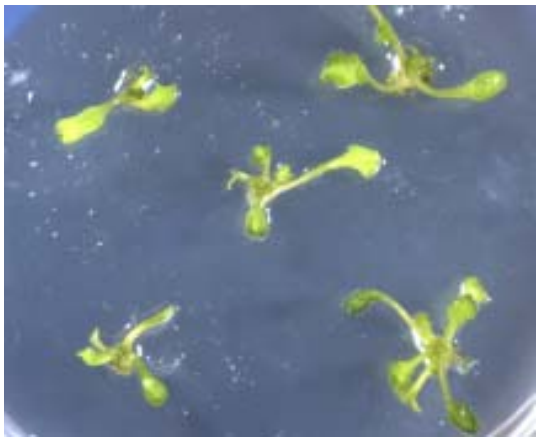
PLANT GERMPLASM PRESERVATION RESEARCH UNIT

2006 ANNUAL REPORT

CRYOPRESERVATION OF *Arabidopsis*

Problem: Cryopreservation method development is usually time-consuming and highly specific to plant species and accession. *Arabidopsis thaliana* is a model plant species for genetics and biochemical analysis since it has a sequenced genome, well characterized mutants, and available RNA expression tools. We developed a cryopreservation protocol for *Arabidopsis* so we can take advantage of the available molecular tools to better understand the process of cryopreservation.



Arabidopsis shoot tips were cryopreserved using PVS3 after 14 days of cold acclimation. Recovering shoot tips were plated onto medium and allowed to regenerate plants.

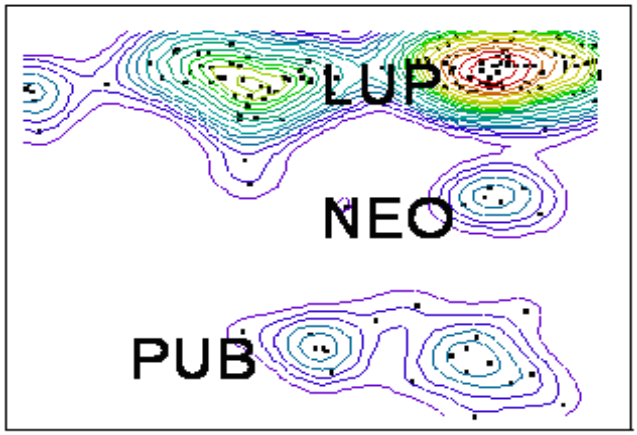
Findings: We demonstrated that *Arabidopsis* could be successfully cryopreserved using either plant vitrification solution 2 (PVS2; 30% glycerol, 15% ethylene glycol, 15% dimethyl sulfoxide, 15% sucrose) or plant vitrification solution 3 (PVS3; 50% glycerol, 50% sucrose) as cryoprotectants prior to rapidly cooling shoot tips in liquid nitrogen (LN). In addition, shoot tips could be cryopreserved using a two-step cooling procedure with PGD (polyethylene glycol-glucose-dimethyl sulfoxide) as a cryoprotectant. Shoot tip regrowth after PVS2 cryoprotectant treatment was improved after cold acclimation treatments of 8 or 18 days. The high levels of recovery after LN exposure makes *Arabidopsis* an ideal model system for further studies on molecular changes that occur during cryopreservation.

Interpretation and Recommendations: The successful cryopreservation results for *Arabidopsis* have provided an excellent opportunity to use microarray gene expression analyses to determine the molecular changes that occur during the pretreatment and recovery steps of cryopreservation procedures. Our collaboration with Dr. Chhandak Basu (University of Northern Colorado, Greeley, CO) seeks to use *Arabidopsis* to identify these molecular changes.

- G. Volk
- L. Towill (retired NCGRP)

METHODS TO DETECT HYBRID HISTORY AMONG ACCESSIONS

Problem: The accurate identification of distinct historical lineages is important for delimiting species, understanding genetic relationships among populations, and prioritizing groups of organisms for in situ and ex situ conservation programs. This study used computer simulations to evaluate the relative performance of some of the most common methods for identifying lineages, collectively termed "cladistic phylogeny reconstruction methods." We then applied this approach to an AFLP data set of North American hops.

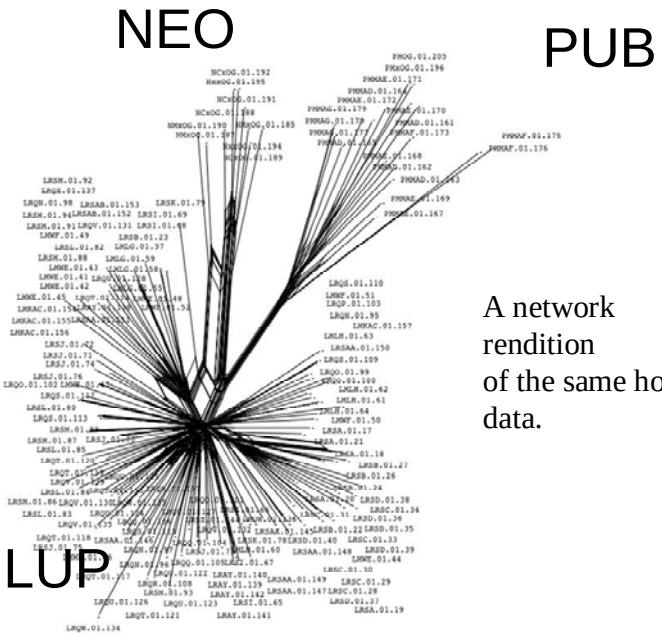


Principle coordinate analysis using modal clustering as a way of identifying hybrid history in hops subspecies *lupuloides, neomexicana* and *pubescens.*

In particular, it is critical to understand the limitations of phylogenetic methods which typically identify sharing a single common ancestor vs. population genetic methods that can identify phenetic groups that may have interrelated individuals that are not hierarchically defined.

Findings: The study shows that these methods are biased, promoting the incorrect conclusion that a group of organisms is a distinct historical lineage, when in fact it is an amalgam of two or more lineages (a "hybrid taxon" of reticulate ancestry). This study concludes that it may be very difficult to distinguish hybrid taxa from distinct historical lineages using common methods for phylogeny reconstruction.

Interpretations & Recommendations: A new application of a nonparametric clustering procedure and network method was shown to perform better than cladistic methods for distinguishing real historical lineages from hybrid taxa. The latter statistical procedure may be particularly useful for discovering NPGS seed accessions likely to contain novel alleles relative to other accessions from the same species. The procedure might also be used to identify accessions that have been contaminated with genes from other accessions.



A network rendition of the same hops data.

- C. Richards
- P. Reeves

GENETIC DIVERSITY OF *Malus Sieversii* IN KAZAKHSTAN

Problem: Since 1988, seeds have been collected from more than 800 wild *Malus sieversii* trees in Kazakhstan. Trees representing many of these accessions have been planted in the orchards at the USDA-ARS Plant Genetic Resources Unit (PGRU) in Geneva, NY. A collaborative research project between PGRU and PGPRU seeks to use molecular data to estimate the genetic structure in this collection.

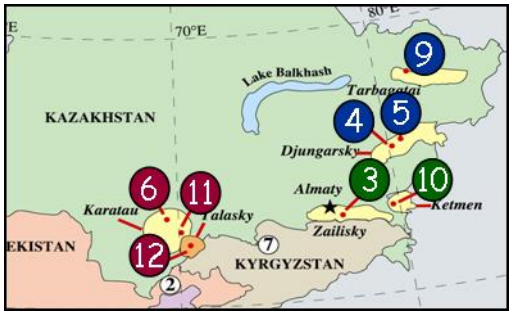


Fig.1. *Malus sieversii* collection sites in Kazakhstan are labeled with identifier numbers.

Findings: Eight collection sites (Fig. 1) were represented by 949 trees in the PGRU orchards. These trees were genotyped using 7 microsatellite genetic markers. A population graph (Fig. 2) demonstrated that there is genetic differentiation according to collection site. The large node for site 3 demonstrates the high level of variation found in the wild populations near the population center of Almaty. Additional data revealed high levels of variation among seeds collected from the same tree and an alternative structure based on clustering of individuals that also has some geographic correlations.

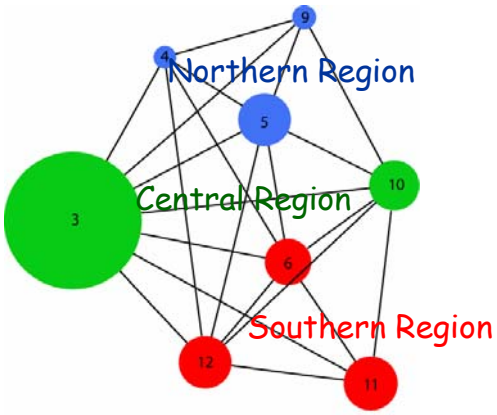


Fig.2. Population graph displaying the within site (nodes) and among site (edges) genetic variability among *M. sieversii* collection sites.

Interpretation and Recommendations: *Malus sieversii* seed populations collected from 8 sites in Kazakhstan could be genetically differentiated. Further analyses will identify core subsets that can be used to capture most of the genetic diversity in this important wild population of domesticated apple progenitors.

- C. Richards
- G. Volk

UNCONVENTIONAL STORAGE BEHAVIOR

Problem: Freezer storage at -18°C extends lifespans of seeds from many species 2-3 fold compared to traditional refrigerated storage at 5°C. However, many institutions that genebank species from wild-collected populations from warm climates are reporting concerns that freezer storage reduces longevity of some germplasm. ARS is increasingly focused on making collections of germplasm from these areas. It is therefore imperative that temperature anomalies of storage longevity around -18°C be addressed, the basis for susceptibility to these anomalies be identified, and alternative storage protocols be developed.

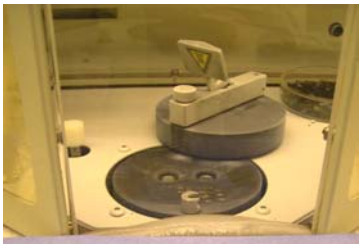
Spores of Esquisetum spp. exhibit unusual physiology that can be used to model seed aging.

Seeds of *Ambronia ammophila* were collected from Yellowstone National Park and are part of a study to understand physiology of wild-collected native species.

Findings: Empirical and biophysical approaches detect anomalous aging kinetics at freezer and cryogenic temperatures and confirm the mechanism for faster than predicted deterioration. Factorial experiments that compare aging time courses in seeds, pollen and spores stored at a range of temperatures and water contents enable us to model predictable behavior and to identify germplasm with behavior outside normal expectations. Collaborations with botanical gardens and Federal land managers provide access to germplasm to test susceptibility of diverse species and germplasm types and to find commonalities among diverse germplasm that would lead to tools that predict susceptibility. Associated experiments to measure biochemical activity in stored germplasm and state-phase behavior of lipid and aqueous domains allow us to hypothesize the mechanism for the faster than predicted aging.

Interpretation and Recommendations: About 10-15% of the incoming germplasm from wild-collected populations appear to be orthodox, but, when tested, do not exhibit the expected benefit from freezer storage. The percentage of nonconforming species is even higher when species native to warm climates are considered. It takes years to empirically detect anomalous temperature behavior and so tools that predict temperature sensitivity will ensure that these valuable genetic resources are properly preserved.

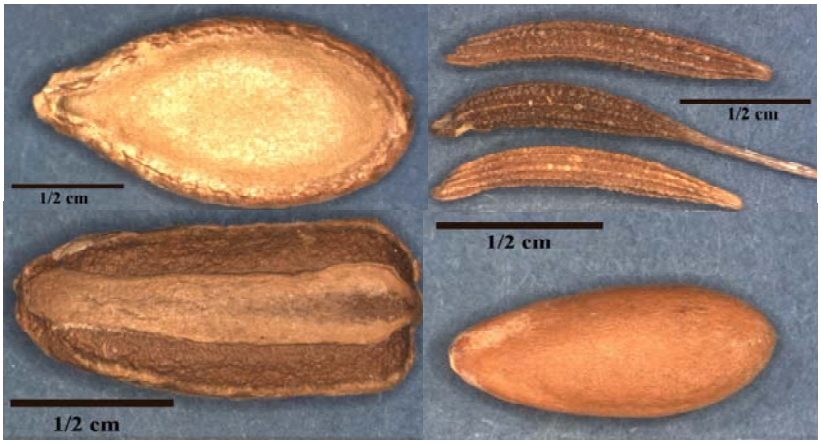
- C. Walters



Differential scanning calorimetry is the main research tool used to investigate biophysical parameters that influence stability of stored germplasm.

SEED BANKS AS DNA BANKS?

Problem: Many biorepositories now include samples that are intended for DNA extraction and analyses. Herbarium samples are commonly used for DNA studies. If NCGRP is to provide these services for plants, what is the most appropriate storage platform and what is the shelf life expected for DNA under various storage strategies?



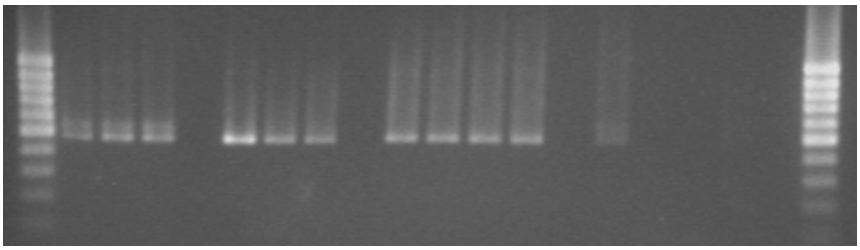
Seeds that are ~135 years old that were found in an historic home in Georgia. These seeds were used as source material to test the hypothesis that DNA could be amplified from deteriorated seeds.

Findings: DNA quality is defined by the length of DNA fragments within cells, the length of fragment that can be amplified, and the fidelity of amplified sequences to the target organism. We have shown that DNA remains stable in seeds long after seeds have lost viability and that identification to species is possible using primer sequences specific for plants. Precautions against amplifying contaminating DNA are required in highly degraded samples. The greatest risk to DNA quality is action of nucleases, and so storage conditions that inactivate nuclease activity provide stable DNA for centuries. DNA appears to be more stable within seeds than in leaf tissues.

Interpretation and Recommendations: Seed banks can reliably serve as DNA banks for many research projects.

DNA extracted from squash seeds harvested in:

2002 1937 1935 1860's



Agarose gel image of PCR amplification products using primers targeted to rbcL.

- C. Walters
- C. Richards

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