



POTATO GENE RESOURCES

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Utilizing the Canadian plant gene resources potato collection for discovery science

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While crops such as maize and rice have seen early adoption of many genomics tools, potato has somewhat lagged behind. This is in part due to the widely grown cultivated potato having a complex polyploid genome (eg. tetraploid) that is highly genetically variable. Nevertheless, potato breeders and researchers alike are keen to employ new technologies to help with developing new potato varieties in a more efficient manner that is better able to respond to changing environmental conditions and consumer preferences. One way forward would be to get whole genome DNA sequences for parents and progeny across breeding populations and use this sequence information to help in the selection of new potato varieties. Unfortunately, it is currently too expensive to fully sequence the entire genome of many different individuals in a breeding program. However, it is now possible to efficiently sequence a subset of a genome across many individuals simultaneously, and identify tens of thousands of spots in the genome that differ among

individuals. These spots in the genome, called single nucleotide polymorphisms (SNPs), are extremely useful in a wide array of applications including pedigree analysis, genetic mapping and gene discovery, and trait prediction. At the Fredericton Research and Development Centre we have been applying the DNA sequence based SNP genotyping on the Canadian Plant Gene Resources (PGR) potato collection to aid in the curation of the PGR collection, and develop the tools needed to gain efficiencies in variety selection in the AAFC potato breeding program.

Part of the motivation to develop the genome wide SNP markers in a “gene bank” such the PGR potato collection was the amount of genetic diversity present; the PGR potato collection is an interesting mix of very old heirloom varieties, modern varieties, and breeding lines that have been important in potato variety development in Canada and abroad. The genetic diversity in the population allows for relatively easy SNP discovery, and to date we have identified ~35000 SNP markers spread across all 12 potato chromosomes. A byproduct of the SNP discovery in the PGR collection is we now have very comprehensive genetic fingerprints

of all the varieties it contains. These fingerprints can be used to help curate the collection and identify potential variety mix-ups during tissue culture propagation, and also give us a means to explore the familial relationships among all the varieties. For example, we can use the large SNP set to estimate the genetic relatedness among all pairs of varieties and determine parent-offspring relationships or sibships. If we restrict our analysis to these first degree relationships, we find that almost 50% of the varieties have at least one close relative in the collection (Figure 1). Preliminary analysis has shown the reconstructed pedigree relationships closely match with historical pedigree data (shown in red in Figure 1) for a subset of the varieties in the collection. Once completed, this analysis will be useful in determining what varieties to maintain in the collection and what new candidate varieties should be added to incorporate novel traits while also maximizing the genetic diversity.

Marker-trait associations and gene discovery

One goal of molecular breeding is to use genetic markers linked to desirable traits as tools to aid the efficient selection of new varieties of agricultural crops. The challenge is to identify the DNA markers that are associated with traits that are important in variety development, such as disease resistance, plant growth and yield. To meet this challenge you need two things: a genetically diverse population that is variable

for the trait you are interested in selecting and a large set of genetic markers (eg. SNPs) spread across the genome. In our case we are lucky in that the PGR potato collection has some historical trait data available for several simple traits such as flower colour, tuber skin and flesh colour, and disease resistance for potato virus X (PVX) and Y (PVY). For some of these traits (eg. potato virus X resistance) it is known where the genes controlling the trait variation are in the genome, so we can use this to validate the effectiveness of our new set of SNP markers in gene discovery. By scanning the genome using our 35000 SNP markers we can identify individual SNPs that are most likely to be near a gene (or genetic locus) contributing to the variation in a particular trait. In the case of PVX resistance our set of SNP markers narrow down a region on the tip of chromosome 12 that should contain a resistance gene (Figure 2 a). In comparison with the truth known chromosomal position of a resistance gene homologue, our top SNP marker is located a mere 50000 DNA bases away (out of a genome of 750 million bases!). The same is true for tuber skin colour, as our SNP set identifies a region on chromosome 10 that is known to control expression of the coloured pigments in the tuber skin (Figure 2 b). In both cases this demonstrates that even with the modest population size of the current PGR potato collection our set of SNP markers have the power to identify individual marker loci are tightly linked to causal genes for traits that can be important in future potato breeding.

Researchers at Acadia University Exploring Potential of Cryogenics for Germplasm Conservation

Dr. Robin Browne Propagation Specialist,
Germplasm Conservation K.C. Irving
Environmental Science Centre and Harriet
Irving Botanical Gardens Acadia University

In recent years, there has been increasing interest in the application of cryogenics for conservation of plant germplasm. Concerns about food security and protection of biodiversity have prompted global initiatives to conserve valuable germplasm of the major food crops and their wild relatives as well as all indigenous native plant species. At the K.C. Irving Environmental Science Centre and Harriet Irving Botanical Gardens (K.C. Irving Centre), located on the campus of Acadia University (Acadia), researchers have been working to save native plant species of the Acadian Forest Region as well as conserve some of the clonal crops of relevance to growers in the Maritimes.

Since 2013, seed from more than 80 native species have been collected or acquired for storage and testing at the Acadia Seed Bank (ASB); approximately 20% of these species are currently considered rare or endangered. Additional trials are underway to utilize tissue culture techniques as a complementary strategy for conservation and provision of research material. In 2018, a major research project was also initiated to develop a repository for grapevine cultivars of interest to vineyards in Nova Scotia.

As part of the research program for developing effective conservation methods for

native plants and grapevine clones, the potential of cryogenics – the storage of tissues in liquid nitrogen at ultra-low temperatures of -150°C to -192°C – is being explored. The longer term objective is to apply cryogenic storage procedures to a wider scope of native plant species and clonal crops, which includes native species that produce recalcitrant seed, which cannot tolerate desiccation and storage at conventional low temperatures (typically from 4°C to -20°C), other native species that do not produce seeds (e.g., ferns and mosses) as well as other clonal crops (e.g., strawberries and potatoes).

The Acadia research team at the K.C. Irving Centre includes Dr. Robin Browne (Propagation Specialist, Germplasm Conservation), Samuel Jean (Acadia Seed Bank Coordinator), Alain Belliveau (Irving Biodiversity Collections Manager, E.C. Smith Herbarium), Dr. Allison Walker (Associate Professor and Director of the E.C. Smith Herbarium and Acadia Seed Bank), Lisa Harkness (Research Associate, Grapevine Cryogenics) and Phyllis Essex-Fraser (Lab Manager, Grapevine Cryogenics). Their work is also supported by many other staff, special volunteers and students who have been involved in various aspects of the ASB and Grapevine Repository projects, including support with collection, processing and testing of seeds for the ASB, greenhouse-based propagation and growing of seedlings and cuttings, as well as tissue culture and cryogenic studies.

In order to enhance the development of the conservation program at the K.C. Irving Centre, the research team has been seeking to

collaborate with other germplasm conservation stakeholders, such as the New Zealand Institute for Plant and Food Research Limited (NZPFR). Since 2017, Acadia has been working in partnership with the NZPFR on applications of cryogenics for conservation of native plants and clonal crops (see photos). NZPFR has a major cryogenics program for kiwifruit, potatoes, grapevines, apples and raspberry. Their potato cryopreservation program currently includes 163 accessions that have shown a threshold recovery rate of over 40% , with a mean recovery rate of 78.0 %. The program is ongoing, with more potato accessions expected to be added steadily. In addition, NZPFR works closely with the New Zealand Indigenous Flora Seed Bank (NZIFSB) for cryopreservation of threatened native species.

In 2017, a formal collaboration was also established with Agriculture and Agri-Food Canada (AAFC), Kentville Research and Development Centre (KRDC) to study grapevine cryogenics. The research team at AAFC-KRDC includes Dr. Harrison Wright (Plant Physiologist), Dr. Shawkat Ali (Plant Pathologist) and Conny Bishop (Technical Officer). The primary purpose of the collaboration is to provide essential expertise with regard to the development of virus detection and eradication techniques as well as to conduct field trialing of plant material recovered from cryogenic treatments. Dr. Ranjith Pathirana (Science Team Leader for Germplasm Conservation at NZPFR) recently visited the Annapolis Valley, Nova Scotia, to follow the alliance of both the Acadia and AAFC-KRDC research teams (Figure 3.).



Figure 1: Research Associate Liya Mathew from NZPFR removing potato selections from cryopreservation dewar

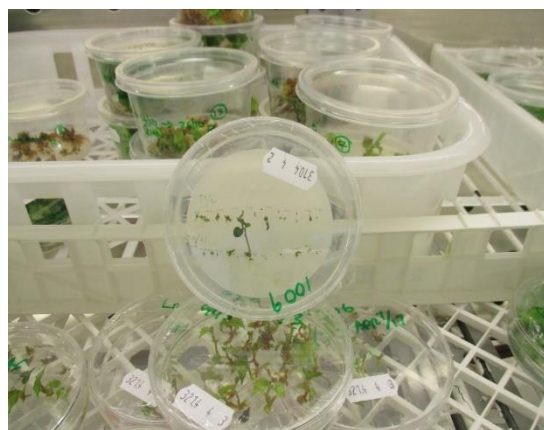


Figure 2: Recovered potato cultures (top plate) from cryopreservation.

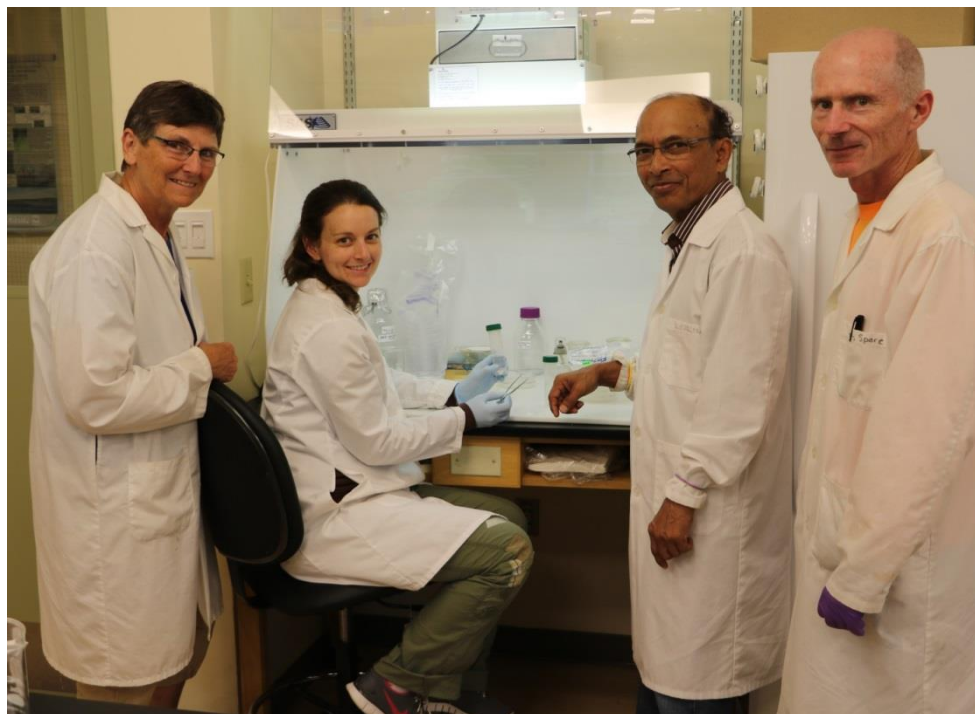


Figure 3: Collaborative cryogenic studies at the K.C. Irving Centre, Acadia: (Left to Right) Conny Bishop (AAFC-KRDC), Lisa Harkness (Acadia), Dr. Ranjith Pathirana (NZPFR) and Dr. Robin Browne (Acadia).

In November 2018, two members of Acadia's research team - Dr. Robin Browne and Samuel Jean - traveled to Fredericton, New Brunswick, to learn more about germplasm conservation programs in that province, and to explore the potential for collaborations with researchers there. During their visit, Dr. Browne and Mr. Jean met with Dr. Benoît Bizimungu (Curator, AAFC-Fredericton Research and Development Centre (FRDC) Potato Genebank) and Malcolm Zwicker (Managing Technical Officer, New Brunswick Plant Propagation Centre (NBPPC)) concerning the conservation of potato genetic resources. Tissue culture is used at both institutes for potato conservation, but cryopreservation is currently not employed to support the long-term storage of valuable

potato cultivars and lines. In addition to visits devoted to conservation of potato germplasm, meetings and tours were arranged with personnel at the Canadian Forest Service (CFS) facility to learn about ongoing conservation activity for native tree and shrub species. While at CFS, Dr. Browne and Mr. Jean met with Donnie McPhee (Manager, National Tree Seed Centre) about seed storage and testing programs as well as John Letourneau (Greenhouse Manager) about current and future planting trials for native tree species. In addition, Dr. Browne and Mr. Jean met with Dr. Martin Williams (Research Scientist), Dr. Tannis Beardmore (Lead Scientist) and Kathleen Forbes (Technical Officer), who are all engaged in butternut conservation studies, which includes the application of tissue culture and cryogenic methods.

In 2019, one member of the K.C. Irving Centre's research team – Ms. Harkness – will visit the NZPFR Germplasm Conservation Science Team as well as the NZIFSB at Massey University to further expand collaborations with these researchers. She will be an Academic Visitor at both institutes, from March 1 to April 12, to learn more about their research for conservation of native species and clonal crops, with emphasis on cryogenics research.

Through the development of collaborative relationships with NZPFR, NZIFSB, AAFC-KRDC, AAFC-FRDC, NBPPC and CFS, the Acadia research team at the K.C. Irving Centre hopes to advance germplasm conservation programs with native tree and shrub species as well as clonal crops, including grapevines and potatoes.

Annual Report 2018

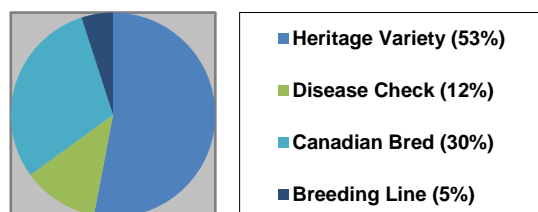
Canadian Potato Genetic Resources

Sylvia Soucy

The Collection

1. Holdings

• The Canadian Potato Genetic Resources is a node of Plant Gene Resources Canada and holds 177 clones within its Genebank. Of this total, 176 are maintained *in vitro*, and 96 clones were grown for tuber production at our Benton Ridge Potato Breeding Substation, Benton, New Brunswick. A full listing of accessions may be found in the request form. The following chart illustrates the types of clones in each category.



2. New Accessions

- “Black Bull”, donated by Eric McCumber of Long Reach, NB, was introduced into the collection in 2017 but is still undergoing virus freeing before we can accept it into the gene bank.

3. Evaluations

- There were no evaluation trials at the Fredericton Research and Development Centre in 2018. The evaluation plots are useful for Total Glycoalkaloid (TGA) Analysis, specific gravity measurement, photographs and culinary evaluation.
- Thirty-two clones were grown in 20-hill plots at the Benton Ridge Potato Breeding Substation, Benton, NB, to provide material for demonstration and cooking quality evaluation throughout the winter and spring.

4. Management

- Passport data for 167 PGR accessions is available online at the Genetic Resources Information Network-Canadian Version (GRIN-CA). GRIN-CA may be accessed through the [Plant Gene Resources of Canada](http://www.plantgenetresources.ca) website. No new information was posted in 2018 due to the inaccessibility of GRIN-CA during upgrades.
- Disease testing was conducted for new *in vitro* accessions and clones which have been maintained *in vitro* for five years. Thirty-five clones were grown in the greenhouse and tested once in 2018. All clones were negative for PVA, PLRV, PotLV, PVS, PVX, PVY and PSTV. Results for BRR are pending. Extra mini tubers from the greenhouse grow out will be offered to Genebank clients in the spring of 2019.
- All *in vitro* clones were screened twice during 2018 for bacterial and fungal contamination using Potato Dextrose Broth and Richardson's Broth. All clones currently

in the Genebank are negative for these contaminants.

- A total of 1485 microtubers were harvested from 171 of the Genebank accessions in 2018. Approximately half of the microtubers were sent to Saskatoon in September 2018 to be stored as back up at Plant Gene Resources of Canada, AAFC. The viability of the collection is protected by this remote location storage arrangement. Dallas Kessler, of Plant Gene Resources Canada, Saskatoon SK, continues to monitor the microtubers. The remaining microtubers are stored at the Genebank in Fredericton, NB.

5. Distribution

- Accessions in the Canadian Potato Genetic Resources fall under [The International Treaty on Plant Genetic Resources for Food and Agriculture](#) which requires the recipient to

sign a Standard Material Transfer Agreement (SMTA) that the material shall be used or conserved only for the purposes of research, breeding and training (education) for food and agriculture. This agreement is included with the request form. For more information and assistance in determining whether your plans fall into this agreement visit: [The International Treaty on Plant Genetic Resources](#) website. By accepting shipment of the requested material you are accepting the terms of the SMTA and recognize that your name will be submitted as a recipient of this material to the Governing Body of the Treaty.

- Twenty-three requests for 385 clones were received in 2018. Of this number, 50 clones were distributed as *in vitro* plantlets, 226 clones as field grown tubers, and 109 clones as greenhouse grown mini tubers. “Marc Warshaw’s Quebec”, “Likely” and “Bintje” were the most requested accessions in 2018.

Distribution of Clones by Purpose – 2018

Purpose of Request	Number of requests	Clones	<i>In vitro</i> plantlets	Field tubers	Mini-tubers
Research	20	301	50	142	109
Teaching or Demonstration	3	84	0	84	0
Conservation	0	0	0	0	0
Total	23	385	50	226	109

Requests by Destination – 2018

Destination	Number of requests
Newfoundland and Labrador	1
British Columbia	2
New Brunswick	6
Quebec	6
Ontario	6
Saskatchewan	1
USA	1
Total	23

Five-Year Compilation of Clone Distribution for Potato Gene Resources 2014-2018

Year	Research	Education	Conservation	Total	Field tubers or mini-tubers	<i>In vitro</i> plantlets	Total
2014	13	2	11	26	492	119	611
2015	14	1	7	22	360	186	546
2016	23	4	5	32	826	195	1021
2017	15	3	0	18	414	98	512
2018	20	3	0	23	335	50	385
Total	73	13	23	121	2427	648	3075

Repository Items of Interest

Communication

- In addition to the requests for clones, many requests for information about the Genebank, the availability of clones, clone descriptions and pedigrees, and techniques for handling *in vitro* material were received throughout 2018.

- The annual Potato Gene Resources newsletter has a distribution list of approximately 300 recipients.

- The current newsletter and several back issues may be accessed on the Weekly Checklist of [Government of Canada Publications](#).

Meetings and Miscellaneous Information

- The third addition of Agri-Science Days on Biodiversity, Bio-resources and Collections were held on January 23-24, 2018 at the Ottawa Research and Development Centre and was attended via videoconference.

- The 2018 meeting of the NRSP-6 project's Technical Advisory Committee (TAC) was held at Sturgeon Bay, WI, on May 30, 2018. Dr. Benoit Bizimungu (curator of the

Canadian potato gene resources) presented a report on utilization of potato accessions imported from the US Potato Genebank by Canadian researchers. Information on the Genebank and minutes of the TAC meetings can be found at the [USDA Potato Genebank](#) website.

Donor Agreement

- Donors wishing to provide plant material to Agriculture and Agri-Food Canada (AAFC) for the purpose of research, conservation and distribution by Plant Gene Resources of Canada must now complete a “donor agreement”. Decisions on accepting material

into the Canadian Potato Genebank are up to the discretion of the curator,

Dr. Benoit Bizimungu
(Benoit.Bizimungu@canada.ca).

Visitors

- June 26, 2018 – Counsellor Wang Junming and second secretary Hu Xuan from the Embassy of the People's Republic of China in Canada along with a small delegation met with Dr. Benoit Bizimungu to tour the Fredericton Research and Development Centre and the Genebank.



Research Scientist/Potato Gene Resources Curator Dr. Benoit Bizimungu (Right) leading tour of Potato Genetic Resources Genebank in June 2018.

- November 22, 2018 – Robin Browne, Propagation Specialist and Samuel Jean, Seed Bank Intern from K.C. Irving Environmental Science Centre at Acadia University toured the Genebank.

Fredericton Research and Development Centre Website

- The Fredericton Research and Development Centre is custodian of the Canadian Potato Genetic Resources. [The Fredericton Research and Development Centre](#) website offers an overview of the Centre's mandate, resources and achievements along with research studies being conducted at the Centre and the staff associated with those studies.

Plant Gene Resources of Canada

- Plant Gene Resources of Canada (PGRC), the national Canadian Genebank, preserves, characterizes and distributes plant genetic resources for food and agriculture. PGRC is based on collaboration between AAFC Research Centres and people dedicated to preserving the genetic diversity of crop plants and their wild relatives. PGRC plays a significant part of AAFC's commitment to the Canadian Biodiversity Strategy in response to the Convention on Biological Diversity and the International Treaty on Plant Genetic Resources.

- [The Plant Gene Resources of Canada \(PGRC\)](#) website includes information on the PGRC multi-nodal system of germplasm conservation in Canada and allows searching for germplasm information on the Genetic Resources Information Network-Canadian version (GRIN-CA). Dr. Axel Diederichsen, Research Scientist and Curator at PGRC can be contacted at the Saskatoon Research and Development Centre of AAFC at Axel.Diederichsen@canada.ca.

The Genebank and the Seed Potato System

- The Canadian Potato Genetic Resources provides *in vitro* plantlets and greenhouse or field tubers for breeding, research and heritage preservation. While extensively tested for freedom from disease, the plantlets and tubers distributed are produced outside the Canadian Seed Certification System and are not eligible for certification.
- The Canadian Seed Potato Certification System operates under the *Seeds Act* and its regulations. Certification begins with tested plantlets established *in vitro* in a facility accredited for this task by the Canadian Food Inspection Agency (CFIA). The plantlets are used to produce greenhouse tubers which then go to the field in a limited generation system, at each step meeting strict standards specified in the regulations. More information on potato seed certification can be found at the [CFIA](#) website.

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The Potato Gene Resources Newsletter is available as an electronic version. If you are still receiving a paper version and wish to receive future Newsletters by e-mail, in pdf (portable document format), please send your e-mail address to: Sylvia.Soucy@canada.ca.

We will continue to send the printed Newsletter to those who do not ask to receive it electronically. Maintaining contact with you is important.

Curator's Note

I am pleased to welcome Ms. Sylvia Soucy to the Canadian potato gene bank as the new potato genetic resources technician in replacement of Ms. Teresa Molen who retired

in July 2018. Sylvia comes with a wealth of knowledge and experience in potato tissue culture and molecular biology. With these valuable skills, Sylvia will be an important player in supporting potato genetic diversity conservation and utilization by gene bank users in Canada and around the world.

Benoît Bizimungu, PhD
Potato Breeder & Gene Resources Curator

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Potato Gene Resources

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