MINUTES

Potato Crop Germplasm Committee meeting

PAA 2014 -- Spokane, WA

7:00AM -- July 31st, 2014

Present: Abad, Bamberg (Chair), Jansky, Ronis, Whitworth, Douches, Cooper, Martin, Ellis, Endelman, Sathuvalli

The meeting was opened by participants introducing themselves. Bamberg then gave a sketch of the history and purpose of the CGCs, and particularly the history and rationale for the composition of the potato CGC as it relates to the PAA. He noted that the full record of past grant awards and meeting minutes is available through the genebank website's links to GRIN.

The agenda items proposed by Bamberg by email on July 14 were addressed:

Quarantine: Dr. Abad reviewed details of his report to the committee (attached). His lab serves the genebank and all other importers. They must detect and clean up infected stocks. A challenge is setting quotas and priorities. However, the committee applauded Dr. Abad's commitment to leading a responsive and efficient program that has eliminated most of the quarantine delays and limitations of the past. Bamberg noted that a published review for an industry or research outlet could help germplasm users understand the rules of potato import as pertains to phytosanitary and germplasm ownership considerations, and the grave consequences that might result from breaking them.

CIP: Dr. Ellis gave an update on the genebank. He is presenting a poster at the PAA14 meeting (attached). CIP germplasm is under the International Treaty. The genebank is making improvements in cryo preservation, the quarantine program, databases, and safety backups. CIP is keen on continuing cooperation with USPG on research, as well as joint efforts to fingerprint germplasm, rationalize the collections, and otherwise improve its health, evaluation, documentation and reliable identification. Funding is a challenge. Collecting is still shut down, but they are hopeful of progress in the near future.

Evaluation grant awarded for FY14. Dr. Cooper was funded this year to study insect and bacterial resistance to address the Zebra chip problem (report brochure attached). With the genebank, he has screened and identified *S. bulbocastanum* that psyllids do not prefer and others that inhibit insect development. Crosses have been made at the genebank to combine the two types of resistance. Work on screening *S. verrucosum* for resistance and using it to introgress *bulbocastanum* is in progress.

Evaluation priorities: Zebra Chip is of high importance. Thus, one reasonable option for FY15 funding would be a second year of Cooper's ZC resistance screening. But all members should be on the lookout for other worthy evaluation projects. We favor new, promising ventures that can be given a start with these small grants. Also, the work should result in evaluation datapoints that can be applied to our potato germplasm accessions in GRIN.

Vulnerability Statement. This year, we responded to the NPL's request for an updated report, which has now been posted. Thanks to Shelley for the considerable effort she put into drafting much of the new document.

Other issues: During travel for these meetings, Bamberg received a report (attached) from the base collection at Ft. Collins (NCGRP), with respect to their long-term backup storage of USPG germplasm.

Meeting adjourned at 8:40.

Respectfully submitted,
John Bamberg

Status of the Potato Quarantine Program, 2014

Presented to the Potato Crop Germplasm Committee

July 31, 2014 in Spokane, WA

by

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Introduction

The mission of the Potato Quarantine Program (PQP) is to test germplasm for pathogens as a condition for the entry of this valuable crop germplasm into the United States. Special emphasis is given to the detection of viruses, viroids and bacteria including phytoplasmas. This program is the first line of defense against the inadvertent introduction of new potato diseases into the USA. Such diseases have the potential to create both economical and environmental burden to the crop. Additionally, in our program, any infected accession is subjected to therapy for the elimination of pathogens and then retested to ensure the success of the treatment. Eventually all the accessions are released to the requesters.

Staff

A slight rearrange occurred in our Lab, last year. Prat Bandla, tissue culture specialist, will continue taking responsibilities in potatoes, sweetpotatoes and in addition to these crops; she started working with tissue culture in sugarcane and other poaceas. Prat was also trained in Cryotherapy at the USDA-ARS Cryopreservation Unit at Fort Collins, CO. Richard Slocum, our senior specialist is focusing in cassava and rice in addition to his crops i.e. pomes, apples, kiwis and small fruits. Crindi Loschinkohl, our crop specialist, continues doing an outstanding job with her tremendous expertise in acquisition, testing, and distribution of potatoes, sweetpotatoes and more recently cassava. Seth Pack continuous to be our gardener, he is doing an excellent job in the greenhouse work and helping Crindi with the biological and molecular tests. We just received the authorization to hire a new Student worker to help in the routine chores at the TC lab. The USDA-APHIS Plant Germplasm Quarantine Program continues under the leadership of our Director, Dr. Joseph Foster.

Accomplishments

Our PQP continue keeping very high standards in pathogen detection tests for potato diseases. We keep on using a sound biological test under optimum conditions that ensures the interception of unknown or unusual viruses. This test includes the mechanical inoculation onto 12 different indicator plants and grafting on healthy potatoes when the accessions are negative for all the tests yet still showing symptoms in the original potatoes. Serology and molecular based methods will not detect the unknown viruses. We routinely use ELISA for PVX, PVT, PVM, PVA, PMTV, potyviruses and *Clavibacter michiganensis*. ImmuoStrips for *Ralstonia solanacearum*. RT-PCR and PCR tests with generic primers for: luteoviruses, carlaviruses, potexviruses, potyviruses, geminiviruses and phytoplasmas, respectively. We are using qRT- PCR (real time) to detect *Potato yellow vein virus,* a potentially damaging and seed transmitted virus**.** Furthermore, in collaboration with the International Potato Center (CIP) in Peru, we are identifying difficult unknown viruses by next-generation sequencing analysis, a new method where no specific primers are needed.

As several viroids have been reported affecting potatoes, we have developed a wide spectrum system of conventional RT-PCR that will detect PSTVd and all

pospoviroids that potentially can infect potatoes. Therapy continuous to be primordial in our program for the elimination of viruses in infected accessions. Thermotherapy and chemotherapy are used in the treatments and our current curing method will be enhanced with Cryotherapy this coming season.

We continue the introduction of true potato seed (TPS) accessions. Testing for this group is slightly different. Ten percent of the seedlings are sacrificed to be grown and mechanically inoculated in a set of five indicator plants that will show symptoms of all seed transmitted viruses in potatoes if present. If the test is negative; the remaining 90 % of the seeds are released.

Our primary stakeholders continue to be potato Breeders from universities, government and the private industry. We are also continuing our collaboration with the NRSP-6 US Potato Genebank by introducing more potato accessions through our quarantine program. This season, we have requested seven accessions for the Genebank from the International Potato Center in Peru. In addition, several clones introduced to the Genebank several years ago are undergoing therapy to eradicate viruses in our tissue culture lab. These accessions were placed in thermotherapy and chemotherapy; the clones will be maintained in tissue culture until they have generated sufficient material for testing. After testing is complete and the accessions are found to be negative to the testing procedures, they will be released to the Genebank.

Potato germplasm acquisition and releases

Our inventory for 2013-2014 consisted of 105 potato clones, surpassing one more time our quota for the year (Table 1). It includes all the acquired and released germplasm as well as the clones in therapy for this season. From those, 98 clones were received this season, all as *in vitro* cultures. The remaining 8 clones were obtained the previous years. After testing, we released 56 accessions. Nine clones either died or did not grow, and 40 clones tested positive for either carlaviruses or *Potato leaf roll virus* all summarized in table 1. Detections were made only in clonal accessions. For true potato seed (Table 2), 48 accessions were received this year in our program. Only 26 were tested and 18 accessions released. The donor requested the destruction of the material due to a probably contamination with PSTVd.

Obtaining foreign germplasm

Federal law (Title 7 of the Plant Pest Act) prohibits the importation of plant parts for use in vegetative propagation of some 50 plant genera, including tuber-bearing *Solanum* spp. Importation of true potato seed (TPS) is also prohibited. The quarantine period for potatoes is typically 6-7 months. Potatoes generally are acquired from foreign donors or institutes or from plant exploration as seed lots of 200 seeds or more, as tubers, or as *in vitro* plantlets. All acquisitions must be accompanied by an import label issued by the PGQP pathologist for potato. Potato slots are filled and processed on a "first-come, first-served" basis. The indexing season for potatoes (based on greenhouse growing conditions for indicator plants) is from September through May. Requests for

potato importation should be submitted between January and May preceding the start of the testing cycle.

The Potato Crop Germplasm Committee is considered an important component in the plant introduction system. The committee can help by taking an active role in developing and submitting an annual prioritized request for potato germplasm to the PGQP.

Acknowledgments

The Potato Quarantine Program is operating nearly without backlogs. This accomplishment would not be possible without the dedicated and outstanding work of our personnel at the PGPQ. I want also to acknowledge Dr. Joseph Foster, our Director for his guidance and encouragement. Also, to Dr. Clarissa Maroon-Lango at PGQP, her great collaboration, the outstanding molecular testing in her lab and her friendship is gratefully appreciated.

Table 1.- 2013-2014 Potato Clonal Testing.

Clonal Potatoes

There were 105 potato clones in the PGQP in the 2013-2014 season.

1	clone was received in 2011		
	1	from Chile	for G. Secor
6	clones were received in 2012		
	1	from Poland	for C. Brown
	1	from Japan	for M. Martin
	1	from Germany	for L. Ewing
	1	from Germany	for Valley Tissue Culture
	1	from The Netherlands	for Valley Tissue Culture
	1	from Germany	for C. Keller
98	clones were received in 2013		
	all were received *in vitro*		
	42	from Chile	for G. Secor
	6	from Ethiopia	for K. Perry
	5	from France	for K. Perry
	11	from Germany	for Valley Tissue Culture
	2	from Germany	for F. Goktepe
	11	from The Netherlands	for Valley Tissue Culture
	10	from The Netherlands	for N. Champouret
	2	from Peru	for M. Martin
	9	from Peru	for R. Shakya
	1	from Scotland	for J. Wallace

Of these 105 clones:		
6	died before testing began	
2	arrived too late for testing	
1	was discarded (a replicate of a released clone)	
96	were tested	
	40 were positive	(Carlavirus, Luteovirus)
	56 were released	

Table 2.- 2013-2014 TPS Testing.

True Potato Seed

There were 48 TPS lots in the PGQP in the 2013-2014 season.

30	from The Netherlands	for J. Bragg
18	from The Netherlands	for J. Debons

Of these 48 seed lots:

22	were discarded at the request of the donor before testing commenced
26	seed lots were tested
	18 were released
	8 were discarded at the request of the donor after testing commenced

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For additional information about the potato quarantine program in PGQP, you may contact:

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The Genebank at the International Potato Center: A Global Asset to Ensure Food Security

Dave Ellis
Nataly Franco[1]
Rene Gomez[2]
Ivan Manrique[3]
Ana Panta[4]
Genoveva Rossel[5]
Alberto Salas[6]
Rocio Silvestre[7]
Fanny Vargas[8]
Rainer Vollmer[9]
Brenda Zea[10]

[1]Safety Back-up
[2]Cultivated Potato
[3]ARTCs
[4]In vitro
[5]Sweetpotato
[6]Wild Potato Species
[7]Breeding Lines
[8]Herbarium
[9]Cryopreservation
[10]Phytosanitary

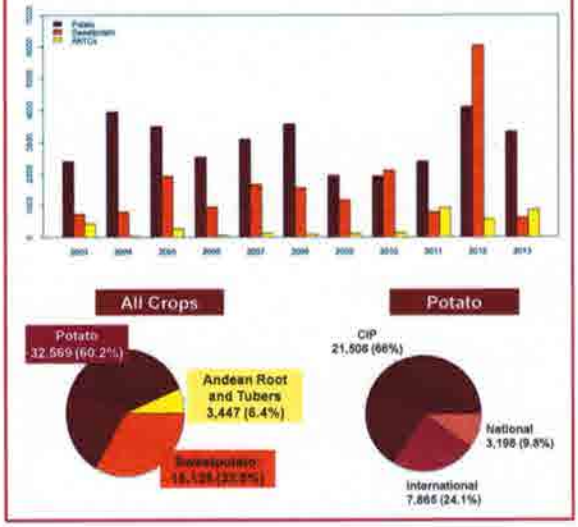
In-Trust Collections for Humanity

The genebank at the International Potato Center (CIP), in Lima, Peru safeguards the global collections of potato (6,768 accessions), sweetpotato (7,503 accessions) and nine different Andean Root and Tubers (ARTC, 2,516 accessions) (Figure 3.). These collections are held in-trust for the Food and Agriculture Organization of the United Nations (FAO) and are distributed for research and breeding world-wide under the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA). The CIP genebank is the largest in vitro genebank in the world maintaining over 14,000 accessions (>90% of the cultivated collection) in vitro. The importance of the in vitro collection is that this is the only way to conserve and distribute disease-free clonal material, a requirement for the international transfer of clonal plant genetic resources. Wild accessions in contrast are maintained as seed with parents of the regenerated material being tested for seed-borne diseases. In the past 10 years, CIP has distributed over 15,000 samples representing over 30% of the diversity (accessions) held in the collections to over 150 different countries. In keeping with the mandate of a Research Center of the Consultative Group on International Agricultural Research (CGIAR), CIP is committed to ensuring global food security and productivity and in this end, 90% of material distributed from the CIP genebank has been shipped to and used in the developing world. In the Andean region, the CIP Genebank partners with numerous indigenous communities, such as the Parque de la Papa in the Cusco area, exchanging knowledge germplasm and research.

World's Largest In Vitro Genebank

Wild accessions are maintained and distributed as seed (populations). Clonal (cultivated) collections are maintained and distributed as in vitro plantlets. CIP maintains over 14,000 accessions by over 70 dedicated people working in the lab and the field. Although all accessions are *theoretically* available for research and breeding, the CIP genebank has undertaken an unprecedented project to confirm that only pathogen-free, true-to-type germplasm is distributed. During this 5-year project, some accessions are not available, yet if desired, they will be prioritized to get this material to potential users as soon as possible.

Figure 1. Germplasm distribution by collection in past 10 yrs.



Parque de la Papa – A Living Laboratory

Parque de la Papa is an association of six indigenous communities high in the Andes who the CIP genebank has been partnering with for almost 15 years. The Parque is a 9,000 hectare valley where potato is currently grown from 13,000 ft.. to over 14,700 ft.). In the past 30 years, Due to warming climates and the associated insect and disease pressures, potato cultivation in this valley is now at elevations 300 ft. higher than just 30 yrs. ago! The effect of a warming climate is very pronounced in the high elevation Andes and CIP is working with these communities to develop strategies to adapt to this rapid change.

International Treaty for Plant Genetic Resources for Food and Agriculture

The CIP collections are maintained *in-trust* for the FAO and distribution and use are governed by the ITPGRFA. Therefore distribution of all material is under the terms of the Standard Material Transfer Agreement (SMTA) of the ITPGRFA.

Figure 2. Number of accessions, species and breeding lines conserved in the CIP genebank (20 May 2014).

	Species	Accessions	Countries Represented	Breeding Lines
Cultivated Potato	7	4,354	21	3,516
Wild Potato	151*	2,414	14	-
Cultivated Sweetpotato	1	5,324	56	715
Wild Sweetpotato	67	1,179	19	-
Cultivated ARTC	11	2,018	13	30
Wild ARTC	35	497	4	-
Total	272	16,876	62 unique	4,261

* Hawkes

Figure 3. Composition of cultivated potato collection by taxa.

Spp.	# of accessions	% of collection	Ploidy
S. stenotomum subsp. *stenotomum*	301	6.9	2x
S. stenotomum subsp. *goniocalyx*	99	2.3	2x
S. phureja	206	4.7	2x
S. x chaucha	117	2.7	3x
S. tuberosum subsp. *andigenum*	3218	73.9	4x
S. tuberosum subsp. *tuberosum*	179	4.1	4x
S. x ajanhuiri	14	0.3	2x
S. x juzepczukii	36	0.8	3x
S. x curtilobum	6	0.1	5x
Hybrids	178	4.1	n/a

Global Asset Freely Available

All accessions are freely available upon request for research and breeding for use in food and agriculture and can be ordered at the CIP website (www.cipotato.org – genebank tab). All accessions are shipped under the terms of the SMTA (www.planttreaty.org). Due to the delicate nature of the in vitro material, a small shipping and handling fee may be requested to cover the cost of overnight courier for delivery. We do attempt to track use and are always looking for reliable results about the use of our germplasm.

• CENTRO INTERNACIONAL DE LA PAPA •
CIP
A member of the CGIAR Consortium

Summary

We are currently screening populations of wild potatoes, *Solanum bulbocastanum, S. verrucosum, and S. hjertingii* for resistance to potato psyllid and *Liberibacter*, the pathogen that causes zebra chip disease of potato. To date, we have identified five populations of *S. verrucosum* that are putatively resistant to potato psyllid. Assays to screen *S. bulbocastanum* for resistance to *Liberibacter* are currently underway.

USDA ARS

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REPORT ON CURRENT CGC GRANT

Screening for Resistance to Potato Psyllid and Zebra Chip Disease among Wild Potatoes

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Significance

- Zebra chip disease of potato has recently become a major concern for potato growers in the western U.S.
- Zebra chip is caused by the bacterial pathogen, *Liberibacter solanacearum*, which is transmitted by the potato psyllid.
- Currently, zebra chip is controlled by insecticide applications to target the psyllid.
- Development of new potato varieties that are resistant to *Liberibacter* or potato psyllid would provide a cost-effective management tool for zebra chip disease.
- Using choice and no-choice assays, we recently identified psyllid resistance in *Solanum bulbocastanum*.

Zebra chip disease of potato

Objectives

- Screen populations of *S. verrucosum* and *S. hjertingii* for resistance to potato psyllid
- Screen populations of *S. bulbocastanum*, *S. verrucosum*, and *S. hjertingii* for resistance to Liberibacter.

Approach: Psyllid Assays

- Combined use of choice pre-screening studies followed by no-choice performance assays.
- For prescreening assays, one plant from each population will be placed in each of 10 cages, and 100 adult psyllids will be dispersed in each cage. The numbers of eggs, nymphs, and adults on each plant will be counted after three weeks. Data from these prescreening assays will be used to identify populations with putative resistance to potato psyllid for inclusion in the performance assays.
- For performance assays, a single mated female will be confined to each plant (10 per plant population) and the number of eggs will be counted after three days. Plants will be maintained for another three weeks before counting the number of living offspring.

Approach: ZC Assays

- Three *Liberibacter*-infected psyllids will be confined to each of 10 plants per population for 24 hours. Non-infected adult psyllids will be confined to plants for controls.
- Plants will be tested for *Liberibacter* using PCR three weeks after removing the insects and will be monitored weekly for symptoms associated with *Liberibacter*. Assays will be terminated after 12 weeks or when the above-ground portions of the plants die.

Progress

- Results of choice assays on *S. verrucosum* identified five populations with putative resistance to potato psyllid. These include PI 195170, PI 195171, PI 251756, PI 545745, PI 275259.
- No-choice assays on *S. verrucosum* are currently underway.
- *S. bulbocastanum* plants have been inoculated with *Liberibacter*. Initial results are expected by the end of August.

Future

- Determine whether resistance traits from different populations can be combined for enhanced psyllid resistance.
- Determine how much of the resistance from wild potatoes can be transferred to the background of marketable potatoes.

More Details

- Cooper WR and JB Bamberg. 2014. Variation in *Bactericera cockerelli* (Hemiptera: Triozidae) oviposition, survival, and development on *Solanum bulbocastanum* germplasm. American Journal of Potato Research. In press. DOI 10.1007/s12230-014-9384-x

National Center for Genetic Resource Preservation (NCGRP) CGC Report 2014

The NCGRP, in Fort Collins, CO is a part of the National Plant Germplasm System (NPGS), and provides safety backup of NPGS collections and conducts research to improve gene bank functioning.

Plant and Animal Genetic Resources Preservation Unit stores a broad range of plant, animal and microbe diversity not only for the NPGS, but for other organizations here and abroad. 2014 seed and clonal activities:

- Received 8,371 seed packets from NPGS active sites. The PAGRP now provides safety back up for 82% of seed collections and 14% of vegetatively-propagated collections in the NPGS
- Conducted 7,448 germination tests. Over 63% of incoming seed from NPGS sites had > 85% germination.
- Conducted 1,019 monitor tests. 68% of the accessions had > 85% viability, indicating they are storing well. For accessions with declining viability, efforts are underway to get this information back to NPGS sites so they can send us fresh seed.
- A total of 170 clonally propagated plant accessions were placed into long-term storage. Significant progress was made in developing cryo protocols for dormant buds. Efforts this year bring our total number of cryopreserved vegetative accessions to 3762.
- Staffing changes- Dr. Stephanie Greene came on board in May 5 as seed curator, filling the Vice Ellis position. Dr. Dave Dierig retired from the ARS on May 30.

Cruciferous Vegetable CGC

NCGRP-unique accessions		% Backup of active collection (seed)	
Ames	Geneva	Ames	Geneva
54	329	99	44

† Vegetable and oilseed

Root and Tuber CGC

Crops	NCGRP-unique accessions			% Backup active collection (sd)			# cryopreserved
	Ames	Geneva	Pullman	Ames	Geneva	Pullman	
Beet†	-	-	12	-	-	75	-
Carrot	100	-	-	85	-	-	-
Garlic	-	-	-	-	-	-	98
Onion	-	3	0	-	56	30	-

†sugar and table

Potato CGC	NCGRP-unique accessions	% Backup active collection (sd)	# cryopreserved
Potato (seed)	7	96	-
Potato PVP	-	-	223
Solanum CWR	-	-	38

Plant Germplasm Preservation Research Unit develops state-of-art tools to improve genebank capacity and efficiency. Objectives of the Unit include: i). detecting gaps and redundancies in collections using statistical genetics and spatial analyses and comparing diversity in germplasm conserved *ex situ* and *in situ* ii). enhancing long-term viability of stored germplasm; iii). developing metrics to monitor and validate viability, health and genetic integrity; iv). using genomic annotations and methods to locate genes for key agricultural traits. Projects in 2014 included developing better methods to eradicate graft-transmissible pathogens to protect the Citrus collection from Huanglongbing (HLB) (Dr. Gayle Volk); analyzing population structure of *Helianthus pumilus*, a sunflower CWR, to inform effective conservation (Dr. Chris Richards); and developing new methods to detect seed aging that are non diestructive (Dr. Chris Walters).