Apple CGC Meeting/Teleconference February 28, 2017, 1-3 EST

Attendees: Gayle Volk, Thomas Chao, Susan Brown, Kate Evans, Gennaro Fazio, Cameron Peace, Jim McFerson, Phil Forsline, Awais Khan, Jim Luby, Jay Norelli, Michael Wisniewski, Kenong Xu (minutes provided by Gayle Volk).

Welcome to new member Awais Khan.

Minutes from Sept 2016 teleconference: approved

Plant Evaluation Proposals: Submission deadline is 2/24/2017. Jim McFerson is chairing the evaluation subcommittee and has identified 5 members to review the proposals. He will forward the selected proposal to National Program Staff by 3/10/2017. A question was asked regarding the number of CGCs with evaluation proposals funded each year. After the meeting, clarification was sought and received from Gary Kinard. The evaluation proposal system currently excludes commodity/row crops (grains, soybeans, cotton, corn, etc). HQ increased the maximum about of each request from 15K to 30K this year, so fewer proposals will be selected for funding.

Plant Exchange/Evaluation Proposals: New proposals are due to Karen Williams by 7/21/2017. Please submit information to Gayle and Thomas two weeks before that date so that letters of support can be provided. In 2016, the following exploration proposals were submitted from the Apple CGC and were selected for funding (pending budget approval): "Plant Exploration in Taiwan to Collect Wild *Malus, Pyrus*, and *Juglans*" (Chao), "Plant Exploration in Pennsylvania to Collect *Malus coronaria* Germplasm" (Chao), and "Plant Exploration in Western Europe to collect *Malus sylvestris* germplasm for crop improvement" (Volk). Additional explorations are planned for submission in 2017 (see Chao report).

Report: Apple Collection, Current Status (See Chao report for details)

- Recent Acquisitions: Seed and clonal accessions have been received from multiple sources, including native US species, 'Hauer Pippin' hard cider cultivar, and *M. halliana* (PI38231) from USDA Chico Plant Introduction Station. Budwood from ~20 crabapple cultivars will soon be received from Arie Den Boer Arboretum, Iowa. These trees will be removed from the arboretum once the materials are established in the NPGS. The arboretum has ~1000 accessions in total.
- Cryo back-up: 15 tart cherry and 55 Malus accessions were sent to the Plant and Animal Genetic Resources Preservation Unit at NLGRP on 1/9/2017 for cryo back-up processing. This included both Priority 1 and Priority 2 accessions on the apple cryo back-up inventory that was developed in 2016. 66 Malus accessions from 2016 cryoprocessing were received on 2/15/2017 for viability testing.
- Outreach: A number of interviews and tours of the collection were provided in 2016. It was recommended that Thomas forward any products (magazine articles, etc) from these tours to the CGC, when they are available.
- Future explorations: An extensive analysis of future exploration opportunities for *Malus* species was provided as part of the curator report. The CGC expressed interest in continuing to collect seeds from native US Malus species for inclusion in the collection.

China does not allow any exports of wild Malus or Vitis germplasm. However, a list of ~1000 cultivars (released 16-18 years ago) from China that can be imported is available from Thomas. If there is a lot of interest in importing some of the cultivars on the list, the CGC may be able to

help coordinate request (because only 50 pome fruit cultivars can be imported through APHIS each year).

Legacy datasets: Datasets on the natural infection of fireblight, individual shoot inoculations, as well as core collection data collected in Illinois have not yet been made public. CGC members will help find a method whereby this information can be made publicly available without introducing an excessive number of new descriptors. Jay Norelli and Awais Khan expressed specific interest in this effort. Note: See follow-up email/CGC report on this topic.

Malus germplasm collection related activities in the next 5-6 years

- 1. <u>Complete 7 'Royal Gala' x *M. sieversii* populations trait characterization and QTL mapping.</u> Will use GBS data. Population 4593 has some potential QTLs relating to disease resistance.
- 2. Continue cryo back-up storage of Malus in Fort Collins. Although the report states that it will take about 7 years to back-up the accessions on the priority list, many of those accessions in the Priority 3 category may be challenging (difficult species), and other back-up strategies may be needed (see Securing the collection, below).
 Related Discussion: Jay Norelli has transgenic fast-plant apple trees that he would like to make publicly available. The NPGS currently doesn't have policies for clonal transgenic collections. It was proposed that perhaps the transgenics could be cryopreserved in Jan 2018 and incorporated into the living collection once procedures are in place. Thomas suggested that this topic be included in the next PGOC meeting (Nov 2017).
- 3. <u>Complete GBS marker for entire Malus collection</u>. Due to ploidy and the transfer of markers across distant species, only 1200 accessions have GBS data available. New algorithms may make it possible to expand this dataset to include the rest of the collection.
- 4. <u>Identify, update, and correct information relating to Malus collection (in GRIN-Global), using SSR, GBS, and morphology characters.</u> Morphological evaluations will be used to confirm information on hybridization, duplicates, and mislabeled materials in the NPGS apple collection. In 2016, SNP chip data were used to confirm morphological observations that suggested that some of the grafted M. orientalis trees were not correctly identified. They will be repropagated.
- 5. <u>Continue evaluation of seedlings in wild Malus seedling block (W3).</u> The wild seedlings in this block should be assessed to determine whether they should be incorporated into the main grafted collection. Available GBS and SSR data will aid in this assessment. See "Securing the collection" below.
- 6. Continue evaluation of the Malus collection for potential hard cider production.
- 7. <u>Update Malus Vulnerability Statement.</u> In collaboration with CGC members.
- 8. <u>Best management practices for clonal germplasm collection.</u>
- 9. <u>Collection of apomictic seeds of wild Malus species as another means for germplasm preservation.</u> Apomixis was identified in bagged flowers/fruit of *M. hupehensis*, *M. coronaria*, and *M. sikkimensis*. These seeds could be used to back-up wild species materials in a cost efficient manner.
- 10. Screening Malus collection for possible virus infection. Use of deep sequencing.
- 11. <u>Identify the ploidy of unknown Malus accessions</u>. Considering the purchase of a \$40K flow cytometer for data collection.

Orchard Space Utilization/Maintenance Issues (Chao)

The former K1 orchard (trees removed 12/2015) has space for 391 accessions. At this time, 150 accessions are in the nursery and have been designated for placement in this orchard. This leaves space for approximately 240 accessions. In addition, there is wild grape planting next to K1 that is getting repropagated, and approximately 100 additional apple trees can be put into this future space (when available). There is also some space next to the B9 planting (squeeze in another 100 accessions?). The G1 block (Gala x M. sieversii seedlings) may be removed in 5-6 years, which will make additional space available.

At this time, 2 grafted trees are planted in the field for each accession. When established, one of those trees is usually removed, leaving a single tree in the field for each grafted inventory. In the future, the two trees for each accession may be left in the field for a longer time period.

The Black stem borer continues to be a concern. In addition, trees were lost 2 years ago due to the standing water in the hard-pan region of the orchard. Trees have been lost due to the Black stem borer as well as the standing water. All but 2 of these trees can be replaced by using cryopreserved materials. Lorsban is an effective insecticide for use against the black stem borer, but it can only be sprayed once per year. Infested trees must be removed.

Discussion: Thomas has proposed that some trees not be continually retrieved from cryopreservation and re-established in the field. If trees have been recovered and have 6 previous inventories, he proposes that those trees not be replanted. They may be particularly susceptible to pest/environmental pressures in Geneva. Alternatively, materials may be multiplied, and additional budwood be placed into cryo for primary inventory storage.

Irrigation was added to the Malus nursery plot this past year, because it was so dry.

Securing the Collection (Volk)

As part of the NLGRP-PGPRU project plan, we will assess the security of each Malus inventory in collaboration with Thomas, particularly those in the W3 orchard. We will develop plans to secure valuable accessions either by way of cryopreservation, seed back-ups, or pollen storage. Pollen/anther cryostorage research is underway for Prunus. This could easily be implemented for wild Malus accessions that produce flowers. The assessment will be discussed at a future CGC meeting.

Updates on ongoing research collaborations involving NPGS Apple Collection (insufficient time for updates).

- Malus sieversii from Kyrgyz Republic: (2015 CGC funded project, Miller)
- Malus sieversii in Arkansas (2016 CGC funded project, Garcia & Rom)
- Rootstock breeding program (Fazio)
- Fruit breeding programs (various)
- Cornell apple genomics (Xu)
- Appalachian Fruit Research Laboratory (Norelli and Wisniewski)
- RosBreed 2 (Peace)
- Hard cider varieties (Peck)
- Additional participants/projects

Comments: This teleconference focused exclusively on the NPGS apple collection/curator report and was limited to two hours. The next CGC meeting/teleconference will include National Program Staff, Quarantine, and DBMU reports as well as updates on research collaborations.

REPORT for APPLE CGC February 28, 2017 Teleconference Meeting

Plant Genetic Resources Unit, USDA-ARS Geneva, NY 14456

Prepared by C. Thomas Chao, Horticulturist/Curator Email: c.thomas.chao@ars.usda.gov

Recent Acquisitions

Since the previous Apple CGC report (9-21-2016), we have received 3 M. angustifolia seed accessions from Missouri, 1 M. angustifolia seed accession from Kentucky through Arnold Arboretum, 3 M. fusca seed accessions from Washington, budwood of 'Hauer Pippin', a M. x domestica and popular hard cider variety from California, and a M. halliana (PI38231) budwood from former USDA Chico Plant Introduction Station that rescued by Karen Williams and Gary Kinard of the USDA National Germplasm Resources Laboratory. This M. halliana accession was originally collected by Frank N. Meyer at Sianfu, Shensi (ShanXi), China on January 30, 1914, then sent to Chico Station on March 30, 1914. Following is the description of the accession by Frank Meyer according to the Plant Inventory Book: "A flowering crab apple, of low-branching, wide-spreading growth, said to bear masses of small double flowers of rosy red color. Scions obtained from the garden of the English Baptist Mission Hospital." Two plant exploration proposals titled "Plant Exploration in Taiwan to Collect Wild Malus, Pyrus, and Juglans Germplasm" and "Plant Exploration in Pennsylvania to Collect Malus coronaria Germplasm" should be funded by the National Germplasm Resources Laboratory, waiting for final budget decision. The exploration trip to Taiwan should take place in late August to early September 2017. The trip to Pennsylvania should take place in late September into early October 2017. We are expecting to receive budwood of crab apples from Arie Den Boer Arboretum, Iowa soon. We are also expecting M. fusca seed accessions from the University of British Columbia, Botanic Garden soon. Currently we have M. fusca seeds from Alaska, Washington, Oregon, and California, but none from British Columbia.

Cryo backup

We shipped 15 tart cherry accessions and 55 *Malus* accessions to Plant and Animal Genetic Resources Preservation (PAGRP), USDA-ARS, Fort Collins on 1-9-2017 for cryo backup storage. We received 66 *Malus* accessions that we cryo-preserved in 2016 from PAGRP on 2-15-2017 for viability testing. We are in the process of re-hydrating these cryo-treated budwood before budding.

Personnel, Fiscal, and Operational Resources

Mr. Julian Koob joined the clonal field team in November 2016. We are also planning to hire a 6-months part time laboratory assistant to help with DNA isolation and biochemical characterization of fruit samples.

Publication since September 2016

Volk, G.M., A.D. Henk, P.L. Forsline, A.K. Szewc-McFadden, G. Fazio, H. Aldwinckle, and C.M. Richards. 2016. Seeds capture the diversity of genetic resource collections of *Malus sieversii* maintained in an orchard. Genet. Resour. Crop Evol. doi:10.1007/s10722-016-0450-8

Outreach Activities since September 17, 2016

- Tour of the *Malus* collection, book club from Rochester, Geneva, NY., September 23, 2016 (8 members).
- Tour of the *Malus* collection, "Sustainable living and learning" classes, Dr. Tom Drennen, Chair Economics and Environmental Studies, Hobart and William Smith College, Geneva, NY., September 29, 2016 (60 students plus 4 professors).
- Tour of *Malus* collection, Jenn Smith plus two, Executive Director, New York Cider Association, Geneva, NY., October 3, 2016.
- Tour of the *Malus* collection, John Bunker, apple guru, author, and heirloom apple expert, and Cammy Watts, Geneva, NY., October 3, 2016.
- Interviewed by U.S. Department of State/Kyrgyzstan TV about the *Malus* collection, Geneva, NY., October 4, 2016.
- Tour of the *Malus* collection, Robyn Mello, Philadelphia Orchard Project and Experimental Farm Network, Geneva, NY, October 24, 2016 (2 members).
- Tour of the *Malus* collection and discussion, writer Kerry Wilkins, Geneva, NY., November 17, 2016.
- Phone interview, DRAFT magazine, Kate Bernot, November 18, 2016.
- Tour of the *Malus* collection, Chinese Academic of Agricultural Science delegation, Geneva, NY., December 2, 2016 (17 members).

Future Malus germplasm exploration target

According to the Apple (Malus) genetic vulnerability state of 2015, several wild Malus species are currently absent or underrepresented in the *Malus* collection, therefore they are targets of future exploration and/or exchanges. These include species such as M. baoshanensis (China), M. chitralensis (Pakistan), M. crescimannoi (Italy, Sicily), M. doumeri (China, Taiwan, Laos, Vietnam), M. florentina (Turkey, Albania, Greece, Italy, Macedonia, Serbia), M. jinxianensis (China), M. leiocalyca (China), M. maerkangensis (China), M. muliensis (China), and M. spontanea (Japan). Current Chinese policy does not allow these wild Malus species to be exported outside China. Unless the status changed, it is not possible to collect these five species that only present in China. We currently have one accession of M. spontanea from Japan in the field and 9 seed accessions from 9 locations in Japan (through Arnold Arboretum in 2013), with a total of 1,039 seeds. M. spontanea might be highly apomictic and several M. spontanea trees at different locations in Japan might be apomictic in nature (personal communication). We need to confirm the apomictic origin of *M. spontanea* through genetic testing. No further exploration is needed as now. An exploration proposal for collecting wild Malus, Pyrus, and Jaglans in Taiwan is provisionally funded for fall 2017. The three target wild Malus species are M. doumeri Bois, M. koidzumi Chang, Tseng, Ou & Lu (a new Malus species, previously classified as M. doumeri in Southern Taiwan), and M. hupehensis (Pamp.) Rehder. The feasibility of exploration for these Malus species are listed in Table 1.

PGRU has the largest collection of the four North America wild *Malus* species, *M. angustifolia*, *M. coronaria*, *M. fusca*, and *M. ioensis*. These four wild *Malus* species are located in the U.S. and some area in Canada. PGRU should take on the responsibility of collecting and preserving these four wild *Malus* species in North America. These four wild *Malus* species also could offer new traits of interest, such as major fire blight resistance. However, we still have gaps in our collection of these four wild *Malus* species. For example, we have low or zero representative sample of *M. angustifolia* from Kentucky (1 seed accession), Tennessee, Missouri (3 seed accessions), Kansas, and Oklahoma. For *M. coronaria*, we do not have samples from Pennsylvania, Ohio, and West

Virginia. For *M. fusca*, we do not have sample from British Columbia, Canada. For *M. angustifolia*, *M. coronaria*, and *M. ioensis*, many of the seed accessions collected by E. Dickson over 25 years ago have very low seed count and they should be re-collected if possible. In the exploration of *M. angustifolia* in 2015 and 2016 in the Southern US, this species is observed facing threats, especially from urban development and extreme weather. PGRU also has the largest collection of *M. sieversii* from Central Asia, most were from Kazakhstan. If all possible, additional samples of *M. sieversii* should be collected from Kyrgyzstan, Tajikistan, and Uzbekistan, as all crop wild relatives (CWR) of all fruit germplasm are under threat in the region.

Please also remember that PGRU is also responsible for the collection of cold-hardy wild *Vitis* species from North America and collection trips are in planning in the next 5 years.

Malus germplasm collection related activities in the next 5-6 years

1. Complete 7 'Royal Gala' x *M. sieversii* populations (G1 block) trait characterization and QTL mapping.

PGRU maintains 7 F1 populations of 'Royal Gala' x *M. sieversii* which have been extensively used by the apple research community for various genetic and physiological studies. To better serve the community and provide a common resource, we are constructing a consensus genetic map as well as individual maps using GBS-based SNP markers. To make the map(s) useful to the RosBreed community, we have also genotyped one of the populations with the RosBreed 8k chips. In 2017, we will also collect the 2rd-year data of flowering time and some growth traits for potential QTL mapping of those traits. We will collaborate with Awais Khan of Cornell University to evaluate fire blight and scab resistance of some of these populations.

2. Continue cyro backup storage of *Malus* at Fort Collins.

We will continue the cryo back up storage of the *Malus* collection and (tart cherry collection) with PAGRP, USDA-ARS. It would take about 7 years to complete the cryo back up of current list of *Malus* accessions. Some wild *Malus* species have lower success rate going through the cryo process and large quantity of budwood might be needed to ensure availability of viable cryo treated buds.

3. Complete GBS marker for entire Malus collection.

We previously reported that we had genotyped most of the *M. x domestica* accessions and some of the wild *Malus* species accessions with GBS-based SNP markers. The coverage of the SNPs for many of the wild *Malus* species accessions is low due to a variety of reasons, including poor read alignment with the reference genome, shallow depth coverage, error rate of GBS, and polyploidy issues. With the decreased cost and increased capacity per GBS run, novel sample bulking strategies and improved bioinformatic tools, we should be able to generate GBS-based SNP profiles for wild species in a cost effective and efficient manner. Since 2012, we have added new *Malus* accessions in the collection and these collections need to be gentyped too. Comprehensive marker profiles for the entire *Malus* collection would help better to curate and characterized the collections (i.e. genetic comparison, tracking, identification, and other purposes).

4. Identify, update, and correct information relating to *Malus* collection (in GRIN-Global)(using SSR, GBS, and morphology characters).

SSR markers were used in the past to identify hybrids and duplicates in the *Malus* collection. The GBS markers provided additional polymorphic SNP markers for differentiate the *Malus* accessions. The GBS data, for example, able to identify possible species hybrids in *M. x domestica* accessions. However, genetics alone could not be 100% proof that an accessions was mis-identified. We like to confirm the mis-classification of these *Malus* accessions by examining their leaf morphology and fruit morphology in the future season, if we do not have those information available already. Once the morphology confirms the mis-classification, then we will correct the information in GRIN-GLOBAL database.

In fall 2015, six young *M. orientalis* trees (3 accessions, two trees each) recently propagated from the W3 block selection were observed to have different leaf morphology than other *M. orientalis*. Upon further comparison of the leaf and fruit morphology with the mother trees in W3 block, it also confirmed observation. It was possible that these 6 *M. orientalis* trees were not *M. orientalis*, and mistakes occurred in propagation of original 28 *M. orientalis* selections. In 2016, leaf DNA were isolated from these 3 accessions and from their parental trees in W3 block. The RosBREED 10K chip testing confirmed that these propagated trees had complete different genotypes than their parents. We will re-propagate these *M. orientalis* from W3 and remove those 6 trees in M7 block. This example shows that mistake could happen during the propagation process. Review of the propagation process and improved labeling/tracking system could enhance the accuracy of propagation.

5. Continue evaluation of seedlings in wild *Malus* seedling block (W3).

W3 block is a block of wild *Malus* seedling block includes *M. bhutanica*, *M. hupehensis*, *M. kansuensis*, *M. orientalis*, *M. prattii*, *M. sieboldii*, *M. sieversii*, *M. sylvestris*, *M. toringoides*, *M. transitoria*, and *M. zahaojiaoensis*. They were planted in 2002. SSR markers were used to study the all the *M. orientalis* and *M. hupehensis* in W3. GBS markers were used to study the *M. hupehensis* in W3 also. 28 *M. orientalis* seedlings were propagated and included in the permanent collection based on SSR profiles. Genetic marker should be apply to all other wild *Malus* species seedlings and identify individuals that could represent the maximum genetic diversity and propagate them into the permanent collection. Seedlings have been screened for fire blight and scab resistance. General fruit characterization of the seedlings should be carry out and identify those with larger fruit size and unique fruit quality.

6. Continue evaluation of Malus collection for potential hard cider production.

In 2016, we began the evaluation of the *Malus* collection for hard cider production. Greg Peck, Horticulture section, Cornell University and his Ph.D. student began collecting fruit from new Spanish cider apples and other accessions in the collection. PGRU also began tasting of the Malus collection, searching for accessions with high juice content, high acid, high tannin content, or combination of all characters. Test batches of cider using fruit of selected *Malus* accessions were made by Empire Cider company LLC. We are also looking for accessions could potentially be used as "single varietal cider" in hard cider making as some hard cider currently in the market using varieties like Baldwin, Gravenstein, Golden russet, Kingston

Black, Hidden Rose, and Newtown Pippin. These 'single varietal cider" varieties in general have high juice content, with certain acidity, high tannin content, and unique flavor.

7. Update Malus Vulnerability statement.

The latest version of Apple (*Malus*) Vulnerability Statement was updated and published in 2015. The revision and update of the statement will be part of the next cycle of project plan for the clonal collection. We will complete the revision and update the statement in 2019 and 2022. The update version of the Vulnerability Statement is now linked to the funding eligibility of CGC evaluation proposal, starting in 2017.

8. Best management practices for clonal germplasm collection.

Currently, there is a "Manual of Operation for Clonal Collection" dated October, 2014. This manual details the procedure we maintain, regenerate, backup, and distribute the clonal collection. We will update this manual by 2020 and rename it "Best Management Practices". All issues related to the *Malus* collection would be a major component in this update.

9. Collection of apomictic seeds of wild *Malus* species as another mean for germplasm preservation.

Bags were placed on several wild *Malus* species before the bloom in 2015 and seeds from the bagged branches were harvested and germinated. Leaf DNA of seedlings from several wild *Malus* species were tested using RosBreed 10K chip. The results showed identical genotypes between the seedlings and parents of some wild *Malus* species and confirmation of existence of apomixis in M. *hupehensis*, *M. coronaria*, and *M. sikkimensis*. More bags were placed on the same accessions and additional accessions in 2016, and the seeds were harvested and stored at -20°C freezer. The confirmation of apomixis and the use of bags would also us alternative method for germplasm preservation than preservation of the genotype in the field. We will expand the testing of apomixis in the wild *Malus* species, and collect apomictic seeds for germplasm preservation in the future.

10. Screening *Malus* collection for possible virus infection, and investigate method to improve the health of the collection.

Our *Malus* field collection is vulnerable to all sort of diseases including viruses. Potential new virus that could cause fast and higher mortality rate is also a concern. Virus could be spread much faster among trees and propagation materials. We are considering using deep sequencing approach to detect possible virus infection in the *Malus* collection. The NY apple growers also are considering future clean budwood regulation. Input and suggestion from Apple CGC on this issue is welcome.

11. Identify of polyploidy of unknown Malus accessions

We are considering the purchase of a flow cytometry machine that would allow us to determine the ploidy levels of unknown *Malus* accessions, new *Malus* accessions, and progenies. This machine would be used for the *Vitis* collection plus vegetable germplasm collections.

 ${\bf Table~1.}~{\it Malus}~{\bf germplasm~exploration/exchange~target, feasibility~and~timing.}$

Genus	Species	Location	Current holding	Feasibility	Timing or comment
Malus	baoshanensis	China	0	No	
Malus	chitralensis	Pakistan	0	Challenged	More feasible through exchange
Malus	crescimannoi	Italy, Sicily	0	Possible	2019 or 2020?
Malus	doumeri	China, Taiwan, Laos, Vietnam	1	Yes/Possible	Fall 2017 Taiwan; Vietnam 2018? Laos or Myanmar in the future
Malus	florentina	Turkey, Albania, Greece, Italy, Macedonia, Serbia	3	Possible	2019 or 2020 for Albania, Greece, Italy, Macedonia or Serbia
Malus	jinxianensis	China	0	No	
Malus	koidzumi	Taiwan	0	Yes	Fall 2017
Malus	leiocalyca	China	0	No	
Malus	maerkangensis	China	0	No	
Malus	muliensis	China	0	No	
Malus	spontanea	Japan	1 plus 9 seed accessions	Yes	Not needed as now
Malus	angustifolia	KS, KY, MO, OK, TN	Few or none	Yes	2019 or 2020
Malus	coronaria	OH, PA, WV	0	Yes	2017 for Pennsylvania; 2018 for Ohio; 2020 or 2021 for West Virginia?
Malus	fusca	BC, Canada	0	Yes	2022 in combination with trip to Alaska or through exchange with local arboreta or through more exchange
Malus	angustifolia, coronaria, ioensis	Eastern US (Dickson collection)	Many have low seed counts	Yes	2022-2025?
Malus	sieversii	Kyrgyzstan, Tajikistan, Uzbekistan	few	Possible	Joined exploration for CWR with other fruit crops are more practical
Malus	sieversii	China	0	No	