**Minutes**

**2018 Sugarcane Crop Germplasm Committee Meeting**

**Bonita Springs, FL**

**June 25, 2018**

In attendance:

Niranjan Baisakh (LSU AgCenter)

Yong-Bao Pan (USDA-ARS)

Jorge da Silva (Texas A&M)

James Todd (UF/USDA-ARS)

Wayne Davidson (FSCL)

Herman Waguespack (ASCL)

Collins Kimbeng (LSU AgCenter)

Charley Richard (C. Richard & Assoc.)

Mike Grisham (USDA-ARS)

Jeff Hoy (LSU)

Philippe Rott (UF)

Jim Shine (SCGC)

Hardev Sandhu (UF)

Chris LaBorde (USSC)

Michael Pontif (LSU AgCenter)

Dimitre Mollov (USDA-ARS)

Martha Malapi Wright (USDA-APHIS) Jianping Wang (UF)

Lifang Qin (USDA, Canal Point) Sushma Sood (USDA, Canal Point)

Claudia Kaye (US Sugar) Duli Zhao (USDA, Canal Point)

Alexander Sanchez (USDA-ARS) Himaya Mula-Michael (USDA, Houma)

Anna Hale (USDA, Houma) Michael Winterstein (USDA, Miami)

The meeting was chaired by Dr. Anna Hale. Meeting was called to order at 9:06 AM.

* Introductions, passed out sign in sheet (See members in attendance above)
* Claudia motioned, Collins seconded approval of 2017 CGC minutes. Motion carried.

**USDA-ARS NGRL 2017 Report presented by Dimitre Mollov**

* Gary Kinard was absent. Group informed about a three day workshop. Tomas (the formal curator of the World Collection of Sugarcane Germplasm at Miami, FL) was in attendance. Tomas now works with Banana and Bamboo and seems to have recovered from Hurricane Maria.
* NGRL has three units and the presenter (Dimitre) is involved with the Plant Disease Unit and not as involved with the other two units.
* John Wilson retired and now works for the Smithsonian.
* A lot of retired people not being replaced.
* Anna wanted to know if anyone had dialed in by phone. There was no response.

**Report on the Status of the World Collection of Sugarcane Germplasm presented by Mike Winterstein**

* A total of 1400 accessions planted in a 10 acre block and regenerated every three years.
* Peter Bretting called in.
* Staffing situation: 4 staff with 2 vacant positions. Hiring freeze still in place.
* Good plan to have ArcGis map coordinates of where germplasm are located as this will be good for incoming staff especially with current lack of full staff.
* Program at Fort Collins was terminated.
* Good idea to increase resources at a new site with lower disturbance.
* Good to have a backup collection with a core collection of about 300 genotypes.
* Collaborating with Angelique D’Hont (CIRAD, France) by supplying lyophilized leaf samples.
* Good to have local Data Base web application.
* There was a lot of discussion on the Crop Vulnerability Status which continued after the NPL report.
* Jianping asked about the core collection work James did in 2012 which removed redundant accessions and ended up with about 1000 accessions
* James: a core collection exist in Canal Point.
* James was asked how the accessions were being (will be) backed up. He said by cuttings.
* James highlighted problems with having *S. Spontaneum* at Canal Point.
* Duli: a permit is expected to be in place within three months.
* Jorge wanted to know how many *S. Spontaneum* clones they were talking about and if Canal Point has the resources to support this.
* Phillip asked if it was not too risky to have both the World Collection and the Core Collection in the same state.
* Jorge offered Texas as an alternative location and informed the group that Texas has concrete slab in place.
* Jeff said the lack of resources was more important than the location
* Jianping: one in LA and one in Texas will make sense
* Niranjan: why not replicate the Whole Collection in Puerto Rico?
* Charlie: lack of resources is a bigger problem. How will we take care of two when we cannot take care of one?
* Chris: split the responsibility between Texas and Canal Point.
* Jorge: Texas has infrastructure but no labor.
* Someone wanted to know how it is being backed up. J
* Niranjan working with a collection of 1227 after removing redundant accessions and adding 179 accessions from Houma. Also added some LA clones and ended up with 1400 clones.
* Jiaping asked about the collaboration with Angelique (CIRAD, France)
* Someone responded that they want access. Leaf sample is being provided for DNA analysis.
* Niranjan: Angelique has funding or is seeking funding to work on Energy Cane. Ray Ming has funding too.
* Peter was still available on the phone.

**NPL Report presented by Peter Bretting**

* Remote presentation
* Slide 1 showed location of gene banks.
* Slide 2: partnerships with universities.
* Slide 3: increase in samples over last couple of years. Most of the increase came from filling gaps in the existing collection.
* Slide 4: on average, about 250,000 accessions are distributed per year. 2/3 with in the US and 1/3 internationally. Within the US, 2/3 are requested by public institutions with faculty at universities being the largest single users. The remaining 1/3, requested by a broad section of users and varies with crop.
* Slide 5: The budget is in red. Peaked in 201, 2011 and 2012. Still not recovered from the 2013 government shut down and sequestration and hovering around 44 million US dollars for all the programs.
* Slide 6: the dollar will buy less in today’s market compared to 2012
* Slide 7: Demand for samples has increased from 125,000 to 250,000. The capacity to meet these challenges has been exceeded and can only be managed by setting priorities.
* One third (30-36%) of staff will retire in the next 5 years.
* Issues that will affect the sugarcane community: No personnel to continue work on In Vitro methods. A breakthrough is needed. GxE issues, cryproservation methods need to be revamped.
* Slide 8: Collins took a restroom break.
* Slide 9: Personnel changes, need to hire additional staff
* Slide 10: Need for program to train new PGR managers as 1/3 of managers likely to retire within 5 years. USDA/NIFA Grant secured for a workshop at Fort Collins in April 2018 which discussed among other things designing and developing a PGR training program to be delivered through distance learning.
* Jeff asked about the NPL’s priority to USDA? How do we fund the backup collection? The Miami location will be closed under the current budget so it might be prudent to look for a backup site.
* Martha: if moved to Puerto Rico as suggested earlier accessions would have to go through quarantine and APHIS cannot handle a large number of imports at this time.

**Sugarcane Importation and Quarantine-Related Activities presented by Martha Malapi-Wright**

* Works with bamboo, and grasses including sugarcane and rice.
* Facilitates safe movement of propagative plant parts.
* Cost about $3 - $4000 per test but no fee is charged to stake holders. Others do charge a fee. No fee is charged to avoid the need for smuggling but smuggling still occurs.
* Organizational chart: Included Poaceae, Vegetables, Pomes (Fruit trees), Prunus (Ornamental plants)
* Two and a half (2 ½) labs. For all 4 programs.
* Molecular lab., tissue culture lab, next gen seq. lab.
* Over 100 sugarcane clones in program. Over 60% were infected with a pathogen, mostly (53%) with the sugarcane yellow leaf virus (ScYLV).
* Infected plants not discarded. Sent to tissue culture lab. for cleanup. Plants cleaned after two growth cycles which takes two years. Plants retested again to make sure they are disease free.
* Up to 22 tests (for viruses and bacteria) are run on each clone and all tests are run twice at a cost of about $3 to $4000. Some pathogen show up in fall not spring and vice versa.
* Let her know if you don’t want a clone you imported so she can prioritize.
* Use several methods. RTPCR, ELIZA, PCR, sequencing etc.
* With Next Gen. Seq. new tests cost $1000. No individual testing for specific pathogens. Millions of fragments of DNA sequenced in parallel. ID multiple pathogens at the same time. Takes 5 days to run tests.
* Developing new diagnostic techniques with Next Gen Seq. so will be able to shorten testing time. Working with several different countries. No country has yet to accept Next Gen Seq. but we don’t want to be left behind.
* Phillip: how many reads are needed for RNA viruses – 25 million reads, DNA viruses – 1 to 5 million reads. 10 million for bacilliform.
* Humans need 90% coverage so at least 90 % coverage will be needed for sugarcane.
* To validate viral identification positive control is sequenced multiple times. Up to 15 good positive controls are sequences throughout the year.
* FDA requires a positive control for every single run.
* The validation process includes sequencing and data analysis with testing done using different concentrations.
* Issues affecting the NGS-Poaceae Program:
* One hundred twenty-two clones quarantined in last 12 months. Up to 50% with ScYLV as identified with Next Gen Seq.
* 8-10 genotypes (isolates?) of ScYLV reported. Tough to ID because of recombination. Not all 8-10 genotypes were reported in the US. Whole genome sequencing ongoing to ID all 8-10 genotypes.
* Samples preserved in freezer since 2012. All show heat map for ScYLV with high rates of ScYLV on clones from Brazil, Argentina, China (2), Peru (9), and Guatemala.
* New virus from *Miscantus sinensis* clone imported 2 years ago. Closely related to YLS virus.
* Letter of support will be helpful in getting more funding for this work.
* James: if you find a virus but you don’t know it is a pathogen then what? Martha: we have no standard procedure for that scenario. But likely if it is not a known plant pathogen we will not replicate the test. In the case of a new plant pathogen the plant was showing symptoms.
* Jeff: 3-4 viruses of unknown origin? Martha these turned out to be human viruses.
* Dimitris: 23% may be sugarcane proteins.
* Martha: the software analyzes for everything single type of virus and it takes 5 days and it is reevaluated by a biometrician.
* Duli: if imported varieties are 60% infected with ScYLV, do our varieties (FL, LA, TX) also have a 60% infection rate?
* Phillip: referred to a paper in Plant Disease published in either 2014 or 2015 showing 75% of samples with YLS with a wide range of genotypes of the virus.
* Martha: but clones are always cleaned up before release.
* Phillip: the Cuban genotype of YLS is here in FL. In FL the Cuban genotype is more important and is present in over 50% of infected sugarcane clones. Two majoy groups of YLS genotypes are from Brazil and Cuba.
* What is found in India maybe a variation of both.
* Dimitris: it is important to note that since testing started no new strains have come from outside.
* Martha: APHIS releases clean material what happens after that is out of their control.
* Phillip: Be careful with Next Gen. Seq. because sometime the technique will detect one genotype but not the other when both might be present.
* Martha: No intention to completely replace all testing with Next gen. seq. Backup testing using the old method is still going on.

**Nomination of Committee Members**

* New members were nominated and ‘old’ members removed from the committee
* Steve is in Australia.
* Tomas is moving to Puerto Rico.
* Swap Mike with Chris.
* Ben Legendre?
* Per McCord?
* What do we want to do with Hawaii?
* Anna: Leave Jack. Jack will represent Rio Farms.
* Gail?
* Replace Jack (Canal Point). Keep Per’s vacant.
* Chris replaces Mike Irey.
* Leave Susan.
* Jack replaces Andy Scott.
* Martha replaces Clarissa.
* Jeff replaces Ben Legendre.
* Hold Tomas’s spot open.
* No replacement for Steve
* Duly replaces Jack.

Nominations for committee members from the 2018 meeting were motioned by Jim Shine, Seconded by Jeff Hoy. Motion carried.

**Sugarcane Streak Mosaic Virus presented by Dimitri Mollov**

* Has presented on this subject before.
* This project was initiated after last year’s meeting.
* Anna found symptoms on some samples. Sent samples to Dimitri and they turned out to be SSMV.
* Different family from sugarcane and sorghum mosaic virus so we need to use different approaches.
* Primers from India did not work.
* Using gene bank information, 4 sets of primers detected the virus in these sugarcane clones.
* The good news is only 2 out of 14 clones from Thailand were infected.
* Clones were sent to both Fort Pierce and Houma all the clones sent to Fort Pierce died.
* The positive clones have been removed from the carts. The two clones were sent to Martha for cleanup.
* Twenty years ago all testing came out to be negative.
* Martha: the symptoms are very obvious.
* Phillip: was Next Gen. Seq. used? Did you find any others? Martha: No. But sometimes two viruses combine to give symptoms.
* Dimitri is sure no other viruses were involved.
* Phillip: maybe some viruses show no symptoms because titre is low and the environment promotes another one.
* Phillip: low titre may preclude one from detecting a virus so one cannot confidently exclude the presence of a virus. Sorghum plants infected with SMV show no symptoms.
* Mike: old streak mosaic virus primers did not work on a collection of ornamental Miscantus that obviously had the symptoms.
* James: since strains slipped through quarantine, will it be worthwhile to check other accessions brought in over the years (probably before testing was perfected)?
* Anna: among the MPTH clones that were sent back to FL half were not sent because of the mosaic (probably died from other causes).
* Dimitri: willing to share the protocol if anyone is interested in testing/using it or simply send me the plant of concern and he can test it for you.
* Dimitri: Cathy sampled/tested progeny of infected clones and they turned out negative.
* Phillip: what is the natural vector? Dimitri: unknown.
* Dimitir: Thompson took a few leaves back to Australia. He found it to be widespread in Thailand and virtually every sample from Pakistan has it. Maybe they have an effective vector.
* Dimitri: Isolated cases reported in China and Japan.
* A related virus Triticum **mosaic virus** (TriMV) is transmitted by mites.
* At this point we know what it is not but not what it is.
* South East Asia has a lot of streak mosaic viruses.
* Dimitri will continue to improve protocol and Martha is happy to share primers.

**Open Discussion on crop Vulnerability report on Sugarcane**

* This issue had previously been discussed at length.
* Anna: are the collections in Hawaii and Texas A and M still there?
* Peter: talked about cryopreservation. Would like to identify gaps so we know where the emphasis lie.
* Mike: was able to grow out plants from In Vitro technique so it can be done (cryopreservation).
* The person doing the work left and has not been replaced. It was being funded by EMBRAPA.
* Martha: interested in visiting the issue if funding provided or available.
* Anna: urged members to look at the report and send her updates on where we are with cryopreservation.
* Anna: Is David Kuhn coming back? Mike: he retired.

**Open Discussion**

* Niranjan: 90% coverage what is the depth of coverage? Martha: when you don’t detect anything? When sequencing RNA million reads/ sample 75 single. We refer to FDA guidelines.
* Phillip: sequencing is important but amplification is also important as one virus can be amplified over the others. So depth is important. Random is not as random as you think.
* Martha: yes it depends on the replication rate of that virus because the titre could be so high that it contaminates the next round relative to other viruses.
* Jiaping: If it has disease why clean it up? (probably asking doesn’t this mean the clone is susceptible?).
* Collins: maybe a different strain that we are not concerned about and besides the clone may be important for other traits.
* Mike: the variability is higher in Fl. We picked up sorghum mosaic virus strain E on 2086.

Jim shine motioned, Claudia seconded to adjourn the meeting. Motion carried.

Meeting was adjourned by the Chair Dr. Anna Hale at 12:05 PM.