

**S-009 Regional Technical Advisory Committee  
Minutes of Annual Meeting, July 27, 2010**

**Ramada Geneva Lakefront  
Geneva, NY**

***Members present:***

Florida (University of Florida).....Ann Blount ([paspalum@ufl.edu](mailto:paspalum@ufl.edu))  
for Kevin Kenworthy  
Guam (University of Guam) .....Mari Marutani ([marutani@uguam.uog.edu](mailto:marutani@uguam.uog.edu))  
Kentucky (University of Kentucky).....Tim Phillips ([tphillip@uky.edu](mailto:tphillip@uky.edu))  
North Carolina (North Carolina State University).....Tom Stalker ([tom\\_stalker@ncsu.edu](mailto:tom_stalker@ncsu.edu))  
Oklahoma (Oklahoma State University).....Yanqi Wu ([yanqi.wu@okstate.edu](mailto:yanqi.wu@okstate.edu))  
South Carolina (Clemson University).....Emerson Shipe ([eshipe@clemson.edu](mailto:eshipe@clemson.edu))  
Southern Association of Agric. Exp. Sta. Directors ..Gerald Arkin ([garkin@uga.edu](mailto:garkin@uga.edu))

***USDA, ARS, PGRCU representatives present:***

PGRCU, Griffin, GA .....Gary Pederson ([gary.pederson@ars.usda.gov](mailto:gary.pederson@ars.usda.gov))  
PGRCU, Griffin, GA .....Noelle Barkley ([elle.barkley@ars.usda.gov](mailto:elle.barkley@ars.usda.gov))

***Others present:***

USDA-ARS, College Station, TX .....James Frelichowski  
([james.frelichowski@ars.usda.gov](mailto:james.frelichowski@ars.usda.gov))  
USDA-ARS, Beltsville, MD.....Peter Bretting ([peter.bretting@ars.usda.gov](mailto:peter.bretting@ars.usda.gov))  
USDA-NIFA, Washington, DC .....Ann Marie Thro ([athro@nifa.usda.gov](mailto:athro@nifa.usda.gov))

The meeting was called to order at the Ramada Geneva Lakefront Hotel at 1:00 pm on July 27, 2010 by Tim Phillips, Chair. Those present introduced themselves. Due to weather problems and flight delays, Don LaBonte (LA), Paul Raymer (GA), and Tom Zimmerman (Virgin Islands) were unable to make it to Geneva in time to attend the S-009 meeting. Ann Blount agreed to serve as acting S-009 Secretary and take minutes for Paul Raymer.

Dr. Gerald Arkin, the administrative advisor for the S-009 RTAC, welcomed the group to the S-009 meeting in Geneva. He gave an overview about the role of the CGCs, citing their responsibilities. He stressed the need to report back to the state administrators about the PI collections and GRIN if there are any short comings or needs. He mentioned the importance of letting clientele know where plant germplasm comes from and inform the public more about the role of NPGS in our presentations and writings.

The minutes from the 2009 meeting were approved as previously circulated and posted on the website. There were no changes to the agenda. Two new Crop Germplasm Committees (CGC), Medicinal Plants CGC and Specialty Nut Crops CGC, have been created and are being organized.

Ann Marie Thro gave a NIFA update and stressed that we remember to recognize and cite that Hatch funding was used to support various research projects. Hatch funding support should be mentioned in any relevant CRIS reporting, resulting publications, annual reports, and presentations. Also it should be

reported in plant registration articles in the Journal of Plant Registration and Journal of Horticulture Science where Hatch funding supported the development of cultivars or germplasm. Hatch funding will be flat this next year. AFRI funding is up 20% and genetic resources will be up under AFRI. The only way to get Hatch funding increased is to promote the fact that Hatch funds directly support our programs. We need to ask the editors of the various journals to remind authors to mention Hatch funding support where applicable. Ken Quesenberry might be willing to contact the CSSA editorial chair and suggest this to authors.

Ann Marie also mentioned CRIS reporting, particularly the outcome and impact sections. The “non-technical summary” is a mini publicity effort and needs to be taken more seriously; especially noting how the research involves other researchers and how the outcome impacts clientele/farmers. Soon reporting will be done through “REReport” within a two year period.

Gary Pederson discussed the Plant Genetic Resources Conservation Unit accomplishments (Appendix 1) over the past year. This included a general discussion about what is housed at the Griffin, GA location. There was some discussion of seed storage at -18 C and a building addition to the ARS Seed Processing building that will house a new 4 C cold room for seed storage at Griffin. The addition of this cold storage room will enable previous 4 C cold storage to become -18 C storage enabling bulk seed of the entire Griffin collection to be housed at -18 C. Gary also reiterated Ann Marie Thro’s concern about mentioning the Hatch funding that we use to support our research and also that we should recognize NPGS and its impact on our research. More specifically, we should indicate in publications and presentations what percent (%) NPGS germplasm contributed to the development of any new variety.

Gary discussed changes in curation of collections located at Griffin. John Erpelding, sorghum curator at Mayaguez, Puerto Rico, has taken a new position at Stoneville, MS. Currently, Gary is serving as the acting sorghum curator. He also reported that Roy Pittman will become the Vigna curator as soon as a new peanut curator is hired. The plan is to complete the search and selection of a new peanut curator shortly after October 1, 2010. There was discussion about the amount of accessions that had low germination rates and how to better report that information at S-009 and CGC meetings. Tom Stalker encouraged more reporting of regeneration plans from the curators, especially regeneration plans from the new peanut curator. Gary noted that he will have the curators present their regeneration plans to the S-009 committee at the meeting next year in Griffin.

Noelle Barkley reported on “Detection of sweetpotato leaf curl virus (SPLCV) in the U.S. germplasm collection via real-time PCR”(Appendix 2). Mari Marutani asked how difficult the assay was to run and discussed the possibility of learning how to run the assay during the next S-009 meeting in Griffin. She would like to have a better understanding of the viral status of sweetpotatoes in Guam.

Peter Bretting discussed GRIN Global and stated that it is 90% accomplished. He stated that Griffin will have a modest increase in its budget. Overall budget increases of \$40 million dollar will mostly go into salaries, cost of living increases, and STEP increases across the agency. Also, the Foreign Affairs committee has to approve the germplasm treaty and there is still a hold up.

State reports for Florida, Guam, Kentucky, North Carolina, Oklahoma, and South Carolina were presented orally (written reports at the S-009 website). Written reports were distributed for Alabama,

Georgia, Hawaii, Louisiana, Texas, and Virginia. James Frelichowski reported on the status of the cotton germplasm collection at College Station, TX.

The 2011 S-009 meeting will be held in Griffin, GA, on August 2-3, 2011 starting at 1:00pm on Tuesday, August 2<sup>nd</sup>. The incoming S-009 Chair will be Paul Raymer. Kevin Kenworthy was nominated to be the incoming secretary. There were no other nominations and Kevin was elected. S-009 members were encouraged to attend the joint RTAC/CGC Chairs/PGOC session tomorrow morning and the tour at Geneva in the afternoon.

The meeting was adjourned at 5:00pm.

Ann Blount  
Acting S-009 Secretary

Appendix 1

DR. GARY PEDERSON

PLANT GENETIC RESOURCES:  
CURRENT STATUS

# Plant Genetic Resources: Current Status

Gary A. Pederson

USDA, ARS, Plant Genetic Resources  
Conservation Unit

Griffin, GA

# Outline

- PGRCU mission
- Current status of each crop
- Progress made
  - Distributions
  - Funding
  - Staffing
  - Equipment and facilities
- Needs

# What is the mission of PGRCU?

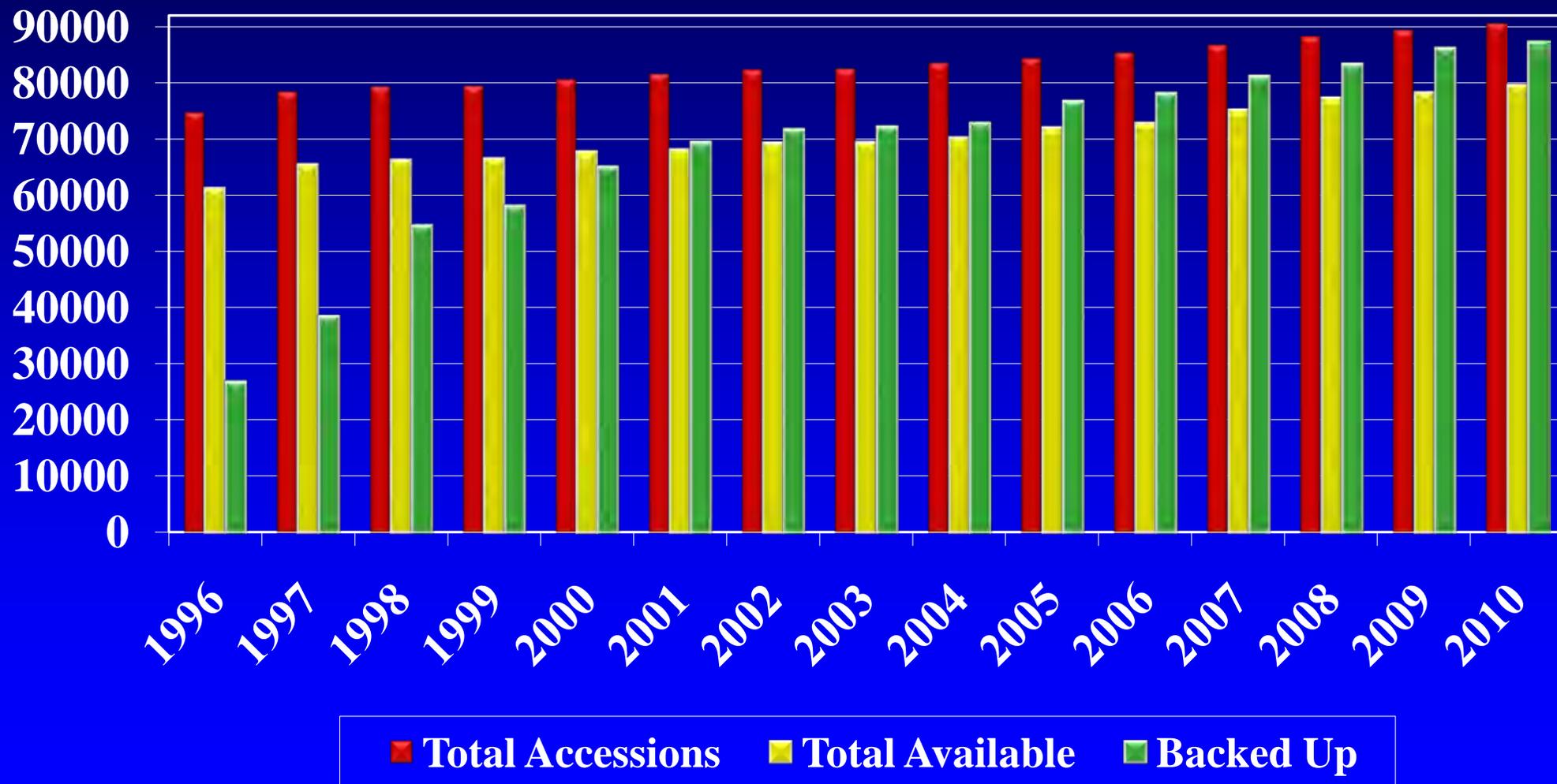
- Plant Genetic Resources Conservation Unit (PGRCU) exists to conserve plant genetic resources for users today and for future generations.
- Mission: "acquire, characterize, maintain, evaluate, document, and distribute plant genetic resources".
- This is what users of the genetic resources maintained at Griffin expect from the Unit.

# PGRCU Collection - June 2010

- Total Accessions
  - 90,668
- Total Available
  - 79,684 (87.9%)
- Backed Up
  - 87,355 (96.3%)

Acknowledgement: Merrelyn Spinks and Lee Ann Chalkley, PGRCU, compiled and summarized all numbers shown in this presentation.

# PGRCU Collection 1996 - 2010



# Vigna

<b>CURATOR</b>	<b>CROP</b>	<b>TOTAL ACCESSIONS</b>	<b>TOTAL AVAILABLE</b>	<b>NUMBER BACKED UP</b>	<b>ITEMS SHIPPED IN 2009</b>
<b>Gary Pederson</b>	Cowpea	8,043	6,404	8,019	596
	Mung bean	4,203	3,822	4,203	15
	Other Vigna spp.	599	352	452	157

# Vegetable Crops & Sweetpotato

CURATOR	CROP	TOTAL ACCESSIONS	TOTAL AVAILABLE	NUMBER BACKED UP	ITEMS SHIPPED IN 2009
<b>Bob Jarret</b>	Cucurbits	2,065	911	1,889	1,316
	Eggplant	1,018	915	1,015	507
	Okra	2,971	1,548	2,935	243
	Peppers	4,699	4,589	4,694	<b>8,913</b>
	Sweetpotato - tissue culture	754	711	667	<b>1,383</b>
	Other Ipomoea spp.	469	210	410	142
	Watermelon	1,898	1,649	1,898	1,646

# Legumes and Misc. Crops

<b>CURATOR</b>	<b>CROP</b>	<b>TOTAL ACCESSIONS</b>	<b>TOTAL AVAILABLE</b>	<b>NUMBER BACKED UP</b>	<b>ITEMS SHIPPED IN 2009</b>
<b>Brad Morris</b>	Castor bean	374	179	373	<b>1,021</b>
	Hibiscus	340	288	338	62
	Legumes	3,647	2,710	3,481	<b>1,635</b>
	Miscellaneous	136	100	130	79
	Sesame	1,216	1,195	1,216	128

# Warm-Season Grasses

<b>CURATOR</b>	<b>CROP</b>	<b>TOTAL ACCESSIONS</b>	<b>TOTAL AVAILABLE</b>	<b>NUMBER BACKED UP</b>	<b>ITEMS SHIPPED IN 2009</b>
<b>Melanie Harrison- Dunn</b>	Bamboo	98	98	2	3
	Grasses	<b>7,221</b>	6,125	6,882	1,499
	Pearl millet	1,319	1,272	<b>1,319</b>	1,579

# Clover and Sorghum

<b>CURATOR</b>	<b>CROP</b>	<b>TOTAL ACCESSIONS</b>	<b>TOTAL AVAILABLE</b>	<b>NUMBER BACKED UP</b>	<b>ITEMS SHIPPED IN 2009</b>
<b>Gary Pederson</b>	Annual Clover	2,218	1,772	2,156	463
	Sorghum	<b>37,252</b>	36,062	35,923	<b>17,566</b>

# Peanuts

<b>CURATOR</b>	<b>CROP</b>	<b>TOTAL ACCESSIONS</b>	<b>TOTAL AVAILABLE</b>	<b>NUMBER BACKED UP</b>	<b>ITEMS SHIPPED IN 2009</b>
<b>Roy Pittman</b>	Cultivated Peanuts	9,306	8,083	9,009	784
	Wild Peanuts	815(?)	689(?)	378	487

# Number (and %) of accessions that are unavailable

<b>Crop</b>	<b>2009</b>	<b>2010</b>	<b>Crop</b>	<b>2009</b>	<b>2010</b>
Cowpea	1,740 (22%)	1,639 (20%)	Hibiscus	55 (16%)	52 (15%)
Mung bean	381 ( 9%)	381 ( 9%)	Legumes	947 (26%)	807 (26%)
Cucurbits	816 (58%)	848 (60%)	Sesame	21 ( 2%)	21 ( 2%)
Eggplant	101 (10%)	103 (10%)	Grasses	986 (14%)	1,096 (15%)
Okra	1,421 (48%)	1,423 (48%)	Pearl millet	271 (21%)	47 ( 4%)
Peppers	101 ( 2%)	110 ( 2%)	Annual clover	386 (18%)	446 (20%)
Sweetpotato (TC)	40 ( 5%)	43 ( 6%)	Sorghum	1,191 ( 3%)	1,197 ( 3%)
Watermelon	232 (12%)	249 (13%)	Cultivated peanuts	1,223 (13%)	1,223 (13%)
Castor bean	182 (49%)	195 (52%)	Wild peanuts	125 (16%)	126 (15%)
			<b>TOTAL</b>	11,102 (12%)	10,984 (12%)

# Digital photos

Crop	Images
Sorghum	7,457
Pepper	1,806
Cowpea	1,699
Watermelon	1,484
Grasses	549
Peanuts	549
Pearl millet	445
Cucurbits	285
Other crops	441
<b>Total</b>	<b>14,715 (16.2%)</b>

# Germination Testing

(Accessions with seed only, tested since 2002)

Crop	Accessions	%	Crop	Accessions	%
Sorghum	27,582	74.0	Okra	1,756	59.1
Peppers	4,650	99.0	Watermelon	1,744	91.9
Cowpea	4,461	55.5	Grasses	1,622	22.4
Mung bean	3,899	92.8	Pearl millet	1,297	98.3
Peanuts	3,798	40.8	Sesame	1,211	99.6
Legumes	2,357	76.6	Eggplant	937	92.0
Clover	1,858	83.8	Cucurbits	655	47.1
			<b>TOTAL</b>	<b>60,207</b>	<b>67.5</b>

# Accessions in -18 C storage

(Accessions with seeds only, seed splitting initiated in 2001)

Crop	Accessions	%	Crop	Accessions	%
Sorghum	26,288	70.6	Watermelon	1,898	100.0
Grasses	6,919	100.0	Cucurbits	1,399	99.1
Peppers	4,690	99.8	Sesame	1,216	100.0
Mung bean	4,045	96.2	Eggplant	1,018	100.0
Legumes	3,074	100.0	Cowpea	838	10.4
Okra	2,971	100.0	Gourds	487	99.4
Peanuts	2,681	28.8	Ipomoea spp.	456	97.2
Clover	2,139	96.4	<b>TOTAL</b>	<b>62,524</b>	<b>70.1</b>

# Requested for regeneration in CY2009

<b>Crop</b>	<b># accessions</b>	<b>Crop</b>	<b># accessions</b>
Cowpea	142	Grasses	185
Sorghum	1,557	Legumes	166
Cucurbit	44	Wingbean	28
Clovers	131	Other Vigna	39
Pearl millet	6	Cult peanut	478
Peppers	68	Wild peanut	10
Sesame	13	Hibiscus	30
Ipomoea sp.	2	Castor bean	11
Watermelon	7	Misc crops	46

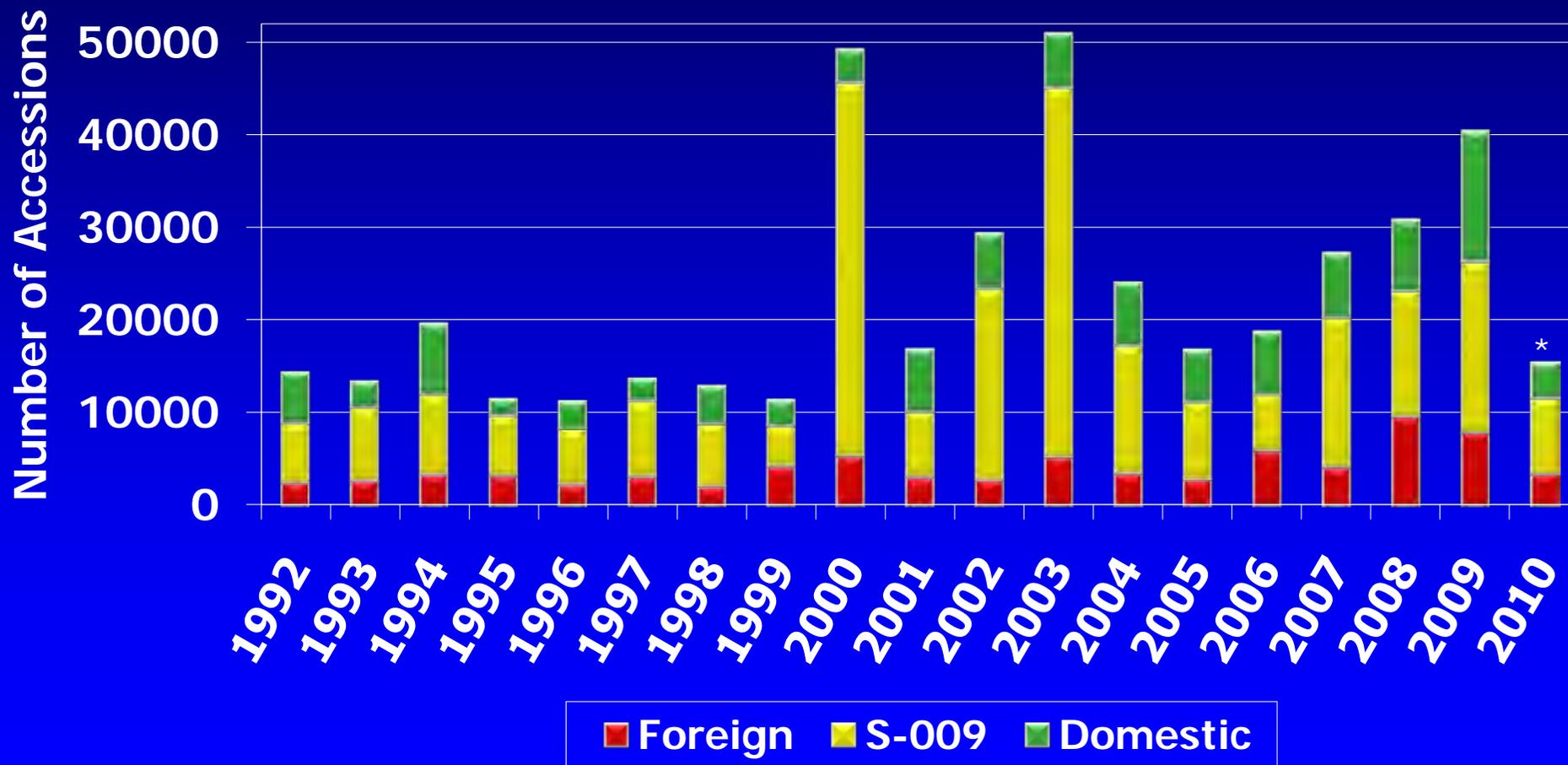
# Regenerations by others in CY2009

- 52 cowpea, 4 cucurbits, 4 watermelon accessions
  - Ricardo Goenaga, USDA, ARS, Puerto Rico
- 20 wing bean, 13 Leucaena accessions
  - Adolfo Quiles, USDA, ARS, St. Croix
- 15 pepper, 9 cucurbit, 1 watermelon accessions
  - Jerry Serimian, USDA, ARS, Parlier, CA

# Distributions in CY2009

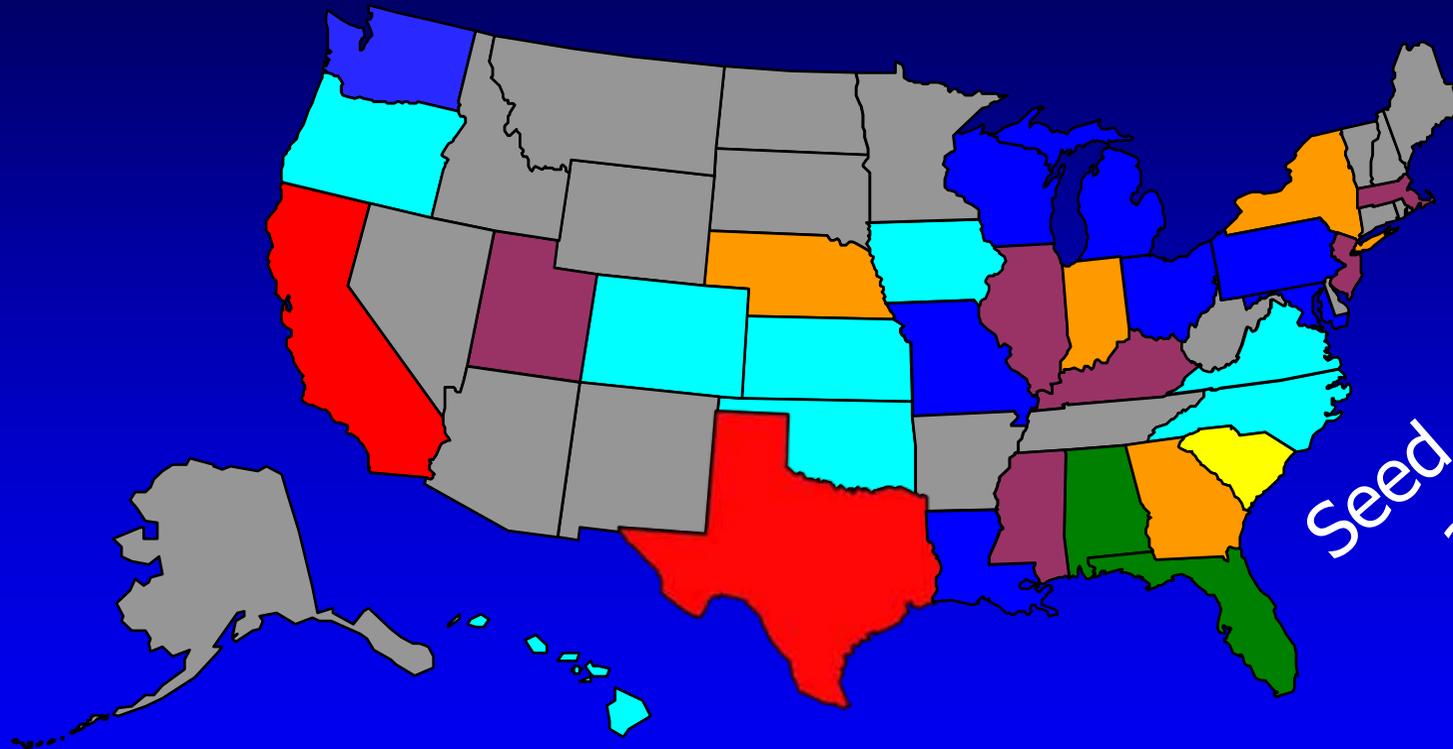
- Domestic = 32,582 items in 735 orders
  - S-9 region = 18,442 items
- Foreign = 7,867 items in 164 orders
- Total CY2009 distributions = 40,449 items

# Distributions



\* As of July 15, 2010

# Domestic Distributions in CY2009



Seed distributed to all 50 States!

Total number of accessions by state



# Distributions outside of 50 U.S. states in CY2009

<b>Argentina</b>	<b>Denmark</b>	<b>Italy</b>	<b>Puerto Rico</b>	<b>Thailand</b>
<b>Australia</b>	<b>Egypt</b>	<b>Japan</b>	<b>Singapore</b>	<b>Turkey</b>
<b>Belarus</b>	<b>Finland</b>	<b>Mexico</b>	<b>South Africa</b>	<b>Ukraine</b>
<b>Bolivia</b>	<b>France</b>	<b>Netherlands</b>	<b>South Korea</b>	<b>United Kingdom</b>
<b>Brazil</b>	<b>Germany</b>	<b>Niger</b>	<b>Spain</b>	<b>Uruguay</b>
<b>Canada</b>	<b>Greece</b>	<b>Peru</b>	<b>Sweden</b>	<b>Virgin Islands</b>
<b>China</b>	<b>India</b>	<b>Poland</b>	<b>Switzerland</b>	
<b>Czech Rep</b>	<b>Israel</b>	<b>Portugal</b>	<b>Taiwan</b>	

# Total PGRCU Funding

- ARS base funding
  - FY2010 = \$2,315,670
- S-009 base funding
  - FY2010 = \$413,298

# PGRCU Funding

- ARS base funding
  - FY2010 = received Pay Act increase
- ARS temporary funding increases
  - FY2009 = \$9,500 (vacuum evaporator)
  - FY2009 = \$25,600 (real time PCR upgrade)

# Staffing - ARS

- 20 ARS full-time employees
- Richard Crawford moved to ARS Unit in Charleston, SC and position terminated
  - Agricultural Research Technician (farm manager)
    - Currently S-009 state employee (Donnie Hice) serving as farm manager

# Staffing – ARS curator changes

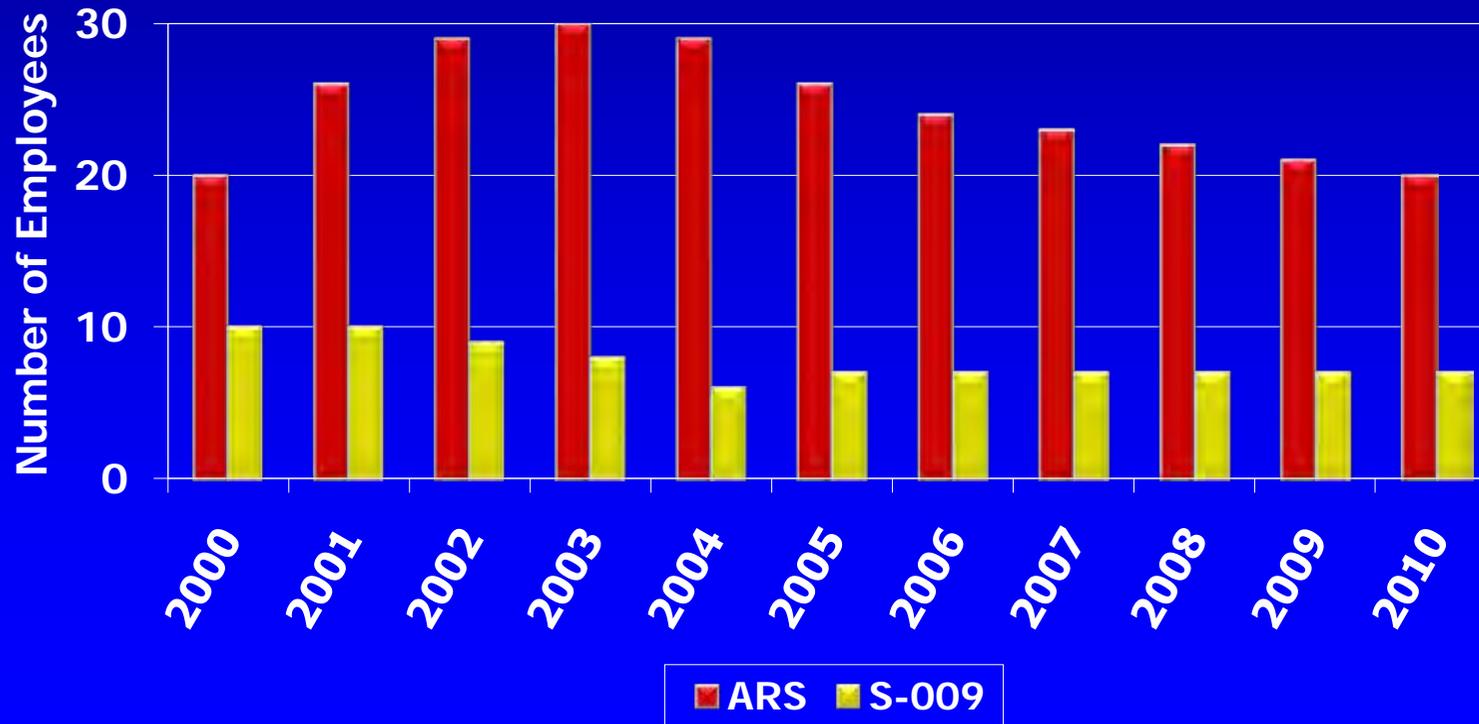
- Sorghum
  - John Erpelding (PR sorghum curator) moved to a new research geneticist position in Stoneville, MS
  - Gary Pederson serving as acting sorghum curator
- Peanut
  - Roy Pittman (current peanut curator) being moved to Vigna curator
  - New peanut curator position will be advertised shortly and filled early in FY2011 (after Oct. 1, 2010)
- New curator position at GS 12/13/14
  - Other curator positions will also be changed

# Staffing - S-009

- Seven permanent S-009 employees
- 13 temporary full-time and part-time employees were hired during FY2010 to handle specific labor needs.

# Staffing summary

- Current staff is 27 employees (20 ARS and 7 S-009)
- Thirteen S-009 temporary labor positions



# Equipment purchased

- Farm operations
  - Bushhog box blade
  - Zero turn mower (bamboo)
  - Irrigation water reel, pump, and sprinkler gun



# Equipment purchased

## Laboratory

- Vacuum evaporator
- Nanodrop spectrophotometer
- Partec flow cytometer (obtained ARS surplus from Raleigh location)



# Vehicles

## American Recovery and Reinvestment Act

(Federal vehicle replacement program)

- Two 2009 Jeep Patriots
  - Traded in 1996 Dodge truck and 1993 Dodge Caravan



# Proposed Building Construction

- S-009
  - Pole barn (50 x 100') – located on Westbrook next to existing S-009 machine shed
- ARS
  - Addition to ARS Seed Processing building
    - Will contain new 4 C cold room (20 x 30')
    - Existing 4 C cold room in State Seed Storage building will be converted to -18 C
    - Current -18 C seed storage space of 1,061 sq ft will be increased to 1,897 sq ft to house bulk seed of entire Griffin collection in -18 C



# Facility Repair and Maintenance

- Replaced roofing and repaired soffit, siding, and eaves on State S-009 Project building (Jarret).
- Installed new control panel for -18 C cold room in State Seed Storage building.
- Replaced doors, moldings, and trim on original State PI building.
- Installed automatic hand dryers in ARS headhouse bathrooms.
- Equipment repair
  - Air compressor for seed processing
  - Air handling system for seed processing
  - Irrigation pump at Byron
  - Irrigation reel and pump at Westbrook



# 3<sup>rd</sup> Curators workshop (Feb. 2010)

- Hosted by UGA Continuing Education at Atlanta
- Topics included regeneration issues, saving money and resources, GRIN/data management issues, characterization and evaluation issues, and distribution and maintenance issues
- Customer service workshop
  - “Give ‘em the pickle” video shown
  - Video has been distributed to 8 germplasm locations throughout the country, National Program Staff, and ARS Human Resources for viewing by local personnel.
- Tours of Griffin and Byron locations



# Needs

- Technical support
  - Peanut: Field support
  - Forage legumes: HPLC characterization
  - Grass: labor to support grass tissue culture
  - Seed storage: seed processing
  - Field crew: weeding, irrigation, harvesting

# Needs

- Equipment

- Tissue culture chamber (grasses) \$ 8,000
- Ultra low temp freezer \$ 9,500
- Peanut thresher \$14,000
- Seed storage trays \$24,000
- Forklift \$25,000
- DNA fragment imager and DNA extractor \$35,000
- Moveable storage shelves for new 4 C room \$45,000
- 4 C cold room for new building addition \$55,000
- Moveable storage shelves for cold room to be converted from 4 to -18 C \$93,000

# Plant Genetic Resources Conservation Unit



April 2007

# Plant Genetic Resources Conservation Unit



Appendix 2

DR. NOELLE BARKLEY

DETECTION OF SWEETPOTATO LEAF CURL VIRUS (SPLCV)  
IN THE U.S. GERMPLASM COLLECTION VIA REAL-TIME PCR

# Sweet Potato: *Ipomoea batatas* (L.) Lam.

- Originated tropical parts of Central and S America, domesticated > 5000 yrs
- Cultivated in many countries- versatile- storage roots, vines, and leaves food source
- Staple food – vitamin A deficiency
- Major producer (~80% world supply) China- livestock feed
- US production- N.C.-38%, CA, LA, MS
  - Average per capita consumption decreasing in US from 31lb in 1920 to 4 lb / year

# USDA Sweet Potato Germplasm Collection

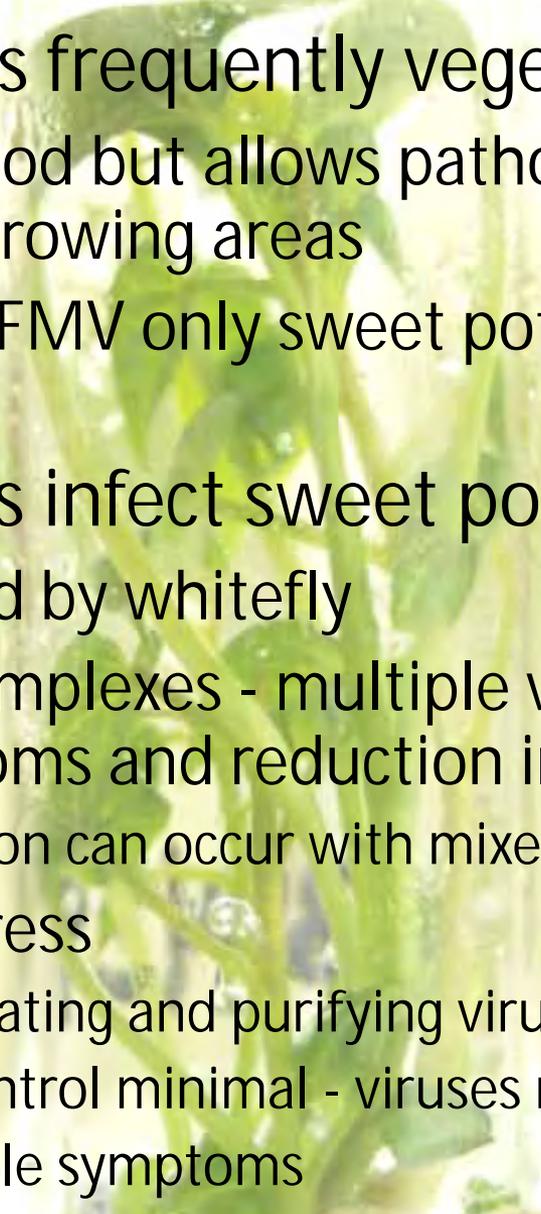


All maintained in vitro

20 C 10 hours light

Sub-culture every 4-6 months

# Sweet Potato Disease



- Sweet potatoes frequently vegetatively propagated
  - Efficient method but allows pathogens to spread among seasons and growing areas
  - 1978-1998 SPFMV only sweet potato virus reported in U.S.
- Over 20 viruses infect sweet potato
  - 15 transmitted by whitefly
  - Synergistic complexes - multiple viral infection - exhibit visible symptoms and reduction in yields
    - Recombination can occur with mixed infection
  - Delayed progress
    - difficulty isolating and purifying viruses –mixed infection
    - pathogen control minimal - viruses not identified yet
    - few noticeable symptoms



# SPLCV

- DNA virus classified genus: *Begomovirus*
- Over 100 viruses classified Begomoviruses
  - Vectored by whiteflies: *B. tabaci* and *B. argentifolii* – spread disease to dicots
- SPLCV - yield decline and can effect quality traits
- SPLCV reported Taiwan, Japan, China, Italy, Kenya, Peru, Spain, and U.S.
  - Distribution and host range unknown
  - Leaf curl symptoms not common and may not persist

# SPLCV



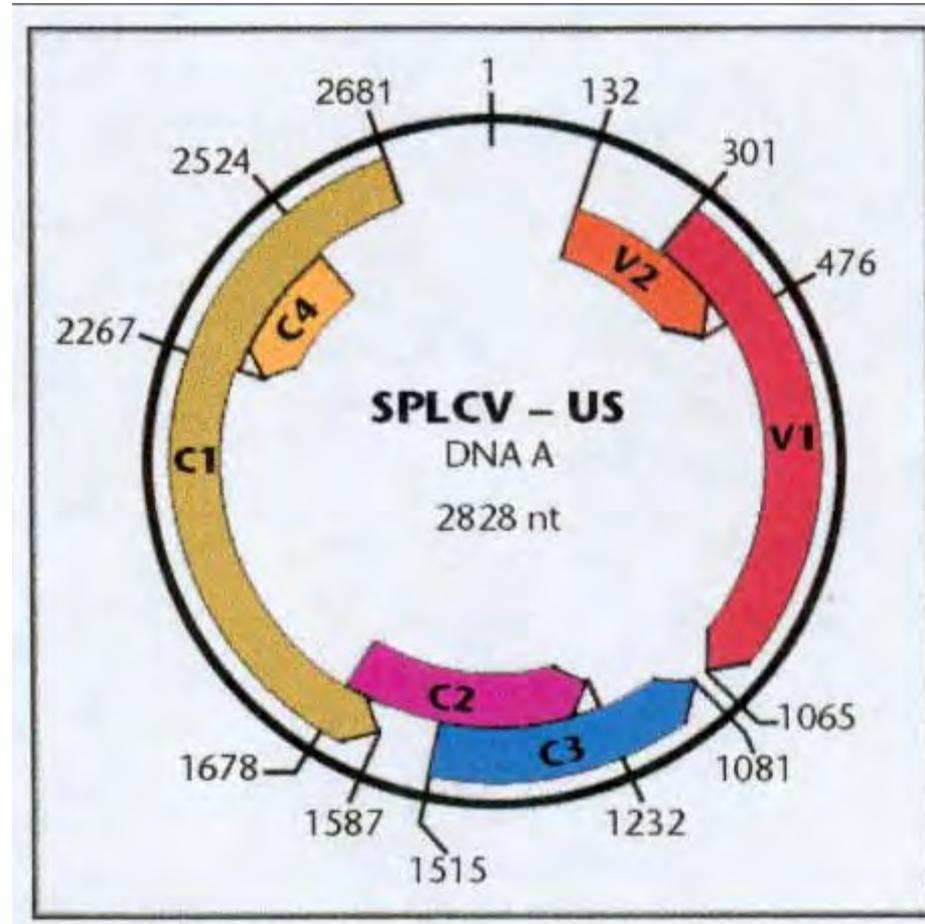
# SPLCV - Begomoviruses



- Numerous strains
  - Difficult to determine strains vs. new virus species
- ICTV
  - 6 species –SPLCV, SPLC-China virus, SPLC-Canary virus, SPLC-Georgia virus, SPLC-Lanzarote virus, SPLC-Spain virus
  - Demarcation is based on 89% sequence identity
    - > 89% - strain
    - < 89% - species

# SPLCV Genome

- 6 ORF's-2 sense; 4 complementary sense
  - V2 and C4 f(x) ?
- ssDNA-rolling circle replication-host
- Can have mono or bipartite genomes: DNA A and DNA B
- DNA A largest component-replication, gene expression, transmission; DNA B inter and intra cellular movement



Lotrakul P. and Valverde R.A. 2009 Mol Plant Path

# Objectives

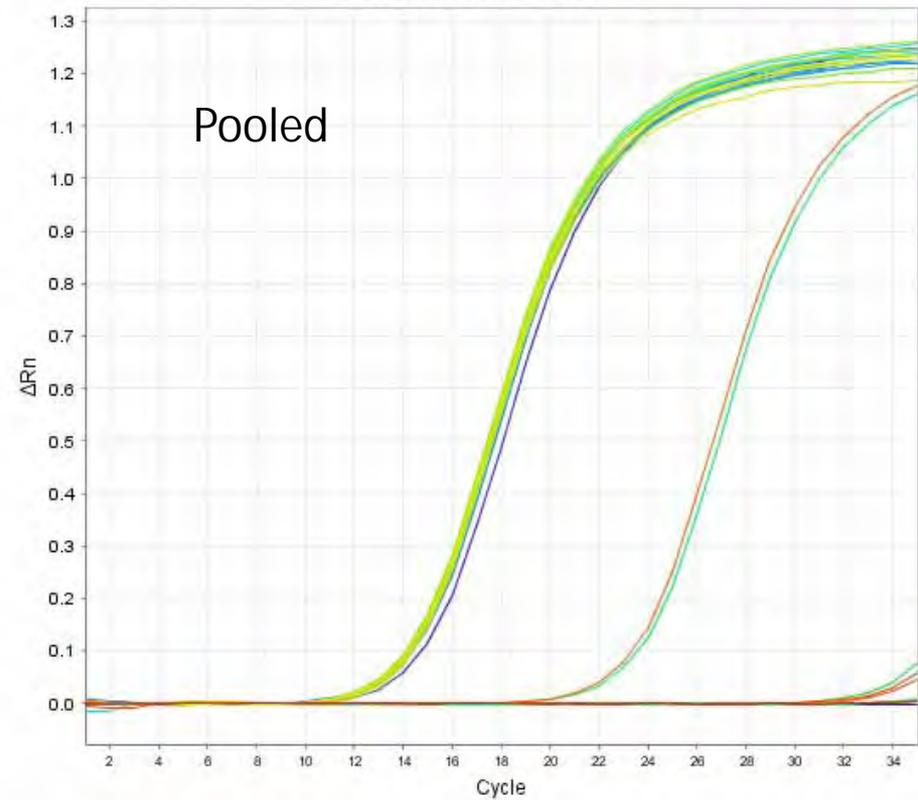
- Empirically test all germplasm accessions for SPLCV utilizing Real-Time PCR
  - 2/70 positive in previous test ~ 3 %
  - Avoid distribution of infected material
- Determine the level of infection in the collection
- Perform bioassay on positive samples and record symptoms
  - Determine “best” indicator plant/species
- Sequence virus isolates to examine the variability in positive samples
  - Evaluate their relationship to other SPLCV strains/species

# Real-Time PCR

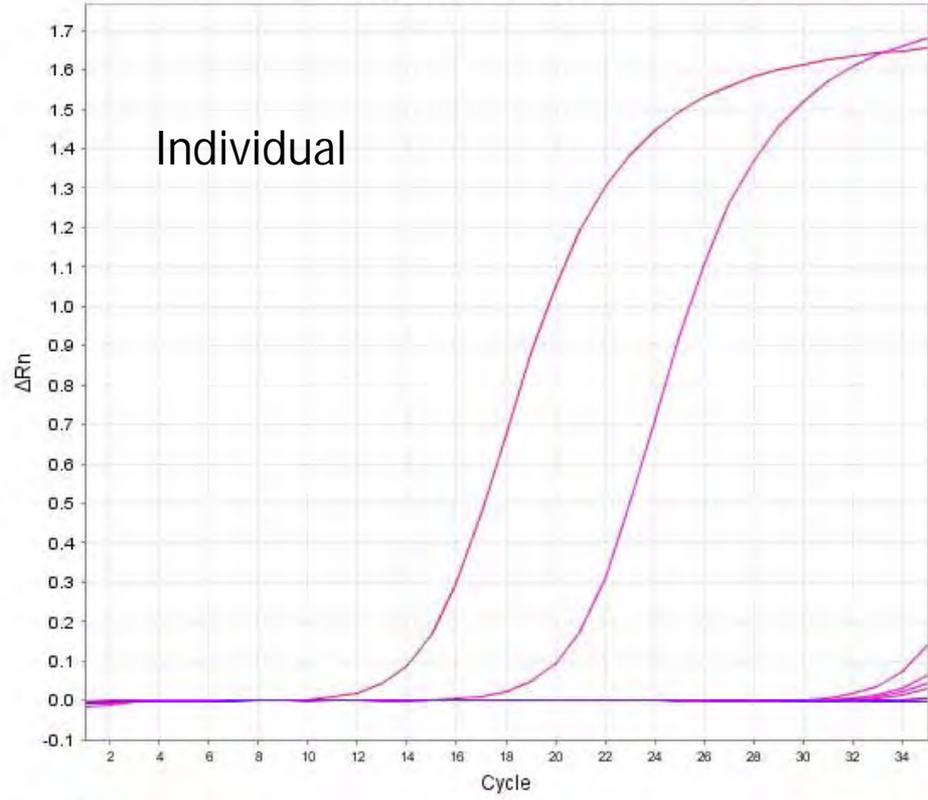
- 2 primers and 1 dual end labeled probe
  - 6-Fam
- Target coat protein – conserved
  - Product size 67 bp
- Pooled Samples 4 fold
  - Spiked each pool with positive control



**Amplification Plot**



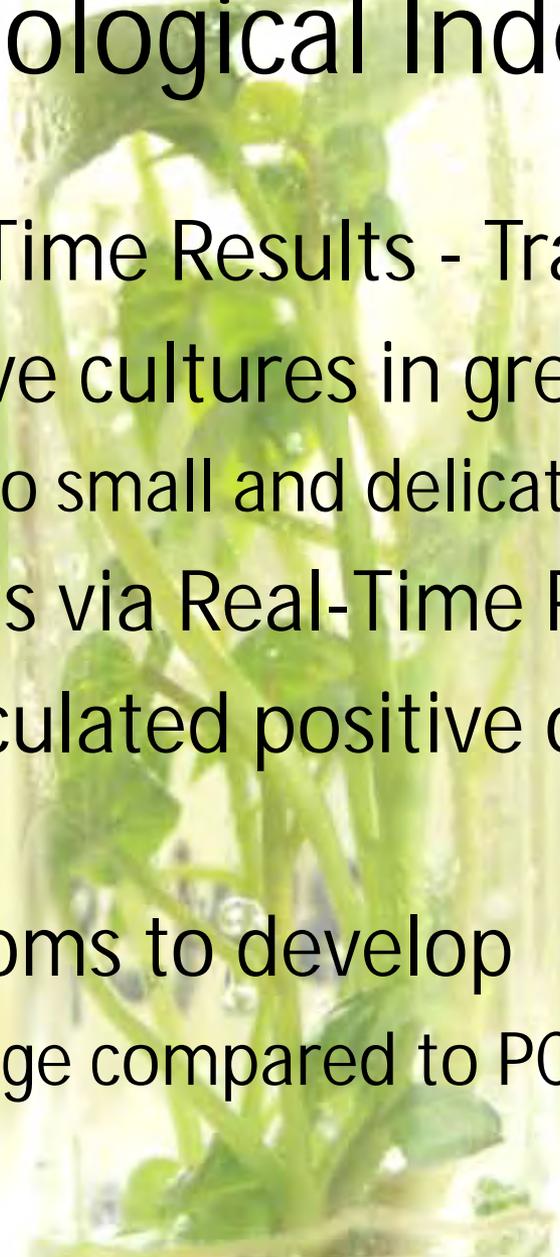
**Amplification Plot**



# SPLCV Results

- 701 *in vitro* accessions tested for SPLCV
  - 47 were positive (6.7%)
    - Received 1987-2007
  - $C_T$  ranged 14.78-29.95
  - 15/47 went through quarantine – tested?
    - 1998
- Greenhouse plants
  - 85 tested
  - 26/85 (30.85%) positive
  - $C_T$  ranged 12.47-29.9

# Biological Indexing



- Verify Real-Time Results - Traditional Method
- Grew positive cultures in greenhouse
  - Cultures too small and delicate to use
- Tested plants via Real-Time PCR for SPLCV
- Grafted inoculated positive onto *I. nil* and *I. turbinata*
- Wait symptoms to develop
  - Disadvantage compared to PCR assay

# Biological Index

*I. nil* –over 10.5 weeks to develop symptoms

PI 16175  $C_T=15.58$



*I. turbinata* – 3.5 weeks to develop symptoms

PI 16175  $C_T = 15.58$



# Biological Index

Hanna  $C_T = 29.5$



*I. nil* –over 10.5 weeks to develop symptoms

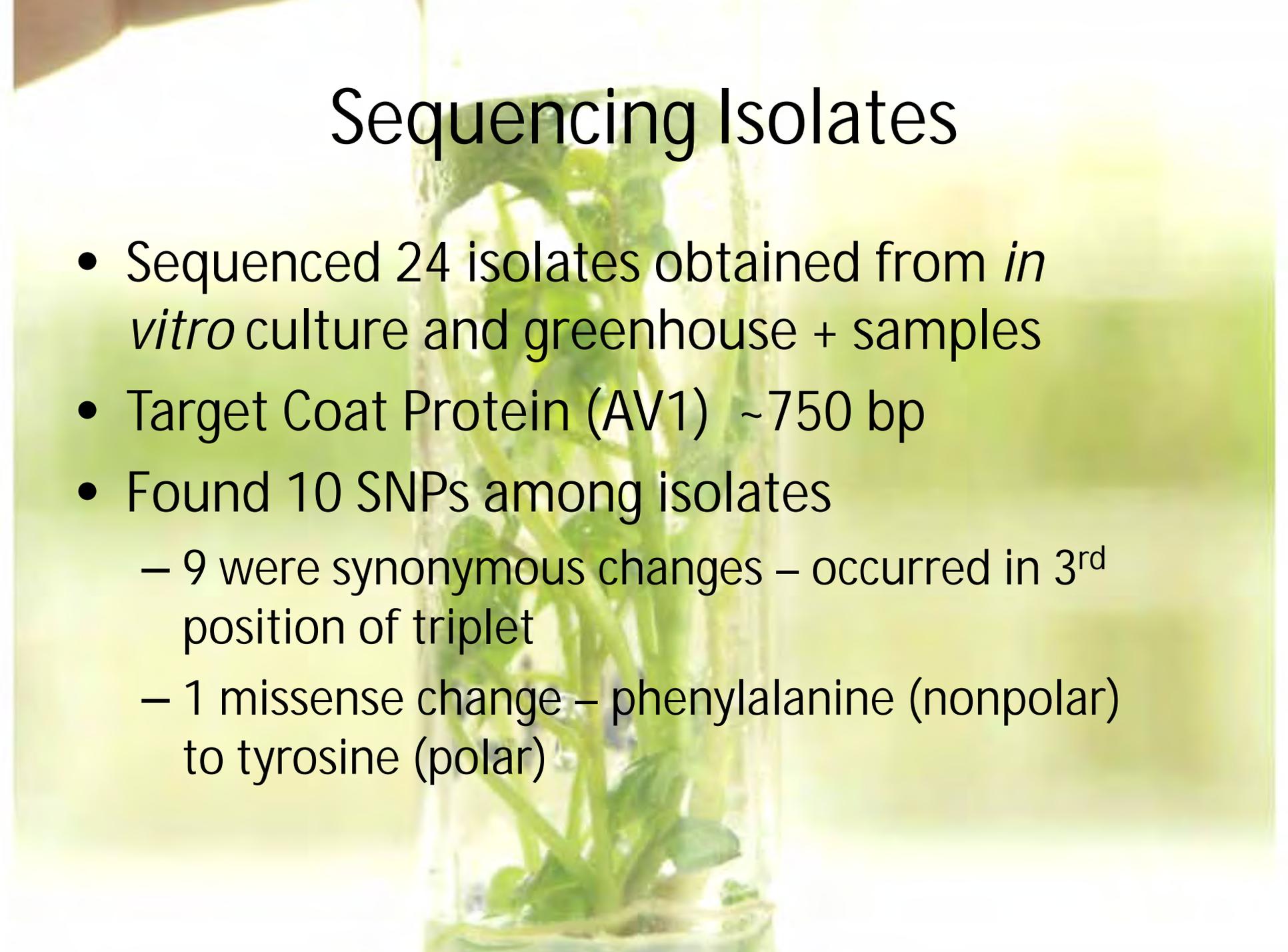


*I. turbinata* – 3.5 weeks to develop symptoms

Hanna  $C_T = 29.5$



# Sequencing Isolates



- Sequenced 24 isolates obtained from *in vitro* culture and greenhouse + samples
- Target Coat Protein (AV1) ~750 bp
- Found 10 SNPs among isolates
  - 9 were synonymous changes – occurred in 3<sup>rd</sup> position of triplet
  - 1 missense change – phenylalanine (nonpolar) to tyrosine (polar)



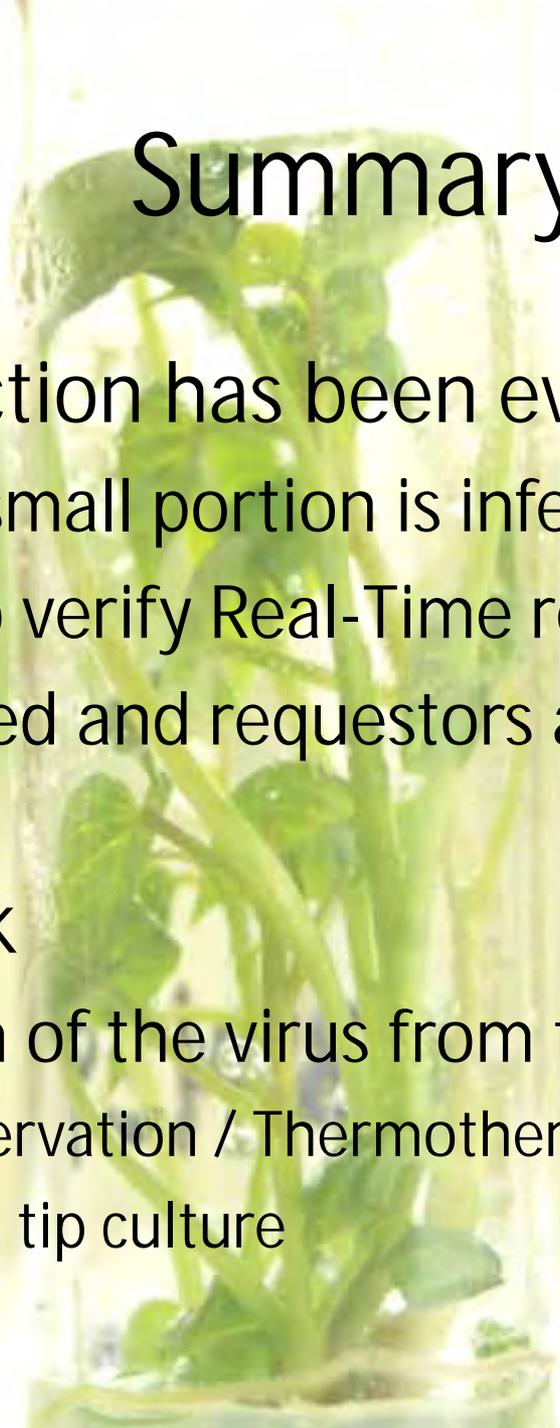
# Sequence Analysis

- 24 isolates ~750 bp sequence AV1
- Phylogeny - Evaluate relationships among SPLCV strains/species
  - Obtained AV1 sequences of strains/species GenBank
  - Model Test –model of nucleotide substitution test 88 i.e.-JC=equal subs. Or GTR = unequal rates of subs.
  - ML using “best model”



0.1

# Summary



- Entire collection has been evaluated for SPLCV
  - Relatively small portion is infected
  - Indexing to verify Real-Time results
  - Documented and requestors are informed on status
- Future Work
  - Elimination of the virus from the *in vitro* collection
    - Cryopreservation / Thermotherapy
    - Meristem tip culture

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