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MINUTES OF THE MEETING OF THE S-9 TECHNICAL ADVISORY COMMITTEE ON THE INTRODUCTION, MULTIPLICATION, AND EVALUATION OF NEW PLANTS FOR AGRICULTURAL AND INDUSTRIAL USES AND THE PRESERVATION OF VALUABLE GERMPLASM

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE - SOUTH ATLANTIC REGION
GENETIC RESOURCES UNIT
UNIVERSIY OF GEORGIA
GRIFFIN, GEORGIA

JUNE 13-14, 1995

SUBMITTED BY

DON R. LA BONTE, SECRETARY
S-9 TECHNICAL ADVISORY COMMITTEE 1995

1. CALL TO ORDER

The Regional S-9 Technical Advisory Committee (TAC) was called to order at 8:00 a.m. on June 13, 1995 by chairman David Coffey in the Conference Room of Stuckey Building of the Georgia Experiment Station, Griffin. Dr. S. Kresovich amended the tentative agenda to include an on-site facility tour.

2. INTRODUCTION OF ATTENDEES

NAME	ADDRESS	PHONE & FAX
*William D. Branch	University of Georgia Coastal Plain Expt. Stn. Tifton, GA 31793-0748	912-386-3561 912-386-7293 (fax)
*Jorge Mosjidis	Auburn University Agronomy Dept. 202 Funchess Hall Auburn, AL 36849	334-844-3976 334-844-3976 Jmosjidi@AG.Auburn.edu
*Don R. La Bonte	Louisiana State Univ. Dept. of Hort. 131 J.C. Miller Hall Baton Rouge, LA 70803	504-388-1024 504-388-1068(fax) dlabont@unix1.sncc.lsu.edu
*Francisco Vazquez	USDA-ARS Box 70 Mayaquez, PR 00681	809-831-3435 809-832-1025(fax)
Brad Morris	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-229-3253 404-229-3323 bmorris@grinARS
*Norman Taylor	University of Kentucky Dept. of Agron N-122 Agric. Sci. Bldg. N Lexington, KY 40546	606-257-5785 606-258-1952 AGRO79@UK.EDU
*Ken Quesenberry	University of Florida Dept. of Agron. Gainesville, FL 32611	904-392-1811 904-392-1840 clover@GNV.IFAS.UFL.EDU

Allan Stoner	USDA/ARS NGRL Room 215, Bldg. 003, BARC-West Beltsville, MD 20705	301-504-6235
David Pinnon	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-229-3254
James H. Chalkey	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-229-3255
*Carlos E. Ortiz	University of Puerto Rico Dept. of Agron & Soil Sci. Agric. Expt. Stn. P.O. Box 21360 Rio Piedras, PR	809-767-9705 809-758-5158(fax)
Roy Pittman	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-229-3252 Rpittman@gaes.griffin. peachnet.edu
Gil Lovell	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-228-7255 glovell@ars-grin.gov
Mark S. Hopkins	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-412-4775 mhopkins@gaes.griffin. peachnet.edu
Merrelyn Spinks	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-229-3297 mspinks@ars-grin.gov
LeeAnn Chalkley	USDA-ARS University of Georgia 1109 Experiment St.	404-229-3334 lchalkley@ars-grin.gov

	Griffin, GA 30223	
Steve Eberhart	USDA-ARS National Seed Storage Lab Ft. Collins, CO	970-495-3212 303-221-1427(fax) naslse@ars-grin.gov
Bob Kleinman	USDA-ARS NCAUR Peoria, IL	309-681-6533
*Bill Rhodes	Clemson University	803-656-0410
Stephen Kresovich	USDA-ARS University of Georgia 1109 Experiment St. Griffin, GA 30223	404-228-7207 skresov@gaes.griffin. peachnet.edu

*Members of the S-9 TAC

3. OFFICIAL WELCOME

Dr. Alan Arkin, Director, Georgia Experiment Station, welcomed the TAC committee. Dr. Arkin stressed the importance of the S-9 project at the GES and the support each state experiment station contributes to the activities at Griffin ($200-300,000). The S-9 project is expected to have another review in 18 months by ARS to assure the project meets the needs of the southern region. Dr. Arkin commented on plans by the ESCOP Plant Germplasm Subcommittee (R.Lower, Chair). Dr. Arkin charged the committee with examining what we need to do, not to do, and what to do differently.

4. APPROVAL OF MINUTES

Minutes of the 1994 S-9 TAC meeting at North Carolina State University, were approved as previously submitted by the secretary.

5. APPROVAL OF AGENDA

The agenda for the 1995 meeting was circulated and approved.

6. APPOINTMENT OF COMMITTEES

A.) Nominations: Bill Branch (Chairman) and Norman Taylor
B.) Next Meeting: (Time and Place) Jorge Mosjidis (Chairman) and Carlos Ortiz
C.) Resolutions: Billy Rhodes (Chairman) and Francisco Vasquez

7. ACTIVITIES OF THE U.S. NATIONAL GENETIC RESOURCES PROGRAM

A national program staff report (*Attachment #1*) prepared by Henry L. Shands was presented by Alan Stoner. For three straight years (1993-95), the budget has been frozen. Dr. Arkin commented on the need to have the National Program leader position filled. Stoner replied that few acceptable candidates have applied after several cycles of advertising. Kresovich added that it is essential to have this position filled to enable greater coordination of efforts and provide insight and support to units within the system, i.e. leadership.

8. ACTIVITIES OF THE NATIONAL GERMPLASM RESOURCES LABORATORY

A copy of a report of the National Germplasm Resources Laboratory to the Regional TACs was prepared by Alan Stoner and others and presented by Alan Stoner (*attachment #2*). Karen Williams is the contact person for plant collecting.

Floor motion: Plant collecting proposals approved by the appropriate CGC will be prioritized by the S-9 TAC beginning in 1996. The Operations Group that selects proposals for funding should have one representative from each regional TAC. It is also requested that firm submission and notification dates are stipulated nationally. Motion-Coffey, Second-La Bonte. Passed

9. ACTIVITIES OF THE NATIONAL SEED STORAGE LABORATORY

A copy of a report of the National Germplasm Resources Laboratory to the Regional TACs was prepared by Steve Eberhart, E. Roos, and L. Wiesner is attached (*attachment #3*).

10. ACTIVITIES OF THE NATIONAL CENTER FOR AGRICULTURAL UTILIZATION RESEARCH

A copy of a report of the National Center for Agricultural Utilization Research was prepared by Robert Kleiman is attached (*attachment #4*). Kleiman highlighted research efforts on meadowfoam. Kleiman further offered to provide seed for evaluation purposes and for storage at the NSSL.

11. CSRS ACTIVITIES ON GENETIC RESOURCES

A copy of a report of the Regional Research Project - 009 "Plant Genetic Resources Conservation and Utilization" prepared by cooperating state AES and USDA representatives is attached (*attachment #5*).

12. OVERVIEW OF UNIT ACTIVITIES

Steve Kresovich outlined the current budget of the unit:

-$1.8 million in ARS support (unchanged since 1988)

-$250,000 in S-9 state contributions
-$100,000 in GAES support-in-kind
-Collaborative Research and Development Aggrements
    -$30-40,000 from Pioneer Hybrid
    -$20-50,000 from Applied Biosystems

In general about 60% of funds go to salaries and 40% for research support. Dr. Kresovich desired to have a greater input of the TAC on funding allocations within the Griffin unit.

Besides the lack of a national program leader, there is also a need to fill the area director position for the South-Atlantic region. An animal scientist has taken over temporarily (Dr. Breeze).

A major thrust of the unit is genetic analysis, i.e., the development of molecular markers for a broad range of national and international needs. The funding for this thrust came directly from Carter, Plowman and Shands. An effort is now underway to develop molecular markers to determine race and diversity in sorghum in association with IPRI.

13. OVERVIEW OF CURATION ACTIVITIES AT GRIFFIN

On the afternoon of June 14, the TAC committee toured facilities and were given an overview of curation activities on peanut, cowpea, clovers and special-purpose legumes, grasses, and vegetable crops. Additionally, the plant pathology and therapy services group and the database group lead by Merrelyn Spinks gave overviews of their activities. Copies of reports in these areas are attached (attachments # 6-11).

14. DISCUSSION AND REVIEW OF UNIT ACTIVITIES

A general discussion period was initiated on Wednesday morning. High points of this discussion were as follows:

1. Rhodes recommended that the TAC become more involved in the unit budget process. Kresovich replied positively. A detailed breakdown of the budget will be provided. Arkin further requested that a matrix pertaining to funding be provided to the TAC before the budget cycle. We (SAES) need to better understand the returns of our investment.
2. Kresovich noted that a strong effort is underway to move as much germplasm as possible from -4C to -20C. Eberhart commented that it is difficult to categorize storage life on a genotypic basis. Quesenberry suggested that Pitman and Eberhart compare long-term peanut samples stored at -4C and -20C to determine the proper storage temperature.
3. A discussion ensued on out-crossing of summer legumes and pollination biology. Mosjidis added that there are two approaches to germplasm maintenance 1.) trait based and 2.) genotypic. Quesenberry questioned Morris on how much outcrossing exists. No generalities could be made. Kresovich responded that there is a strong

need for curators to lead and consult with the CGCs to determine what they should do.

4. Eberhard questioned why certain plant numbers/generation are used. For instance, increasing the population size to 100 plants per generation instead of 20 will increase the frequency of retaining genes. Another approach is to increase plant densities on the same acreage of land and take equal quantities of seed from each plant. Kresovich responded that there is a need to consult with population biologists (possibly at Ames) and CGCs regarding increase size.
5. Stoner and Kresovich commented that there is a great need to coordinate research specialization within ARS. A meeting was proposed (system wide) to further coordinate these activities at the Operations Committee level.
6. Lovell asked what do we do with genera that are underutilized, i.e., can we dispose of it or deposit the whole collection at Ft. Collins. This question needs to brought up at the Operations Committee level.
7. *A motion was made by Mosjidis that annual TAC meetings should be at Griffin in most years and only periodically should this deviate. passed*

15. COMMITTEE REPORTS AND ACCEPTANCE

A.) Nominating committee nominated Bill Rhodes from Clemson University as secretary for 1996. Don La Bonte of Louisiana State University will become chairman of the 1996 TAC.

B.) At the invitation of Kresovich, the 1996 meeting will be held at Griffin, GA during the month of June.

C.) The following expression of thanks and resolutions were submitted by the Resolution Committee.

1.) We express our thanks to the USDA-ARS speakers for their presentations and participation in the meeting.

2.) We express our thanks to S. Kresovich and others for hosting our annual meeting.

3.) Let it be resolved to thank David Coffey and Don La Bonte for their service to the S-9 TAC as president and secretary, respectively, for the year.

4.) Let it be resolved to congratulate Don La Bonte and Bill Rhodes for their selection as new chairman and secretary, respectively, for 1996.

16. ADJOURNMENT

The meeting was adjourned at 11:00 a.m.

Attachment 1

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National Program Staff Report
June 12, 1995
Henry L. Shands

Budget. The FY 1995 budget had no increase for plant germplasm. In the 1995 final action, Miami, FL and Brownwood, TX were retained subject to reexamination during the year. The Oxford, NC location's closure resulted in the loss of funds for the tobacco genetic resources. However, an agreement with North Carolina is under development that, for the term of the agreement, the same program activity will be carried on at Oxford under the direction of Dr. Verne Sisson. The FY 96 budget calls for an increase of $750,000 for plant germplasm. The Miami and Brownwood locations are again proposed for closure. It is planned that the Brownwood repository will be consolidated to the College Station, TX area with no loss of funds for the repository. It is proposed that the germplasm from Miami will be relocated to Mayaguez, PR or Hilo, HI. Congressional action will be taken later this summer.

Germplasm funding:

Activity (STP Codes)	FY 1993	FY 1994	FY 1995
Acquisition *2111,2112*	$ 4,519,400	$4,453,600	$4,097,200
Preservation *2113*	11,941,200	12,266,900	12,559,325
SUB-TOTALS:1/	16,460,600	16,720,500	16,656,500
Characterization *2114*	3,698,000	3,782,600	3,377,300
New Crops 2115	218,700	309,400	--
TOTALS:	20,377,300	20,812,500	20,073,800

1/ Funding level most consistent with activities relating to National Genetic Resources Program.

National Genetic Resources Program. The newly appointed National Genetic Resources Advisory Council for the National Genetic Resources Program (NGRP) met May 16-17, 1995, in Washington under the leadership of Chair John Barton and Vice-Chair Richard Lower. The Council took particular interest in the renegotiation of the FAO International Undertaking and how it might affect the U.S. It urged that a uniform material transfer agreement be developed for easy and clarity for exchange. The international centers' collections now fall under the auspices of the FAO Commission on Plant Genetic Resources but the MTA has not been developed in detail.

The Council also looked at the animal and microbial collections and databases as well as the needed research to actually have an insect collection. The cryopreservation is standard for animal and microbial germplasm but for insects, only Drosophila has been cryopreserved. That technology needs to be extended to other species and other storage means such as diapause extension needs to be studied. Aquaculture is of considerable interest for the Council at the next meeting.

At the meeting, the Council addressed its previous resolution which recommended that the USDA establish a storage category for protected germplasm at the National Seed Storage Laboratory in cooperation with the Crop Science Society of America (CSSA) for protected germplasm. This germplasm would not be part of the NPGS until the expiration of the protection at a maximum of 20 years. The USDA has agreed to this and now the matter is in the hands of CSSA.

Convention on Biological Diversity. The issue of access to genetic resources and Farmers' Rights will be addressed in November at Jakarta. This may be only an FAO report on the status of the renegotiation of the International Undertaking but the Conference of Parties has interest in pushing it to conclusion.

FAO Commission on Plant Genetic Resources and the International Technical Conference on PGR. The FAO will sponsor the 4th International Technical Conference on Plant Genetic Resources which Germany will host at Leipzig from 17-23 June 1996. Dr. Cary Fowler is the Project Officer at FAO. The meeting's objective is to present a report on the State of the World's Genetic Resources and a Global Action Plan for Plant Genetic Resources to a ministerial level conference.

The Sixth Session of the Commission is to be held June 19-30, 1995 and it will include approximately a week of negotiation on the International Undertaking. The result could be another voluntary agreement, a binding treaty, or a protocol to the Convention on Biological Diversity. In all cases, it will bring change to how we exchange germplasm and how we deal with other countries for collecting.

1996 Beltsville Symposium. The 1996 Beltsville Symposium will be on Global Genetic Resources: Access, Ownership and Intellectual Property Rights, May 19-22. It is co-sponsored by the Beltsville Area and the Association of Systematics Collections. It will deal with plants, microbes and insects, in particular.

Personnel. It is with great grief that we report on the loss of Dr. Calvin R. Sperling this year. He was presented with the Frank N. Meyer Medal for Plant Genetic Resources on May 10 as evidence of his truly outstanding efforts for the NPGS. He will be truly missed. We will also lose another outstanding young botanist, David Williams, who will join the International Plant Genetic Resources Institute in the fall. ARS continues to look for opportunities to fill the position of National Program Leader for Plant Germplasm at Beltsville, Maryland. Contact Henry Shands (301-504-5059) for information. In addition, ARS will be looking for selected personnel in the germplasm system in keeping with needs and available personnel slots, particularly in the area of plant collectors with foreign language experience.

attachment (2)

REPORT OF THE NATIONAL GERMPLASM RESOURCES LABORATORY TO THE REGIONAL TECHNICAL COMMITTEES ON PLANT GERMPLASM JUNE 1995

The programs of the National Germplasm Resources Laboratory (NGRL) support the mission of the National Plant Germplasm System (NPGS), which is; "To effectively collect, document, preserve, evaluate, enhance, and distribute plant genetic resources for continued improvement in the quality and production of economic crops." The NGRL support of this mission involves the management of the NPGS's GRIN database, facilitation of 40 NPGS Crop Germplasm Committees, the Plant Exchange Office, the Plant Germplasm Quarantine Office, research to develop improved methods for detecting viruses and viroids, and tropical biodiversity studies. Specifics of the NGRL activities during the past year are detailed in the individual reports below.

Before moving to the reports, it is with sadness that I report that Dr. Calvin Sperling, a member of the NGRL since 1987, died on May 20, 1995 after a prolonged struggle with melanoma cancer. During his brief career, his excellence in field research and work to conserve biological diversity and improve crop plants worldwide earned him a reputation as one of nation's foremost ethnobotanists and plant explorers.

Dr. Sperling developed and implemented plant collecting priorities for target species of interest to U.S. agriculture. His participation in foreign plant collecting trips resulted in the addition of more than 1,000 fully documented germplasm accessions of cereals, food and forage legumes, fruits and vegetables to the NPGS collections. In addition, he oversaw more then 90 plant collecting trips taken by cooperators. Dr. Sperling also developed exploration guidelines that established expectations for U.S. collectors and serve as a standard for other countries. These guidelines incorporate ethics, cultural sensitivity, and equity. Dr. Sperling also created a prototype model for *in situ* preservation of wild relatives of agricultural crops that could complement biodiversity conservation in protected

areas. Further, he developed proposals funded by the World Bank and the United Nations Development Program for the conservation of genetic diversity in Turkey and Ethiopia.

On May 10, 1995, Dr. Sperling was awarded the Frank N. Meyer Medal for Plant Genetic Resources by officers of the Crop Science Society of America. This medal recognizes distinctive service in exploring and preserving the diversity of the world's plants. Earlier this year Dr. Sperling received the N.I. Vavilov Medal for botany for his efforts in plant collecting which exemplify the contributions of the famous Russian agrobotanist.

Allan K. Stoner

GERMPLASM RESOURCES INFORMATION NETWORK

A new GRIN information system (GRIN3) was completed and installed in August 1994. This culminated an effort that involved a total rewriting of the GRIN software. Germplasm maintenance site personnel participated in a 3 day training session on the functionality and use of the new system.

A new Sun Microsystems computer was installed in November 1994 to replace the Solbourne Inc computer. Movement to the Sun has been slowed by difficulties in using the existing disk drives. A Redundant Array of Inexpensive Disks (RAID) and 2 processors have been ordered to augment the Sun equipment. The GRIN Database Management Unit (DBMU) continues to work closely with the maintenance site personnel and the Crop Germplasm Committees to update descriptors and data in the database.

More than 600 copies of pcGRIN have been distributed to scientists in 50 countries. GRIN data has also been made available through the services of the World Wide Web (WWW) and Gopher. The GRIN DBMU continues to regularly assist many foreign germplasm programs with the computerization of the records on their plant germplasm collections. For example, Dr. Edward Bird, Database Administrator, participated in an on-site review of the Brazilian CENARGEN facility. The

DBMU also continued to assist the Indian National Bureau for Genetic Resources, New Delhi, India. with the development of a national germplasm information system and their central computer facility.

Locally, DBMU personnel chaired 2 Beltsville Agricultural Research Center networking committees charged with developing a campus wide network. Additionally, the DBMU was instrumental in the development of WWW home pages for the ARS and the Beltsville Area. The DBMU staff maintain 2 NGRL LANs and approximately 50 pc's. Two DBMU personnel left the Unit during the year. Gail Juvik was detailed to the animal genome project and David Wood accepted a position with a private data processing company.

Jimmie D Mowder

CROP GERMPLASM COMMITTEE FACILITATION

The name of the Crop Advisory Committees (CAC) was recently changed to Crop Germplasm Committees (CGC). This was necessary because of the Federal Advisory Committee Act which limits the advisory role of non-federal participants to those serving on committees authorized by the Congress. Although the name of our germplasm system committees has changed, their importance, role and responsibilities remains the same. While it will take some time to adapt to the new title, it does more accurately reflect the activities expected from the committees.

The fifth biennial meeting of the CGC Chairs and NPGS personnel was held in Beltsville, MD, June 28-29, 1994. Numerous topics relating to the NPGS and genetic resources maintenance and use in general were discussed. These included; status reports from NPGS sites, the role and expectations of CGC's, impact of the Convention on Biological Diversity, the status of global genetic resource programs, intellectual property rights issues, the molecular characterization of crop genetic resources collections; the possible role of core subsets, the Plant Genome Research Program, plant quarantine issues, and recent research on preservation of seed and vegetative tissues.

All 40 CGC's were active during the last year and NGRL personnel participated in 32 of their meetings. Though not all inclusive, the CGC's are supporting the NPGS by:

* identifying gaps in U.S. collections and developing proposals to fill them through exchanges or collecting trips
* assisting crop curators in identifying duplications in collections
* prioritizing traits for evaluation and developing evaluation proposals
* assisting curators and GRIN personnel in correcting and standardizing passport and evaluation data and ensuring that complete information is entered in the GRIN database
* assisting curators with regeneration projects
* identifying germplasm in breeder working collections that should be incorporated into NPGS collections and assisting with arrangements to accomplish this
* working with quarantine officials to identify and ensure implementation of new techniques for pathogen identification
* maintaining up to date reports on the status of crop vulnerability and the needs for collecting, evaluating, enhancing and preserving germplasm
* evaluating the potential benefits and problems associated with the development and use of core subsets

Mark Bohning

PLANT EXCHANGE OFFICE

During 1994, the Plant Introduction and Plant Exploration Offices were merged to form a new Plant Exchange Office (PEO). Dr. George White, the former Plant Introduction Officer, accepted the curatorship of the Clonal Repository in Davis, California. As stated above, Dr. Calvin Sperling, the Plant Exploration Officer, was on sick leave from June 1994 until his death on May 20, 1995.

Exchanges - During 1994, a total of 43,098 items in 787 shipments to scientists in 81 countries passed through the Plant Germplasm Quarantine Center, Building 580, BARC-East. Additionally, 1055 non-permit items were received from foreign cooperators for use by U.S. scientists.

Shipping Procedures Changes - All NPGS sites are now expected to check with their local APHIS inspectors, or phone 301-504-8141, or FAX 301-504-8539, to the Beltsville Plant Germplasm Quarantine Inspection Station staff to determine the quarantine requirements for foreign germplasm requests received at the sites. Requests forwarded to the sites by PEO personnel will have already been screened and will have any necessary permits. Requests received via the Internet and forwarded through GRIN have not been screened. This prior screening allows the sites to request any necessary import permits, additional declarations statements or other documentation required by the importing country. THIS IS VERY IMPORTANT WHEN REQUESTS ARE FOR VEGETATIVE MATERIALS. If screening indicates that a Phytosanitary Certificate is not required, the sites are encouraged to mail the shipment directly to the requestor, and not through the Beltsville Inspection station. These changes have evolved to help reduce hold-up of shipments received in Beltsville for lack of documentation and to prevent the loss of valuable clonal materials.

NPGS site personnel are urged to read foreign import permits carefully. If Additional Declarations statements regarding disease freedom, seed treatments, etc. are included, these MUST be addressed. These statements must be signed, on letterhead stationery, and forwarded with the packing list, the import permit and the germplasm. Also, foreign-language permits should be translated at the site so that site personnel are aware of the requirements of the permit. The pool of translators at Beltsville is rapidly shrinking.

Plant and Materials Seed Project - This AID-funded project provides larger quantities of seed and plant materials to AID Missions, AID Agricultural Specialists, AID Contractors, Peace Corps volunteers, and others in developing countries. In 1994, 1436 items were provided in 69 shipments to 18 countries. Approximately 1200 forages and vegetables were provided to Albania and Bulgaria

and a large shipment of fruit trees is pending shipment to Pakistan. The emphasis for 1995 is to support the former Soviet Union republics, the Baltic States, and Pakistan.

Maryann Loftus is responsible for processing germplasm requests from foreign scientists, and aiding researchers in acquiring germplasm from foreign sources. She is responsible for notifying NPGS personnel of changes in procedures regarding foreign shipment of germplasm, acts as liaison between U.S. scientists and APHIS wishing to import/export germplasm, and assists plant collectors and others traveling overseas with information about germplasm importation.

Documentation of PI's - During 1994, Becky Norris assigned PI numbers to 8085 germplasm accessions entering the NPGS. Several collections being held at the National Seed Storage Laboratory and active collection sites were documented and assigned PI numbers. These included;

clover from Bulgaria
corn from Columbia, Bolivia, Brazil, Reunion, Uruguay
cowpeas from IITA
millet from Kenya
peanuts from Bolivia, Mexico, India, S. Africa
millet from Zambia
rye and oats from Turkey
sorghum from Uganda
sugarcane from India
soybeans and wheat from Australia

PI numbers were also assigned to 323 Plant Variety Protection applications and 274 Crop Science Society of America (CSSA) registrations which represented 25 of the 54 crop groups and breakdown into categories as follows:

CV - 121	GS - 27
GP - 118	PL - 8

A report of manuscripts and registration numbers forwarded to the CSSA each month can be obtained by contacting Becky Norris at the Plant Exchange Office. The report is available starting with January 1995.

A mail survey was conducted to determine the most effective and economical means of providing access to the Plant Inventory, the annually published list with descriptions of all germplasm entering

Williams has served this year as an ARS representative on the Federal Native Plant Conservation Committee and as a member of that committee's Database and Information Sharing Working Group. A PEO proposal to promote *in situ* conservation of wild cranberry genetic resources in the U.S. was approved by the committee's Native Plant Conservation Initiative program which is funded by the National Fish and Wildlife Foundation. However, the required matching funds could not be obtained from a non-federal source.

Ms. Williams has been investigating computer mapping technology and its usefulness to PEO and NPGS for plant exploration and other activities. She is serving on an NPGS Geographical Information Systems (GIS) Working Group studying how GIS technology can address needs in NPGS.

David Williams participated in two plant explorations in Bolivia that resulted in the introduction of wild relatives of peanut and blackberry. During these visits to Bolivia, Dr. Williams met with representatives of the Bolivian National Herbarium (LPB) and the Bolivian Institute for Agricultural Technology (IBTA) to discuss future collaboration between Bolivian institutions and ARS in germplasm collecting and conservation activities and the compliance with the principles outlined in the Convention on Biological Diversity. Based on those meetings, Dr. Williams drafted a provisional Memorandum of Understanding between ARS, LPB and IBTA which is presently being discussed and revised by the participating institutions.

A draft of Dr. Williams' *Arachis* Ecogeographic Survey is presently in review and has been provisionally accepted for publication by IPGRI as part of their Ecogeographic Surveys of Crop Genepools series. As chairman of the Peanut CGC's *Arachis* Collection Review Sub-committee, Dr. Williams has coordinated efforts to update, complete, and augment the passport and evaluation data for the NPGS Peanut collection in GRIN, with the goal of making Peanut a model genus in terms of the quality of accession data available on the GRIN system. Dr. Williams, together with Mexican and Colombian colleagues and GRIN computer specialists, completed the translation of pcGRIN into the Spanish language to help make this database more easily accessible to foreign users, particularly in

the NPGS. Approximately 285 letters went out requesting that the recipients indicate their interest in continuing to receive the Inventory and to select the format they most desired, i.e., printed, CD ROM, Internet, computer disk, or no preference. Out of the 70% responding, 94% wish to continue receiving the Inventory and of these, 49% preferred a printed copy, 26% preferred CD ROM, 13% preferred computer disk, 7% preferred the Internet, and 5% had no specific preference. Based on the results of this survey, the Plant Inventory will continue to be distributed in printed form. A CD may be developed for distribution at a later time.

Endangered Species Project - Vicki Binstock entered data in GRIN identifying the endangered species listed by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Center for Plant Conservation (CPC), and Fish and Wildlife Endangered and Threatened Species (FWE, FWT).

NPGS sites responsible for distribution of native, rare or threatened species as identified by the FWE and FWT are required to obtain permits from their regional Fish and Wildlife Service office. Those sites distributing endangered species listed by CITES need to apply for a permit from the Office of Management Authority, Arlington, VA. Endangered species accessions in GRIN are as follows:

CITIES	1 taxon	1 accession
CPC	127 taxa	360 accessions
FWE	45 taxa	178 accessions
FWT	23 taxa	65 accessions

Plant Exploration - The Plant Exchange Office provided support for 2 domestic and 8 foreign germplasm explorations in FY 1994 and is supporting 8 explorations in FY 1995. All foreign explorations are in cooperation with the host countries and uphold the intent of the International Convention on Biological Diversity. A proposal to streamline the review of plant exploration proposals is under consideration.

Karen Williams continues to coordinate ARS-funded plant explorations. All questions relating to plant explorations and requests for exploration proposal guidelines should be directed to her. Ms.

Latin America.

Plant Exploration Tracking System - The PEO is developing the Plant Exploration Tracking System (PETS). The primary objective of PETS is to track germplasm through the phases of collection, introduction, and deposition in NPGS and the host countries. This system will allow generation of reports on all aspects of plant explorations. Plans are to have the system augment the GRIN database.

Vicki Binstock, Maryann Loftus, Becky Norris, Karen Williams, and David Williams

USDA/ARS Plant Explorations Undertaken in FY 1994

Plant Exploration	Country	Principal Contacts
Arachis hypogaea	Mexico	D. Williams, L. Arias-Reyes
Ipomoea spp.	Papua New Guinea	D. La Bonte, J. Martineau
Grasses and legumes	Mongolia	D. Johnson, D. Sheehy
Forage grasses and legumes	Tunisia, Algeria, Morocco	W. Graves, P. Cunningham
Arachis spp.	Bolivia	D. Williams, C. Simpson, I. Vargas
Oilseed crops	USA	A. Thompson, D. Dierig
Solanum spp.	Bolivia	D. Spooner, G. Aguirre, A. Devaux
Trifolium spp.	USA	N. Taylor, K. Quesenberry
Helianthus spp.	Canada	G. Seiler, M. Brothers
Juglans spp.	Kyrgyzstan	M. Thompson, G. McGranahan, C. Leslie

USDA/ARS Plant Explorations Planned/Undertaken in FY 1995

Plant Exploration	Country	Principal Contacts
Small Fruits	Bolivia	J. Ballington, W. Messinger, D. Williams
Prunus spp.	USA	C. Weeks, W. Okie, C. Ledbetter
Fragaria virginiana	USA	J. Ballington, J. Payne
Oilseed crops	USA	D. Dierig, J. Rebman
Phaseolus vulgaris	Argentina	J. Steadman, C. Sandlin, R. Neuman
Arachis spp.	Brazil	R. Pittman, C. Simpson
Forage legumes	Russia	S. Greene, M. Rumbaugh, A. Afonin
Solanum spp.	Guatemala	D. Spooner, F. Vargas

PLANT GERMPLASM QUARANTINE OFFICE

During the past year we received for quarantine and pathogen testing 264 accessions of sugarcane, 70 stone fruits, 31 pome fruits, 8 grasses, 10 small fruits, 35 woody ornamentals, 36 sweet potatoes, 120 potatoes, 135 rice and 5 of tropical/miscellaneous crops. This represents 12% fewer accessions than were received the previous year. After January 1, 1995 all Solanum and Ipomoea accessions arriving as vegetative propagules have been secured and held in tissue culture during quarantine processing.

As expected ubiquitous viruses were detected in many accessions of pome and stone fruits, potatoes, sweet potatoes and sugarcane. Exotic pathogens were also detected in a few accessions of potatoes, sugarcane, sweet potatoes, and stone fruits. Most of the virus-infected sweet potatoes have now been through therapy via meristem tip culture and the new plantlets are being re-tested for virus. Likewise ca. 50% of the virus infected potatoes and most infected accessions of apples have been given therapy to eliminate viruses. As a result of successful therapy ca. 70 accessions of apples were released from quarantine this year. Some of the infected pears are receiving therapy by Joseph Postman at the Clonal Repository, Corvallis, OR.

Progress reports detailing the current inventory, indexing and therapy status, and the number of accessions released are prepared for the annual meetings of the various Crop Germplasm Committees. However, in summary the following number of samples (accessions x recipients) were shipped to U.S. germplasm curators and scientists during the past year; 680 stone fruit, 75 pome fruit, 109 potatoes, 533 rice, 376 sugarcane, 87 grasses, 53 sweet potatoes, 83 Ribes, 16 tropical root crops amd 5 woody landscape plants. Most of the germplasm is available to the research community by contacting the appropriate Plant Introduction Station or Clonal Repository.

We continue to update the capabilities of our quarantine information system (QIS) to better track our inventory, to support indexing data collection, and to facilitate distribution of germplasm and automated correspondence with importers. The Quarantine Office inventory can be accessed through

GRIN.

Research objectives continue to focus on finding faster, more reliable and cheaper ways to detect some of the more insidious viruses, viroids, MLOs and other infectious agents. To this end, research was conducted on apple proliferation MLO, pear blister canker viroid and fruit crinkle viroid of apple, prune dwarf virus, plum line pattern virus and currant reversion pathogen. Also, twenty Prunus species were evaluated for sensitivity to hard-to-detect pathogens; among them peach latent mosaic viroid, peach wart agent, little cherry virus, and green ring mottle agent which currently require long testing periods or give unreliable results. In addition, attempts were made to isolate several viruses in exotic genera of Nicotiana, from which the viruses can be manipulated and rapid tests developed.

Another research objective was to determine whether the peach latent mosaic virus is present in imported stone fruit germplasm. Ten accessions were tested using PCR and positive results were obtained with some. The search for a biological test to confirm these results continues.

There are dozens of named virus-like diseases of stone fruits not known to occur in the U.S. for which APHIS requires testing. Questions exist whether the pathogens, or only the names of the diseases, are different from domestic diseases. Cultures of 11 foreign diseases were obtained and are being compared with 11 domestic viruses on 8 standard stone fruit virus woody indicators. Although many have incited symptoms in some of the indicators, it will be a year or more before any conclusions can be drawn.

Last year a 'new' poty-like virus was reported to have been discovered in Canada in stone fruit germplasm. One of many questions was whether this virus would cause any significant disease in commercially-grown varieties of plums, peaches, or apricots. To determine this we have inoculated 3 widely grown varieties of each Prunus fruit type. So far no symptoms have been observed.

If there are questions, problems or suggestions concerning quarantine procedures or specific germplasm in quarantine, please call us at 301-344-3003 (Glenn Dale) or 301-504-8624 (Beltsville).

H.E. Waterworth, S.S. Hurtt, B. Parliman

Publications:

Bliss, W. O., C. L. Sutula, H. E. Waterworth and R. W. Fulton. 1994. An ELISA to detect American plum line pattern virus. Phytopathology 84:1131.

Damsteegt, V. D., H. E. Waterworth, G. I. Mink, W. E. Howell and L. Levy. 1994. Prunus tomentosa, bioindicator for plum pox virus. Phytopathology 84: 1152.

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MOLECULAR VIROLOGY INVESTIGATIONS

Sensitive and rapid methods, based on polymerase chain reaction (PCR) technologies have been developed for detection of apple chlorotic leaf spot virus, apple stem pitting virus from peach and a virus related to grapevine virus A from apples and pears. PCR technologies have also been extended for the identification and detection of prune dwarf virus, banana bunchy top virus, chrysanthemum stunt viroid, and potato spindle tuber viroid from tubers and true seeds without the need to germinate tubers or seeds into seedlings as required by other detection methods. Plum pox virus (PPV) was detected by PCR from all parts of sour cherry trees from Moldova. This virus isolate has been identified by sequencing analysis as a unique member of the severe M strain of PPV or as new strain by itself. It has been transmitted by grafting to both sour and sweet cherries. PPV was also detected from naturally infected sweet cherry trees from Italy and its identity was verified by sequence analysis of the 3' non-coding region of the viral genome. PPV was detected from anthers of infected apricots, peach, and plum trees from Hungary, which suggested that stone fruit anthers could be a source of PPV dissemination and possibly infection during the movement of germplasm. The Asian prunus

latent virus, a PPV-like virus, was further characterized. An in vitro heat or cold therapy combined with apical meristem culture eliminated apple scar skin viroid from infected pear trees. Thus the risk of introducing this pathogen from Asia to the U.S. is reduced. Simultaneous diagnosis of viroid mixed infection is citrus plants has been accomplished by multiplex PCR. A new viroid-like agent has been isolated, cloned and sequences from infected blueberry and a new phytoplasma associated with cherry lethal yellows in China has been detected and identified. The results of research will be used by plant pathologists and plant quarantine officials responsible for keeping foreign plant diseases from entering the U.S.

Ahmed Hadidi

Publications:

Podleckis, E.V., Hammond, R.W., Hurtt, S.S., and Hadidi, A. 1994. Comparison of digoxigenin-labeled and ^{32}P-labeled probes: Dot blot detection of plant viroids. B.M. Biochemica 10:12-13.

Halbrendt, J.M., Podleckis, E.V., Scorza, R., Hadidi, A., and Welliver, R. 1994. Evaluation of Prunus for resistance to nematode transmission of tomato ringspot virus. HortScience 29:1068-1070.

Minafra, A., and Hadidi, A. 1994. Sensitive detection of grapevine virus A, B, or leafroll-associated III from viruliferous mealybugs and infected tissue by cDNA amplification. J. Virol. Methods 47:175-188.

Levy, L., Lee, I-M, and Hadidi, A. 1994. Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroid and MLO nucleic acids. J. Virol. Methods 49:295-304.

Hadidi, A. and Levy, L. 1994. Accurate identification of plum pox virus and its differentiation from Asian Prunus latent potyvirus in Prunus germplasm. EPPO Bulletin 24:633-643.

Levy, L. and Hadidi, A. 1994. A simple and rapid method for processing PPV-infected tissue for use with PPV specific 3' non-coding region RT-PCR assays. EPPO Bulletin 24:595-604.

Nemchinov, L., Hadidi, A., and Verdrevskaya, T. 1994. Plum pox virus in sour cherry: confirmation of its identity by RT-PCR, cloning, and nucleotide sequencing of the genomic 3' non-coding region. Conference on Fundamental and Applied Problems in Phytovirology, Crimea, Yalta, Ukraine, page 11 (Abstr).

Nemchinov, L., Candress, T., Foster, J.A., Verdrevskaya, T., and Hadidi, A. 1994. Sensitive detection of apple chlorotic leaf spot virus from pome and stone fruits by reverse trascription-polymerase chain reaction amplification. Conference on Fundamental and Applied Problems in Phytovirology, Crimea, Yalta, Ukrain, page 57 (Abstr).

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Crescenzi, A., Nuzzaci, M., Levy, L., Piazzolla, P., and Hadidi, A. 1994. Plum pox virus in sweet cherry. 16th Interatioal Symposium of Viruses and Virus Diseases of Temperate Fruit Crops, page 52 (Abstr).

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Parakh, D.R., Shamloul, A.M., Hadidi, A., Scott, S.W., Waterworth, H.E., Howell, W.E., and Mink, G.I. 1995. Detection of prune dwarf ilarvirus from infected stone fruits using reverse transcription-polymerase chian reaction. Acta Horticulturae (in press).

Shamloul, A.M., Minafra, A., Hadidi, A., Giunchedi, L., Waterworth, H.E., and Allam, E.K. 1995. Peach latent mosaic viroid: Nucleotide sequence of an Italian isolate, sensitive detection using RT-PCR and geographis distribution. Acta Horticulturae (in press).

Postman, J.D., and Hadidi, A. 1995. Elimination of apple scar skin viroid from by in vitro thermo-therapy and apical meristem culture. Acta Horticulturae (in press).

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Shamloul, A.M., El-Afifi, S.I., and Hadidi, A. 1995. Sensitive detection of banana bunchy top virus from infected leaves, tissue cultures and viruliferious aphids using polymerase chain reaction. Annual Meeting of the Potomac Division of the American Phytopathological Society. (Abstr.)

Nemchinov, L., and Hadidi, A. 1995. Sour cherry isolate of plum pox virus is a unique member of the viral M strain and it is transmitted by chip budding to sour and sweet cherry. Annual Meeting of

TROPICAL BIODIVERSITY

Following publication of an Amazonian Ethnobotanical Dictionary, an Andean Ethnobotanical Dictionary, including important Andean species that might serve as alternative crops to coca was started. These useful Latin America plants yield non-timber forest products whose values make the standing forest more valuable to native Americans. Databases on folk uses of tropical plants, natural medicines, natural pesticides, and nutritional composition of tropical food species were moved to Internet making them available to other researchers. The computerized bibliography of the coca plant, Erythroxylum coca, was updated and an update on the poppy bibliography was initiated. Germplasm accessions of Erythroxylum spp. and alternative crops were collected as part of a special program.

James Duke

Publications:

Beckstrom-Sternberg, S.M. and Duke, J.A. 1994. Potential for Synergistic Action of Phytochemicals in Spices, pp. 201-223, in: Charalambous, G. (Ed.) "Spices, Herbs and Edible Fungi", Elsevier Science B.V.

Duke, J.A. 1994. Biologically-Active Compounds in Important Spices, pp. 225-250, in: Charalambous, G. (Ed.) "Spices, Herbs and Edible Fungi", Elsevier Science B.V.

Duke, J.A. 1994. Synergies among natural pesticides. Presented at the 13th National Congress of Phytopathology, September 18-23.

Duke, J.A. and Beckstrom-Sternberg, S.M. 1994. "Acceptable" Levels of Flavoring Ingregients?, pp. 741-757, in: Charalambous, G. (Ed.) "Spices, Herbs and Edible Fungi", Elsevier Science B.V.

Duke, J.A. and duCellier, J.L. 1994. CRC Handbook of Alternative Cash Crops (Special Indonesia Printing). CRC Press, Boca Raton, FL 33431. 536 pp.

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Attachment 2

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area National Seed Storage Laboratory
1111 South Mason Street
Fort Collins, CO 80521-4500
Telephone: 303 495-3200
Fax: 303 221-1427

May 11, 1995

SUBJECT: NSSL 1994 Progress Report

TO: Regional Technical Advisory Committees on Plant Germplasm

FROM: National Seed Storage Laboratory
Steve A. Eberhart, Director
Eric E. Roos, Research Leader
Loren E. Wiesner, Research Leader

ADMINISTRATION

Steve A. Eberhart, Director

The final phase of the move into the expanded facility was completed with the relabeling and relocation of 326,359 samples within the cold vault. Year-end funds made available by the Northern Plains Area in 1994 were used to purchase shelving for an additional six racks, which should take care of needs for several years (only six racks remain without shelving). Additional cryotanks were purchased with residual funds from the construction project bringing the number to 36. As this exceeds the capacity of the current liquid nitrogen distribution line, we have added this item as a high priority need if year-end funds become available. We have upgraded the telephone PBX to handle planned changes in area codes. The NSSL telephone and fax numbers changed from area code 303 to 970 on April 2, 1995. Equipment in the expanded facility continues to operate as planned with normal repair and maintenance.

A Memorandum of Understanding (MOU) was completed to continue the security backup storage of rice for IRRI, and one was drafted for the CIMMYT wheat and triticale. CSSA has proposed a new category of registration for varieties and parental lines licensed under the Intellectual Property Rights Trade Secrets. ARS agrees to store such materials as Security Backup Plant Materials provided these accessions are released to become part of the NPGS base and active collections after no more than 20 years of storage plus additional reporting requirements. A MOU has been drafted for consideration by CSSA.

The Latin American Maize Project (LAMP) is nearing completion. "The Stage 4 Results from Homologous Areas 1 and 5" are ready for publication and distribution

on CD-ROM. A joint Maize Regeneration Project Review and LAMP Stage 5 meeting was held at CIMMYT headquarters last April. A project proposal for a LAMP II was discussed. Principal Investigators from the 13 cooperating countries unanimously agreed on the need for LAMP II and that efforts are needed to obtain the required funding.

SEED VIABILITY AND STORAGE RESEARCH UNIT

Loren E. Wiesner, Research Leader

The Seed Viability and Storage Research Unit, during the last 13 months, has received 20,940 new samples. Seed samples included 338 from the Plant Variety Protection Office, 37 of endangered species from Botanical Gardens, 410 from plant quarantine, 16,503 from Active sites, 357 from Crop Science registration, 2,253 from the LAMP maize regeneration project, and the remaining 891 from plant breeders, individuals and organizations. Vegetative propagule samples consisted of 151 apple buds. Of the samples received, 7273 were replacement samples for accessions at NSSL. A total of 15,882 samples were placed into long-term storage and 18,775 quality evaluation tests were conducted. In addition to processing these samples, 326,359 samples were relabeled and relocated during the first 2.5 months of 1994.

Samples of 1,632 rye accessions were regenerated by the Botanical Garden of the Polish Academy of Sciences, Warsaw, Poland, during the period of 1986-1994. Half of each sample shipped to the U.S. is stored in liquid nitrogen cryotanks at the NSSL and the other half is stored in the National Small Grain Collection. Germination exceeded 90% on most of the samples. A portion of each sample was retained at the Botanical Garden in Poland.

In the cooperative USDA/USAID maize regeneration project with CIMMYT and USAID, seed has been received at NSSL from Argentina (57), Bolivia (374), Chile (228), Ecuador (90) and Mexico (268). Additional accessions were regenerated in 1994. In the total project, 7,432 are under regeneration with an additional 5,551 planned for 1995 to 1997, and seed of 2,974 accessions have been deposited in NSSL for long-term preservation. An additional 1,236 accessions were regenerated by CIMMYT and sent to NSSL last year. As a means to improve the quality of seed, a seed dryer was obtained for Mexico, and plans were made for purchasing dryers for other countries.

A total of 4,593 accessions were distributed to 111 Scientists. Sorghum (985) and maize (65) accessions were packaged, washed and sent to St. Croix for increase

under quarantine. Maize seeds from Argentina and a North Dakota Indian tribe, were regenerated in the Greenhouse. Thirty-one Argentina maize accessions and one accession from the Indian tribe were rescued. In addition, 86 other NSSL accessions were increased by Greenhouse staff.

A cooperative study with the Plant Germplasm Preservation Research Unit and the Geneva Plant Genetics Resources Unit to cryopreserve apple buds is continuing. An additional 151 apple accessions were stored in LN2.

Research conducted by Dr. C. Stushnoff under a Specific Cooperative Agreement on the cryopreservation of cold tender apple germplasm indicates that these materials can be successfully stored in LN2. Treatment of cold tender apple buds using alginate gel encapsulation and sucrose imbibition was repeated to verify promising results obtained the previous year. Survival following cryopreservation in LN2 ranged from 20 to 100%, similar to results obtained last year. Anatomical studies of strawberry plantlets regenerated from leaf disks showed cell division and enlargement after 3 days in culture, well developed shoot meristems and leaf primordia appeared after 21 days in culture, and osmolar desiccation resistance to sucrose increased with age from 7 to 28 days in culture. It appears that the role of sucrose before freezing may be an important link to its role as a stabilizer during the drying process. Raffinose is also a very effective cryoprotectant of the cold labile enzymes phosphofructokinase and lactate dehydrogenase.

Because very little information is available for viability testing of endangered species, a study was conducted using *Penstemon pendlandii. Seed* was collected on two different dates from two locations in order to have seed of different maturity levels and environments. The viability of seed collected was determined using tetrazolium staining procedures. Two cutting methods were used and both produced similar results. Mature seed from the southern site did not germinate at any temperature on the thermogradiant plate after 18 days. When seed was scarified and germinated on the thermogradiant plate, two temperatures gave the best results, 15-25°C and 15-30°C. We selected 15-25°C for further germination studies because it produced more rapid germination and had less contamination. Gibberelic acid soaking without scarification did not stimulate germination. GA3 treated scarified seed was equal to scarified seed, but no benefits from GA3 treatments were observed. Prechill treatments at 4°C for 4 to 24 months were conducted. Highest germination was 13% after 24 months of prechill. Hand clipping treatments resulted in 100% germination after 20 days, and mechanical scarification produced germination percentages ranging from 66 to 91% after 21 days of germination.

Work has continued on the evaluation of duplication in the NSSL pea collection. Extraction procedures for obtaining purified pea DNA have been developed and

RAPD's are being run. These data will be compared with morphological characteristics of pea accessions considered to be duplicates.

Lack of space in the old facility, need to barcode and inventory our collection, moving the collection to the new facility, rearranging and reassigning locations for each sample in the new vault, loss of two technician positions, and increased funding for seed increase of maize landrace collections has created a backlog of 30,043 samples in temporary storage that need to be processed. Furthermore, with the expanded facility, NSSL can now accept the 139,447 accessions from the active sites which have not been backed up at NSSL.

The number of samples with and without backup by site is as follows:

SITE	NUMBER OF ACCESSIONS	
	Backed up	Not Backed up
North Central Regional PI Station	18,209	22,359
Geneva Plant Genetics Resources	7,278	7,479
Southern Plant Genetics Resources	26,916	54,979
Western Regional PI Station	37,472	20,384
National Small Grains Collection	92,963	25,805
Potato Introduction Station	3,360	1,881
National Soybean Research Lab.	11,939	2,476
Southern Crops Research Lab. (Cotton)	2,669	3,338
Northern Crops Research Lab. (Flax)	2,654	5
Tobacco Crops Research Lab.	1,336	741
Sub-total	204,796	139,447

Active sites in the next year will be sending NSSL backup samples of accessions. Accessions with adequate seed numbers and viable seed will be handled routinely. For accessions with limited seed numbers, each site will provide NSSL enough seed for two regenerations. These shipments will add 139,447 samples to the NSSL inventory. These backup samples, in addition to the 15,000 to 20,000 samples regenerated each year at the active collection sites, will increase the backlog.

Several steps that we have taken to improve our efficiency of operation and increase our productivity include:

1. Developed computer programs to calculate quality data and upload data to GRIN.
2. Encouraged curators of active collections to thoroughly clean seed samples before sending to NSSL.

3. Requested curators of active collections, who are conducting germination tests, to send their results and seed to NSSL as soon as possible so we can use their germination results and store the seed under sub-zero temperatures.
4. Reduced the frequency of retesting viability based on research conducted by Plant Germplasm Preservation Research Unit and due to reduction in permanent staff positions.
5. Increased the temperature and relative humidity of one equilibration room to accelerate the drying process and reduce energy costs.
6. Reduced the number of moisture tests conducted because seed is dried to equilibrium at a relative humidity that produces a near optimum seed moisture for storage at sub-zero temperatures.

Even with these changes, it is clear that we cannot process for storage all of the backlog and the accessions being regenerated each year without additional financial support. We have been able to hire two temporary technicians with temporary funds from Headquarters and have redirected one technician from the Greenhouse into the Seed Quality Evaluation group. With these changes, it is our goal to process for storage 30,000 samples per year and have a manageable backlog by September 1996.

Presently the NSSL has 349,290 samples in long-term storage which include 265,064 unique accessions representing 2,978 species. The remaining samples are multiples of these unique accessions. The unique accessions also contain several types of materials not in the base collection, including 6,728 genetic stocks, 2,761 quarantine accessions, 338 endangered species, and 3,504 Plant Variety Protection voucher specimens. NSSL has 490 of the 3,504 PVP accessions in the base collection and more will be added as the 20 year protection expires.

NSSL has 42,769 unique accessions in the base collection that are not in the active collections. Of these, 10,756 are below 65% germination. Lists of these accessions were provided to curators of active collections to assist them in setting priorities for regeneration work this year.

PLANT GERMPLASM PRESERVATION RESEARCH UNIT

Eric E. Roos, Research Leader

The following reports summarize research conducted by the scientists in the Plant Germplasm Preservation Research Unit. Each report summarizes a single project and includes personnel assigned, the problem area, approach, and results for the past year. Following the reports is a list of publications for the 1994 calendar year.

SEED LONGEVITY STUDIES

ERIC E. ROOS (Supvry. Plant Physiol.); Don Davidson (Biol. Sci. Tech.); James Young (ARS Collaborator, Reno, NV); Jian Fang (Grad. Student, China); Edward Towers (Student)

PROBLEM: Long-term preservation of plant genetic diversity using the most cost-effective means will usually involve storage of seeds under optimum storage conditions. Although we generally know that this is best accomplished by cold, dry storage, we are unable to reliably predict how long seeds will remain viable under these conditions. Nor do we know how different species will store, or how seed lots within a species will behave. The problem then is to acquire longevity data on seeds stored under controlled temperatures and seed moisture contents for a wide variety of species and seed lot accessions in order to validate theoretical approaches.

APPROACH: Seed longevity data are acquired from several sources including experiments initiated by previous scientists at NSSL or elsewhere. Included in these are seeds from the Rincker study on forage legume and grass seed; various vegetable seed lots from earlier work by James and Bass; and seeds of several desert species from the Went longevity study. Also, seed lots which have been in storage at NSSL since the early 1960's are available for viability assessment. Seed moisture contents are determined along with the germination level. Seedling vigor may also be assessed. Seed lots having very low viability levels are targeted for seed rescue attempts (see report by S.A. Blackman).

RESULTS: No tests were scheduled for the Rincker and James experiments this past year. In 1995 we have scheduled tests of some of the Rincker seed and the Went longevity experiment. A summary of data from NSSL base collection samples across several species has revealed a common trend. Many samples received in the 1960's are dropping rapidly in viability. In particular, tests done in the mid 1980's show a big drop from the last test done in the late 1970's. Is this just the normal decline in viability with storage time or is there some factor in our procedure that is contributing to this general decline? We are currently investigating this question. Another somewhat unexpected result from analyzing these data is that initial germination percentage is not always a reliable predictor of longevity. For example, if we classify samples into three groups according to germination of 90%+, 80 to 89%, and less than 80%, we always find some samples in the lower germination groups performing better than those in the highest germination group. Clearly we need a better predictor of long-term survival that just the standard germination test.

SEED LEACHATE CONDUCTIVITY STUDIES

ERIC E. ROOS (Supvry. Plant Physiol.); Christina W. Vertucci (Plant Physiol.); Don Davidson (Biol. Sci. Tech.); Frank D. Moore (Collaborator, Colo. St. Univ.); Kimberly Davidson (Grad. Student); and Jian Fang (Grad. Student, China)

PROBLEM: Current practices for monitoring germplasm viability utilize the standard germination test. The net result is that valuable seeds are used up in this process and are discarded after each test. A non-destructive test would allow the same seeds to be reused for subsequent tests thus saving valuable seeds, but also providing a true monitor of deterioration on a given sample of seeds.

APPROACH: We have been working cooperatively with Colorado State University in developing the seed leachate conductivity test. The objective has been to shorten the time period of soaking from 24 hr to 2 to 4 hr, followed by a dry-back and then subsequent tests on the same seeds. One factor identified as important is aeration during the soaking period. We have developed a device to do this. Another factor of importance is the rate or speed of dry-back.

RESULTS: Current research is centered on the development of a modified seed leakage apparatus, that, in addition to doing single seed analyses, could also be used to provide oxygen and/or various gas mixtures to the seed during the leaching period. Such treatment could result in extending the number of successive leakage tests performed on a given set of seeds. We have filed a patent application for this device. Rapid drying of corn seeds, after an initial 2 hr soak, to their original moisture content, prior to a second leaching test, has allowed us to do six successive evaluations without loss of either germination percentage or vigor, as determined by root lengths. While these results are encouraging, we still need to apply this to an actual aging test.

RESCUE OF GERMPLASM FROM DETERIORATED CORN SEED LOTS

SHEILA A. BLACKMAN (Plant Physiol.); Eric E. Roos (Supvry. Plant Physiol.); Joseph R. Manalo (Biol. Sci. Tech.); M. Rittner, M. Wagner (Students)

PROBLEM: A serious problem for genebanks occurs when seeds are received in poor condition. This may result from suboptimal field conditions during seed development and harvest, suboptimal harvesting and drying practices, or suboptimal storage or transport conditions following harvest. Occasionally, seeds with high germinability may also deteriorate unexpectedly between viability monitoring tests when optimal storage conditions are not maintained. The very low germination rates of these deteriorated seeds may preclude successful field increases, even though they may

represent valuable germplasm. Thus, the problem of recovering such material is of considerable practical urgency.

APPROACH: Our overall strategy for rescuing germplasm from deteriorated seeds is divided into three phases. These phases correspond to levels of complexity. When a deteriorated seed lot is received by the seed lab, rescue is attempted by moving sequentially through each phase. Phase I (whole seed rescue) is a protocol designed in cooperation with Joe Manalo at the NSSL greenhouse. Phase II was developed in our laboratory. We are currently investigating the feasibility of using tissue culture (phase III) in our laboratory.

RESULTS: Phase I. In order to develop a protocol for seed rescue, the effects of various antibiotics, carbon and other nutrient sources, and hormones were evaluated on the growth of excised maize embryos in tissue culture. Kanamycin and thiram were effective against bacterial and fungal contamination and improved the growth of aged embryos. Amongst the carbon sources tested, only sucrose stimulated growth of isolated embryos, but not whole seeds. Complex nutrient mixtures containing macro- and micro- nutrients as well as vitamins did not stimulate growth of whole embryos but they did stimulate the growth of isolated axes. Amongst the hormones, only gibberellic acid stimulated the growth of the primary root. Both germination and growth of aged embryos were increased by the embryo culture protocol developed in this study when compared with whole seed rolled paper towel methods. We have concluded that this protocol can be used to rescue maize germplasm accessions with a germination percentage below that which results in successful field regeneration.

Phase II. The following table compares the effectiveness of the embryo rescue method with that of whole seed rescue techniques. Seeds used in this study were actual seed lots received by NSSL in deteriorated condition. It is apparent that the embryo culture protocol we designed effectively increased the germination rate of these deteriorated seeds. However, some plants, whether they were grown from isolated embryos or whole seeds, had very low seed production. We attribute this to problems in the greenhouse grow-out of these plants, rather than the germination conditions.

Comparison of Whole Seed Germination (W.S.) and Embryo Culture (E.C.) Techniques for Rescuing Deteriorated Seed of Argentinean *Zea mays* populations.

Serial No.	No. of Seeds		No. (%) of Plants Recovered		No. (%) of Plants With Seed		No. of Seeds Harvested	
	W.S.[1]	E.C.[2]	W.S.	E.C.	W.S.	E.C.	W.S.	E.C.
192866	20	44	18 (90)	35 (80)	0 (0)	1 (3)	0	36
192584	20	43	8 (40)	21 (53)	6 (75)	16 (76)	593	1978
192844	17	44	6 (35)	31 (71)	0 (0)	2 (6)	0	6
192897	15	40	4 (30)	14 (35)	0 (0)	3 (21)	0	267
192549	25	87	3 (12)	29 (33)	0 (0)	3 (10)	0	200
192966	25	87	3 (12)	29 (33)	0 (0)	3 (10)	0	49
193579	0[3]	44	---	21 (48)	---	21 (38)	-	1829
192973	0[3]	30	---	6 (20)	---	2 (33)	-	55

[1]Whole seed germination.
[2]Embryo Culture.
[3]Low seed numbers precluded rescue by whole seed germination.

THE EFFECT OF SEED AGING ON RESPIRATION OF CORN EMBRYOS

SHEILA A. BLACKMAN (Plant Physiol.); Eric E. Roos (Supvry. Plant Physiol.); M. Rittner, M. Wagner (Students)

PROBLEM: The mission of genebanks - to preserve the world's germplasm for future breeding - is often frustrated by resource limitations. Regular seed testing and regrowth requires manpower; environmental controls require reliable electrical power. Tightening budgets dictate that seeds be stored in as cost-efficient manner as possible. For the U.S., this means carefully optimizing moisture content and temperature during storage. An ongoing debate over optimum moisture content hinders our attempts to do this. This debate is fueled by the fact that the seed aging has usually been modeled at unusually high moisture contents and temperatures in order to accelerate the aging process. Results from these studies have often been extrapolated to the lower moisture contents and temperatures that are common in seed storage facilities. Our work attacks the debate over optimum storage moisture

contents from a mechanistic perspective. We wish to determine: a) what mechanisms are responsible for seed deterioration under different storage conditions; b) what are the ramifications of these deteriorative reactions for metabolism and germination and; c) what can be done to ameliorate the effects of these reactions in germinating seed.

APPROACH: Our approach has been to characterize biochemical pathways in imbibing corn embryos by monitoring carbon dioxide evolution (an index of respiratory activity) by infrared gas analysis. The activities of different metabolic pathways were manipulated with hypoxia, metabolic inhibitors and mutants of intermediary metabolism to determine the importance of these pathways to germination. We are examining the status of these basic metabolic pathways in embryos from seeds stored at high temperature and relative humidity, at high temperature but very low relative humidity and under optimal conditions (low moisture content, low temperature). Our premise is that the period preceding radicle elongation is crucial in determining subsequent growth rate of the germling. Therefore, we are focussing our studies on this critical period in germination.

RESULTS: Our results show that all embryos - whether unaged or aged - are remarkably insensitive to low oxygen concentration early in imbibition because they have a low energy demand. As germination proceeds, energy demand increases - presumably to meet increasing biosynthetic requirements. As the demand for energy increases, embryos from high quality seeds evolve the byproduct of fermentation, ethanol. We have found that both carbon dioxide and ethanol production declines in parallel with seed quality when the seeds are aged at high - but not low - moisture content.

An intriguing result of our work is that carbon dioxide evolution actually is higher in embryos from seeds aged at high temperature and low moisture content than in embryos from seeds stored optimally or at high moisture contents. This suggests that aging at very low moisture contents creates different lesions than aging at high moisture content. The high levels of carbon dioxide evolved from embryos of seeds aged under high temperature and low moisture contents is not derived from conventional aerobic or anaerobic pathways. This may be a byproduct from the accumulation of some oxidized carbon compound during storage at low moisture contents. We are presently investigating this possibility.

SEED IMAGE ANALYSIS

PHILLIP C. STANWOOD (Res. Agron.); Lana Wheeler (Biol. Sci. Tech.)

PROBLEM: Long-term preservation of plant genetic resources in the form of viable seeds and vegetative tissues, is needed to assure future availability of plant genetic

variability. Without these sources of genes, development of agronomic, horticultural and medicinal plants for disease and pest resistance, environmental and cultural adaptation and general plant improvement will be greatly restricted. My research is directed at: 1) increasing seed longevity in storage through the use of cryogenic techniques; 2) developing techniques to measure early seed deterioration, before loss of viability; and 3) developing digital imaging techniques to better characterize plant genetic resources and methods to consolidate and distribute that information.

APPROACH: Current research is directed at: 1) documenting and understanding long-term effects of cryopreserving seeds and pollen using liquid nitrogen as a storage medium; and 2) the use of computer digital analysis to measure vigor of seed germplasm and to develop the concept of an image oriented database (electronic seed [plant] herbarium) that can be easily distributed and used by interested individuals and organizations.

Figure 1: Images from a SGR (root growth analysis) robotic system. The image on the left shows lettuce seedlings after 3 days of growth. The image on the right depicts roots that have been identified and measured using the SGR imaging and evaluation software.

RESULTS: A robotic system based on computer image analysis was built to simultaneously conduct 50 seedling root growth vigor tests. Several species have been successfully grown on the slant board including lettuce, onion and sorghum seed. Most of the year was devoted to developing and refining control, imaging and analysis software to evaluate root growth curves. Lettuce seeds were used as the test species. Early results indicate that measurement error rates are less than 1%, most lettuce samples can be evaluated in less than 90 hours, and as few as 50 seeds can provide acceptable data with acceptable levels of statistical error. Data that can be extracted from the growth curves include: hours to root emergence, hours to maximum root growth, length of root at maximum growth rate, maximum root length and percent germination.

A seed image database project was started in conjunction with the Plant Germplasm Regional Station in Pullman, Washington and the ARS-GRIN project in Beltsville, Maryland. The core subset of chickpea (505 accessions) is being used as the first test species. Image sets are developed according to source of materials and objects being imaged. Three primary image sets were imaged, digitized and placed on CD-Rom in 1994. The images sets were: 1) Seed images for each accession from the NSSL base collection; 2) An identical set of seed images from the active collection maintained by the Plant Introduction Station in Pullman, Washington; and 3) Images of mature field grown plants grown at Central Ferry, Washington. A software routine was developed to measure seed parameters in each images. Those seed parameters include: length, width, apparent area, apparent perimeter, two shape descriptions, and average grey level. Parameters to measure the color of the seeds is under development. These image parameters, actual images and numeric information from the GRIN (Germplasm Resources Information Network, USDA-ARS) database have been combined into an object oriented database. This database with supporting software is being developed and should be ready for distribution this year. Test images of seed have been placed on the GRIN computer which are available through 'Internet' to the user community. The full image sets of seed and field grown material will be placed on the GRIN computer in 1995. Some digital imaging assistance has been provided to the curators of the sorghum collection in Puerto Rico, the pear curator in Corvallis, Oregon and Dr. Reid Palmer (soybeans) in Ames, Iowa.

INTERPRETATION: The conservation of plant germplasm requires an understanding of physiological deterioration, methods to measure viability, and techniques to counter deterioration. Application of this information will result in improved maintenance of genetic materials by: 1) reducing the frequency at which a sample has to be tested and/or regenerated; 2) reducing maintenance costs both for equipment operation and support personnel; 3) providing greater physical protection of the specimen; and 4) providing a method of preservation for materials that are not or cannot now be routinely stored.

Computer digital analysis of biological materials is an emerging field that has significant potential for the characterization and evaluation of plant genetic resources. The primary project objective is to characterize seed deterioration using computer digital analysis of seedling growth. Resulting information is then related to seed longevity and physiological process leading to deterioration. A second imaging objective is to develop a digital image system to collect and evaluate morphological characteristics of seed germplasm diversity within a species.

CRYOPRESERVATION OF COLD-HARDY, WOODY GENETIC RESOURCES

LEIGH E. TOWILL (Plant Physiol.); J.W. Waddell (Biol. Sci. Tech.); T. Cooper (Student) and Phillip Forsline (National Clonal Germplasm Repository, Geneva, NY)

PROBLEM: Long-term preservation of clonal stocks is needed to avoid potential loss of germplasm. Cryopreservation allows for safe, long term storage which then gives the clonal repository options for minimizing costs with field or greenhouse maintenance. Apple (Malus spp.) is now the first clonal species to be routinely placed into cryogenic storage at NSSL, but the method needs to be improved. Specific problems are 1. How does the cryogenic protocol apply to diversity within the collection? 2. How can the procedure be modified to be more efficient and effective? 3. What alternatives can be used with particularly cold-tender lines? Some of these studies are ongoing and are summations from 2-3 years of work.

APPROACH: One hundred fifty lines were examined in winter-spring of 1993-4 using a procedure previously shown as generally effective. A budding test, performed at Geneva NY, was used to assess survival. Some variables of the procedure were examined with selected lines, including time held at -30°C, cooling rate between -30°C and -196°C, and warming rates for tender and hardy lines. Two procedures are viable alternatives to the dormant bud technique: a. use of in vitro plants with vitrification or two step cooling techniques b. use of dormant vegetative buds from field materials but retrieve plants after freezing with 'tissue culture' techniques (as opposed to grafting).

RESULTS: The lines used represent a range of cold-hardiness potential. The results were similar to those from other years (139 lines showed some survival after -196°C exposure) and reaffirm that the general procedure is useful, but that modifications are needed to improve percent survival and efficiency, particularly in tender lines. Drying of twig sections to about 30% moisture is laborious and inexact in the sense that the bud and bark moisture levels varied considerably in twigs with similar moisture content. In tests with other lines, cooling rates between -30°C and -196°C did not greatly influence survival, although there was considerable variability. Faster warming rates gave slightly higher viability. Tests with sour cherry and pear showed some survival using the standard dormant bud method. Survival was greater with sour cherry. From an analysis of labor and expenses, we estimate that to cryopreserve a line using the existing method costs NSSL about $26.

Previously we investigated the cryopreservation of buds from in vitro plants. Two cold-tender lines survived in high percentages after low temperature exposure using a vitrification technique. Survival using the dormant bud technique with field trees of these two lines was low in tests in previous years. Respiration rates of apple buds (field materials) were examined after certain treatments to determine if this is a feasible method to measure viability and to determine if buds from lines which normally do not survive cryopreservation are indeed alive. If they are, they could be retrieved by in vitro culture. Results were equivocal. Non-graftable buds do respire and respiration could be substantial, but we could not separate respiration due to microbial sources from that of the cells. Wounding itself enhances respiration. It

seems some cells are alive after treatment and suggests that retrieval after culture may be feasible once adequate surface disinfestation is achieved. Surface disinfestation of buds from field materials was incomplete and considerable contamination occurred in trials.

CRYOPRESERVATION OF SHOOT TIPS USING VITRIFICATION METHODS

LEIGH E. TOWILL (Plant Physiol.); J.W. Waddell (Biol. Sci. Tech.); G. Valdez (Biol. Sci. Tech.); D. Huber (Student)

PROBLEM: Vitrification, a method to cryopreserve diverse cells, tissues and organs, has been shown by us and others to be effective for a range of species. This is a relatively new method and is, as yet, not used as a routine method for cryopreservation. Vitrification is a process containing a series of steps; a number of these steps must be optimized. Modifications of the vitrification process need to be explored to develop a procedure that is efficient and effective. When incorporated, cryopreservation will allow clonal repositories options for cost savings in managing their field, greenhouse or in vitro collections. We continue to investigate aspects of vitrification for several species.

APPROACH: Most of these studies were with axillary buds excised from plants maintained in vitro. Such systems are axenic and minimize contamination when the treated axillary buds are cultured to produce the shoots. Both in vitro stock plants and the buds isolated from them may be treated prior to cryopreservation.

RESULTS: 1. A previous study of about 50 species of Mentha showed that most could be cryopreserved with a single method. We have begun a pilot test with 50 clonal lines from the Corvallis repository that are identified as being part of the core subset. From this we will obtain cost information to allow us to plan for future practical studies at NSSL. This will be the second vegetatively-propagated crop for which practical cryopreservation is being done at NSSL.

2. 'Encapsulation and dehydration' methods for cryopreservation also are a form of the vitrification procedure. Preliminarily, we determined the kinetics of drying and survival for mint. The procedure has some technical advantages and is being examined in more detail with potato. Shoot tips survive considerable desiccation, but do not survive subsequent exposure to -196°C. Preculture treatments are being studied to determine their effect on survival.

3. A short-term storage study at -160°C with vitrified shoot tips from Mentha sp. showed no loss of viability over a 12 month period. This is as expected for cryogenic storage and shows that glasses are, at least in the short term, stable and do not devitrify or crack under usual manipulations. Additional materials were put into

storage for examination in early 1995 (12-15 months of storage). Further studies are planned on glass stability with known vitrification solutions.

4. Although cold pretreatments of stock plants from potato and Arachis glabrata gave a slight improvement in survival after cryopreservation, exposure of the isolated shoot tips to elevated sucrose levels was more effective. It is uncertain what sucrose is doing to enhance survival and further studies are planned to determine its role.

ORTHODOX SEED STORAGE: OPTIMUM CONDITIONS AND MECHANISMS OF DETERIORATION

CHRISTINA W. VERTUCCI (Plant Physiol.); Eric E. Roos (Supvry. Plant. Physiol.); Steve Eberhart (Director, NSSL); Jennifer Crane (Biol. Sci. Tech.); Lisa Hill (Student); Robert Cook (Grad. Student); Melissa Andre (Student); Olivier Leprince (Plant Physiol.); Julia Buitink (Visiting Scholar, Netherlands); Kalyani Srinivasan (NBPGR, India)

PROBLEM: All seeds inevitably lose viability during storage. We do not know the cause of the aging nor do we have models that effectively predict the rate. By understanding the mechanisms by which seeds deteriorate in storage we can determine the optimum conditions for storage and efficiently predict when seeds should be regenerated.

APPROACH: We have taken a biophysical approach to the study of mechanisms of seed deterioration and optimal conditions for seed storage. Our research shows that the metabolic status of seeds is linked to the physical status of water in the tissues. A major portion of our research is directed towards identifying the properties of water and how the nature and kinetics of the aging reactions are controlled.

Our findings indicate that the mechanisms of seed deterioration vary according to the water content and temperature at which the seeds are stored. Lipid composition of the seeds appears to be important when seeds are stored under very dry conditions.

RESULTS: We have shown that the principles used to determine optimum conditions for seed storage hold for most seed species. This has allowed us to develop generalized protocols for storage and to make cost-benefit analyses of various storage practices. Our research provides the basis for more efficient protocols now used at the NSSL. Through our consultations with IPGRI, we are helping to develop more efficient seed storage practices in under-developed countries.

CRYOPRESERVATION OF RECALCITRANT SEEDS

CHRISTINA W. VERTUCCI (Plant Physiol.); Jennifer Crane (Biol. Sci. Tech.); Ervin Oelke (U. of Minn.); Raymond Porter (U. of Minn.); Mirian Eira (EMBRAPA, Brazil); Patricia Berjak (U. Natal, So. Africa); Norman Pammenter (U. Natal, So. Africa); David Mycock (U. Witwatersrand, So. Africa); Lisa Hill (Student); Melissa Andre (Student)

PROBLEM: Many economically important plant species from tropical areas and temperate wetlands produce seeds that do not survive desiccation. We are unable to store these "recalcitrant" seeds in the base collection of the NSSL by standard protocols.

APPROACH: We are studying the mechanisms and interaction of desiccation damage and freezing damage in seed tissues. We have shown that the expression of these stresses corresponds with the behavior of water in tissues. We are learning ways to manipulate water properties with various incubation procedures, cryoprotectants, and cooling treatments.

Our model systems have focused on *Zizania* spp (wild rice) and *Citrus* and related genera. These species are agronomically important to the US and a germplasm collection is either non-existent (wild rice) or exclusively in orchards (citrus).

RESULTS: Using the principle of optimum water levels, we have developed two methods to preserve recalcitrant seeds. In the first method, water content is adjusted to avoid both freezing and desiccation damage. Depending on species and maturity level, we achieve between 50 and 100% survival using this method. Efforts are underway to increase the cost-efficiency of this method and to determine the longevity of seeds stored under these conditions. A second method, called "vitrification," involves a slight and extremely rapid dehydration and then plunging embryos into liquid nitrogen at 2000C/sec. This method is labor intensive and is recommended only in instances where the first method is unfeasible.

ACQUISITION OF DESICCATION TOLERANCE IN DEVELOPING SEEDS

CHRISTINA W. VERTUCCI (Plant Physiol.); Jennifer Crane (Biol. Sci. Tech.); Jill Farrant (U. CapeTown, So. Africa); Olivier Leprince (Plant Physiol.); Lisa Hill (Student); Melissa Andre (Student)

PROBLEM: The ability to withstand almost complete desiccation is an unusual feature in biology but is common in many seeds. This ability is acquired during seed development and lost during germination. We believe that if we can understand how most tissues are damaged by desiccation and how some seeds acquire tolerance to

this stress, then we can learn how to improve seed quality and enhance our success in cryopreserving germplasm.

APPROACH: We are studying the requirement for water in developing embryos of orthodox and recalcitrant seeds in relation to the water properties and the presence of desiccation and cryoprotectants. Associated with these studies are studies of the metabolic status and cellular organization of the tissues.

RESULTS: Profound changes occur during seed development that may lead to increased tolerances to desiccation. One change, which may be associated with the presence of a protective protein, is the appearance of water with unusual thermodynamic properties. Results also suggest that desiccation tolerant tissues also have the ability to switch off metabolism during severe stress. We are now trying to manipulate developmental events *in vitro* using molecular markers and thermodynamic properties of water to indicate maturity status. With this technique, we hope to make recalcitrant seeds or orthodox seeds that were harvested prematurely more amenable to storage.

THE STATE OF WATER AND SEED STORAGE STABILITY

OLIVIER LEPRINCE (Plant Physiol.); Christina W. Vertucci (Plant Physiol.); Christian Rittner (Student)

PROBLEM: A model developed at the NSSL proposes that the characterization of the temperature-dependent changes in the properties of water in seed tissues can predict the water-temperature combination to achieve optimum conditions of storage. Another model considers that the water in dry seeds is in a glassy state. It has been suggested that the occurrence of a glassy state is associated with storage stability. We are studying the nature and properties of the glassy state with respect to the kinetics of degradative reactions underlying seed aging.

APPROACH: Using bean embryonic axes as model and differential scanning calorimetry (DSC) as method of investigation, we are characterizing the thermal properties of water in relation to the presence and stability of aqueous glasses. We have relied upon the food science and polymer science literature to gain insights in how glassy structures contribute to the stability of various materials. We used this information to show how the characterization of the glass properties provide insights on the long-term storage stability of seeds.

RESULTS: We observed that the glass transition exhibits three distinct types of thermal signatures which depend on the water content and temperature. Below 3 % of water no glass transition can be detected. Different types of glasses have also different amounts of energy associated with the transition. According to the literature

such differences observed at Tg reflects differences in structure, stability and fragility of the glass. They result from the conditions that induce the glass formation. We believe that differences in physical and/or structural properties of the intracellular glass in seeds are dependent both on the hydration level and the maturation drying.

PLANT GERMPLASM PRESERVATION RESEARCH UNIT
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Note: Names in bold caps are present or former ARS employee, or are supported on ARS funds.

NEW INDUSTRIAL CROP REPORT

for The Annual Meetings of the Regional Technical Committees on Germplasm
1995

Robert Kleiman
New Crops Research
National Center for Agricultural Utilization Research
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Peoria, Illinois

The year 1994 saw the harvest of 40,000 acres of crambe in North Dakota. This year, due to a change of ownership at the oil processing plant, there will be no crambe planted in that area. Hopefully, crambe planting will resume in 1996. At NCAUR however, we are continuing to develop new uses for erucic acid, the major fatty acid in crambe oil. These uses are being developed through a cooperative agreement with a large industrial firm and though a material transfer agreement with another.

Meadowfoam, *Limnanthes alba,* yielded some 640,000 lbs of seed from about 750 acres. This seed was crushed and refined and marketed by the Fanning Corporation for use in the cosmetics industry. In October 2200 acres of meadowfoam was planted in the Willamette Valley. The goal for October, 1995 is 8,500 acres of meadowfoam to be sown. Researchers at Oregon State University are continuing to develop high oil and auto-fertile varieties. NCAUR has been working with the Fanning Corporation to incorporate new products from meadowfoam into cosmetics and to develop contacts with the lubricant industry.

The progress in the area of lesquerella was accented by the second annual lesquerella field day held on April 15, 1995, in the Phoenix area. After a very interesting tour of the Sossaman Farms 30 acre Lesquerella field trial the group adjourned to the University of Arizona Experimental Farm at Maricopa, AZ. There they heard progress in the area of agronomics including herbicides, irrigation, planting dates and harvesting. Research in the area of seed germination and germplasm were highlighted along with uses for the oil and gums from this unique industrial crop. Germplasm collections are continuing, mainly seed from the Kentucky-Tennessee area, for lesquerella species with high densipolic acid. At NCAUR work is continuing to develop the lesquerella gums both in the area of product development and gum isolation. A number of new oil and fatty acid derivatives have been made to be tested for improved lubricant properties.

We are encouraged by the developments in Oregon by plantings of *Euphorbia lagascae*, a epoxy oil producing crop. These plantings are from non-shattering mutants developed in Spain. Seed increase is going on at this time. NCAUR will process this seed into oil for subsequent product development.

Cuphea development is progressing at Oregon State University. Lack of funds is impeding the rapid development of this new important industrial oil seed crop. Work in developing the non-shattering highbred which was discovered 2 years ago is progressing, along with mutation breeding to change a variety of characteristics including fatty acid composition.

Along with these industrial oil seed developments, work is continuing on a limited basis on the composition of new germplasm. This work includes oil content and fatty acid composition.

Publications:

Abbott, T. P., Wu. Y. V., Carlson , K. D., Slodki, M. and Kleiman, R. Isolation and preliminary characterization of *Lesquerella fendleri* gums from seed, presscake and defatted meal. J. of Ag. and Food Chem. 42:(8)1678-1684. 1994.

Abbott, T. P., Nelson, T. C. and Kleiman, R. Seed surface gums content using the DuBois carbohydrate analysis. Indus. Crops and Prod. Accepted for publication May 25, 1995.

Abbott, T. P., Nabetani, H., Sessa, D. J., Wolf, W. J., Liebman, M. N. and Dukor, R. K. Effects of bound water on FTIR spectra of glycinin. Submitted for publication 1995.

Burg, D. A., Kleiman, R. and Erhan, S. M. Production of Hydroxy Fatty Acids and Estolide Intermediates. U.S. Patent Number 5,380,894. Jan. 10, 1995.

Dierig, D. A., Thompson, A. E., Rebman, J. P., Kleiman, R. and Phillips, B. S. Collection and evaluation of new lesquerella and physaria germplasm. Indus. Crops and Prod. Submitted for publication 1995.

Erhan, S. M., Kleiman, R. and Isbell, T. A. Methods for increasing estolide yields in a batch reactor. Accepted for publication March 30, 1995.

Erhan, S. M., Kleiman, R. and Abbott, T. P. Quantitation of estolides by FTIR. In progress.

Hayes, D. G., Kleiman, R. and Phillips, B. S. The triglyceride composition, structure and presence of estolides in the oils of *Lesquerella* and related species. J. Am. Oil Chemists' Soc. Accepted for publication February 21, 1995.

Hayes, D. G., Kleiman, R., Weisleder, D. Adlof, R. O., Cuperus, F. P and Derksen, J. T. P. Occurrence of estolides in processed *Dimorphotheca pluvialis* seed oil. Indus. Crops and Prod. Submitted for publication 1995.

Hayes, D. A. and Kleiman, R. A detailed triglyceride analysis of *Lesquerella fendleri* oil using supercritical fluid chromatography and column chromatographic fractionation. Submitted for publication 1995.

Hayes, D. G. and Kleiman, R. Lipase-catalyzed synthesis of estolide esters and their properties. In progress.

Hayes, D. G. and Kleiman, R. Supercritical fluid chromatographic analysis of new crop seed oils and their reactions. In progress.

Nabetani, H., Abbott, T. P. and Kleiman, R. Optimal separation of jojoba protein with membrane processes. Indus., & Eng. Chem. Research 34:(5) 1779-1788. 1995.

Roath, W. W., Widrlechner, M. P. and Kleiman, R. Variability in *Cuphea viscosissima* Jacq. collected in east-central U.S. Indus. Crops and Prod. 3:217-223. 1994.

Sessa, D. J., Nelsen, Terry C., Kleiman, R. and Arquette, J. D. DSC Index for estimating level of saturation in transesterified wax esters. J. Am. Oil Chem. Soc. Submitted for publication 1995.

Thompson, A. E. Dierig, D. A. and Kleiman, R. Characterization of *Vernonia galamensis* germplasm for seed oil content, fatty acid composition, seed weight, and chromosome number. Indus. Crops and Prod. 2:299-305. 1994.

Thompson, A. E. Dierig, D. A. and Kleiman, R. Germplasm development of *Vernonia galamensis* as a new industrial oilseed crop for the temperate zone. Indus. Crops and Prod. 3:185-200. 1994.

Attachment 5

ANNUAL REPORT OF COOPERATIVE REGIONAL PROJECTS

Supported by Allotments of the Regional Research Fund,
Hatch Act, as Amended August 11, 1955
January 1 to December 31, 1995

1. **PROJECT: Regional Research Project S-009**

Plant Genetic Resources Conservation and Utilization

2. **COOPERATING AGENCIES AND PRINCIPAL INVESTIGATORS:**

State Agricultural Experiment Station Representatives

AL	J.A. Mosjidis*	NC	W.T. Fike*
AR	T.E. Morelock*	OK	J. Kirby*
FL	G.M. Prine*	PR	R.V. Colon*
GA	W.D. Branch*	SC	B.B. Rhodes*
HA	P.J. Ito*	TN	D.L. Coffey*
KY	N. Taylor*	TX	F. Hons*
LA	D. LaBonte* (Chair)	VA	R.E. Veilleux*
MS	C.E. Watson*		

Administrative Advisor G.F. Arkin

U.S. Department of Agriculture

Co-Administrative Advisor, ARS	R.G. Breeze
National Genetic Resources Program, ARS	H.L. Shands
National Germplasm Resources Lab, ARS	A.K. Stoner*
National Seed Storage Lab, ARS	S.A. Eberhart
NCGR-Subtropical Horticultural Res. Stn., ARS	R.J. Schnell
NCGR-Tropical Fruit, ARS	F.T. Zee
Tropical Agricultural Res. Stn., ARS	F. Vazquez
Northern Regional Res. Center, ARS	T. Abbott
Cooperative State Res., Edu., and Ext. Serv.	D.A. Sleper
Soil Conservation Service	H.W. Everett*

Plant Genetic Resources Conservation Unit,
Cooperative ARS and SAES

Regional Coordinator	S. Kresovich
Research Plant Pathologist	A.G. Gillaspie
Research Horticulturist	R.L. Jarret
Curator, Agronomist	G.R. Lovell
Curator, Agronomist	R.N. Pittman
Curator, Agronomist	J.B. Morris

* Indicates voting member of the Technical Committee

3. PROGRESS OF WORK AND PRINCIPAL FINDINGS:

Genetic resources representing over 1,200 new accessions were received, increasing the Unit holdings to in excess of 85,000. New introductions represented a broad range of genera and species. Over 7,500 seed regenerations were accomplished at Griffin or through cooperators across various sites. In response to approximately 1,000 requests, more than 35,000 distributions were made (approximately 90% were supplied to domestic users). Over 500 oligomers were distributed as molecular markers to profile ('type') accessions and map useful characteristics. In complement, an enhanced effort on database development of the Germplasm Resources Information Network (GRIN) occurred in 1995. More detail on these particular service activities can be obtained directly from the research leader, curators, and/or GRIN database manager.

In collaboration with recognized crop experts, evaluation studies were conducted on: (1) morphological and agronomic/horticultural traits in forage legumes, grasses, sorghum, peanut, watermelon, and pepper; (2) nematode resistance in sweet potato; (3) tomato spotted wilt and other viruses in grain legumes; and (4) watermelon mosaic virus resistance in watermelon. Associated characterizations were conducted in all regenerations by all curators.

Several unidentified viruses have been detected in the 1995 legume regeneration plots and work continues with host range, serology, electron microscopy, and electrophoretic techniques to identify these isolates. Once pathogens are characterized, efforts to sanitize collections is undertaken.

Various genetic analysis techniques, *i.e.*, RFLPs, RAPDs, AFLPs, and SSRs, are being applied to establish genetic identity, relatedness, and structure of accessions held in *ex situ* plant genetic resources repositories in the U.S. (work focused on holdings from Griffin, Mayaguez, Ames, Miami, and Geneva). At present, over 100 SSRs primer pairs are available for use in the crops such as sorghum, peanut, maize, watermelon, sweet potato, pepper, apple, and grape. Based on our experiences to date, we have improved efficiency, simplified protocols, increased the level of automation, and decreased the unit cost of each assay. This genetic analysis is conducted in collaboration with Pioneer Hi-Bred International, Perkin-Elmer, Linkage Genetics, and Sequana Therapeutics.

Significant effort went to infrastructure improvement during FY95. A -20C seed storage facility was obtained with additional support from the South Atlantic Area Office. Computer hardware, software, and peripherals were purchased for installation as part of the local area network. To support controlled pollination efforts, 100 large cages were acquired and will be used for FY96 regenerations of vegetables and annual forage legumes. To support genetic analysis work, the Unit received additional support for the purchase of a DNA sequencer/fragment analyzer and computer workstation for data handling and analysis.

4. USEFULNESS OF FINDINGS:

Results obtained via collaborative efforts among scientists at the regional repository, federal laboratories, state agricultural experiment stations, and industry are mutually beneficial as sources of information and products for all. Through the efforts at the repository, broad genetic representation of crop plants and their weedy/wild relatives is maintained for ready access. Cooperators identifying desirable traits among accessions aid in crop improvement efforts to produce a higher quality product more efficiently and in an environmentally safe manner. Information gained from cropping system studies of potential new crops may lead to greater diversification of agriculture in the southeastern United States.

5. WORK PLANNED FOR NEXT YEAR:

Operational priorities for 1996 include significantly expanded seed regeneration, back-up, and documentation activities, particularly for peanut, sorghum, forage grasses, and vegetables. In complement, genetics and plant pathology efforts will be directed to support curatorial functions.

A continued effort to improve Unit infrastructure also is planned. It is expected that the -20C seed storage facility and the local area network will be completed and operational in 1996. We also expect to access more land and additional pollination cages for regeneration efforts.

6. PUBLICATIONS ISSUED OR MANUSCRIPTS APPROVED DURING THE YEAR:

A list of approved manuscripts, theses, and miscellaneous reports addressing plant genetic resources conservation and utilization in the southeastern U.S. is attached as Appendix I.

7. **APPROVED:**

Chair, Technical Committee	3/7/96 **Date**
Administrative Adviser	3/8/96 **Date**

Publications Related to Evaluation and use of Plant Germplasm in the S-9 Region, 1995

ALABAMA

Qiu, J., J.A. Mosjidis, and J.C. Williams. 1995. Variability for temperature of germination in sericea lespedza germplasm. Crop Science 35:237-241.

Surrency, D.S, C.M. Owsley, M.S. Kirkland, D.V. McCracken, P.L. Raymer, W.L. Hargrove, J.L. Day, and J.A. Mosjidis. 1995. Registration of 'Americus' hairy vetch. Crop Science 35:1222.

Zhang, X. and J.A. Mosjidis. 1995. Breeding system of several Vicia species. Crop Science 35:1200-1202.

Mosjidis, J.A., C.M. Owsley, M.S. Kirkland, and K.M. Rogers. 1995. Registration of 'AU EarlyCover' Hairy Vetch. Crop Science 35:1509.

Mosjidis, J.A. and X. Zhang. 1992. Vicia Breeding System. Proceedings of the 48th Southern Pasture and Forage Crop Improvement Conference. Auburn, Alabama, April, 21, 1992, pp. 21-29.

Sakhanokho, H. 1995. Genotype x environment interactions in red clover (Trifolium pratense L.). M. Sci. Thesis Auburn University, Auburn.

Xie, C. and J.A. Mosjidis. 1995. Influence of sample size on the estimation of genetic parameters in a red clover population. Agronomy Abstracts. p. 92.

Mosjidis, J.A. and C. Xie. 1995. Sampling errors and confidence intervals of genotypic and phenotypic correlations in a red clover population. Agronomy Abstracts. p. 92-93.

ARKANSAS

No publications.

FLORIDA

Prine, G.M., J.A. Stricker, D.L. Anderson and W.R. Windham. 1995. Chemical composition of biomass from tall perennial tropical grasses. Proceedings Second Biomass Conference of Americas: Energy, Environment, Agriculture and Industry, Portland, OR, August 21-24. p. 278-287.

Prine, G.M., K.R. Woodard and T.V. Cunilio. 1994. Leucaena and tall grasses as energy crops in humid lower South USA. Proceedings Sixth National Bioenergy Conference--Bioenergy 94--Using Biofuels for a Better Environment, Reno/Sparks, NV, October 2-6, 1994. Vol. 2:681-688.

Espaillat, J.R., E.C. French, S.H. West, D.J. Mitchell, R.T. McSorley, and G.M. Prine. 1995. Assessment and management of a soil-borne disease complex of sage and lavender. Agronomy Abstracts. 1995 Annual Meeting ASA, CSSA, and SSSA at St. Louis, MO. October 29 to November 2, 1995. p. 127.

Prine, G.M. 1995. Pigeonpea, potential grain legume crop for Florida. Florida Scientist 58(1):3.

Cunilio, T.V. and G.M. Prine, 1995. Leucaena as a short rotation woody bioenergy crop. Proc. Soil Crop Sci. Soc. Fla. 54:44-48.

Prine, G.M. 1995. Field evaluation of crown rust in annual and perennial ryegrasses. Proc. Soil Crop Sci. Soc. Fla. 54:16-23.

GEORGIA

No publications.

HAWAII

No publications.

KENTUCKY

Taylor, N.L. 1995. Characterization of the United States germplasm collection of zigzag clover (Trifolium medium L.) Genet. Res. Crop Evol. 42:43-47.

Taylor, N.L. 1995. Registration of three genetic marker stocks for red clover: TP-RC, TP-LS, and PT-MC. Crop Sci. 35:1241.

Taylor, N.L., S.A. Ghabrial, G.A. Pederson, and M.R. McLaughlin. 1995. Quantification of yield benefits from incorporation of virus resistant white clover germplasm into grass legume systems. Plant Dis. 79:1057-1061.

Taylor, N.L. and S.A. Ghabrial. 1995. Registration of 19-L38-1472, a powdery mildew and virus resistant red clover germplasm. Crop Sci. 35:1721.

Prine, G.M., K.R. Woodard and T.V. Cunilio. 1994. Leucaena and tall grasses as energy crops in humid lower South USA. Proceedings Sixth National Bioenergy Conference--Bioenergy 94--Using Biofuels for a Better Environment, Reno/Sparks, NV, October 2-6, 1994. Vol. 2:681-688.

Espaillat, J.R., E.C. French, S.H. West, D.J. Mitchell, R.T. McSorley, and G.M. Prine. 1995. Assessment and management of a soil-borne disease complex of sage and lavender. Agronomy Abstracts. 1995 Annual Meeting ASA, CSSA, and SSSA at St. Louis, MO. October 29 to November 2, 1995. p. 127.

Prine, G.M. 1995. Pigeonpea, potential grain legume crop for Florida. Florida Scientist 58(1):3.

Cunilio, T.V. and G.M. Prine, 1995. Leucaena as a short rotation woody bioenergy crop. Proc. Soil Crop Sci. Soc. Fla. 54:44-48.

Prine, G.M. 1995. Field evaluation of crown rust in annual and perennial ryegrasses. Proc. Soil Crop Sci. Soc. Fla. 54:16-23.

GEORGIA

No publications.

HAWAII

No publications.

KENTUCKY

Taylor, N.L. 1995. Characterization of the United States germplasm collection of zigzag clover (Trifolium medium L.) Genet. Res. Crop Evol. 42:43-47.

Taylor, N.L. 1995. Registration of three genetic marker stocks for red clover: TP-RC, TP-LS, and PT-MC. Crop Sci. 35:1241.

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Taylor, N.L. and S.A. Ghabrial. 1995. Registration of 19-L38-1472, a powdery mildew and virus resistant red clover germplasm. Crop Sci. 35:1721.

LOUISIANA

No publications.

MISSISSIPPI

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Kamau, C.K., L.M. Nduulu, L.M. Gourley, and C.E. Watson. 1995. Phosphorous utilization of acid-tolerant and intolerant sorghum genotypes. American Society of Agronomy Annual Meeting. St. Louis, MO. 29 Oct. - 3 Nov. 1995. Agronomy Abstr. p. 80.

McPherson, G.R., J.N. Jenkins, J.C. McCarty and C.E. Watson, 1995. Combining ability analysis of root-knot nematode resistance in cotton. Crop Sci. 35:373-375.

M'Ragwa, L.R.F., C.E. Watson, Jr., and L.M. Gourley. 1995. Selection response for seedling root length and coleoptile length in pearl millet. Crop Sci. 35:1032-1036.

M'Ragwa, L.R.F. and C.E. Watson, Jr. 1995. Registration of 'KAT/PM-2' pearl millet. Crop Sci. 35:1504.

Nelson, L.R., T.D. Phillips, and C.E. Watson. 1995. Plant breeding for improved production in annual ryegrass. American Society of Agronomy Annual Meeting. St. Louis, MO. 29 Oct. - 3 Nov. 1995. Agron. Abstr. p. 125.

Nduulu, L., C.E. Watson, W.J. Drapala, and L.M. Gourley. 1995. Diallel analysis of sweet corn inbreds introgressed with pest resistant field corn germplasm. American Society of Agronomy Annual Meeting. St. Louis, Mo. 29 Oct. - 3 Nov. 1995. Agron. Abstr. p. 81.

Odouri, C.O.A. 1995. Effects of divergent selection for anthesis date on agronomic characteristics of annual ryegrass. M.S. thesis, Mississippi State University. p. 75.

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Okora, J.O., C.E. Watson, and L.M. Gourley, 1995. Botanical characteristics of fluorescent and non-fluorescent ryegrasses. American Society of Agronomy Annual Meeting. St. Louis, MO 29 Oct. - 3 Nov. 1995. Agron. Abstr. p. 81.

Ouma, J.P., L.M. Gourley, and C.E. Watson. 1995. Inheritance of chilling tolerance in grain sorghum during reproductive phase. American Society of Agronomy Annual Meeting. St. Louis, MO. 29 Oct - 3 Nov. 1995. Agron. Abstr. p. 80.

Pederson, G.A., and G.L. Windham. 1995. Registration of MSNR4 root-knot nematode resistant white clover germplasm. Crop Sci. 35:1234-1235.

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Saadan, H.M., L.M. Gourley, and C.E. Watson. 1995. Inheritance of manganese tolerance in sorghum. p. 52-61. In S.Z. Mukuru and S.B. King (ed.), Eighth EARSAM Regional Workshop on sorghum and Millets, Wad Medani, Sudan. 30 Oct. - 5 Nov. 1992. ICRISAT, Patancheru 502 324, Andhra Pradesh, India.

Shappley, Z.W., J.N. Jenkins, C.E. Watson, and B. Tang. 1995. Use of RFLP's in predicting agronomic performance. p. 531. In D.A. Richter and J. Armour (ed.), Proc. Beltwide Cotton Conf., San Antonio, TX. 4-7 Jan. 1995. National Cotton Council, Memphis, TN.

Villaroel, D., T.C. Kilen, T.P. Wallace, C.E. Watson, Jr., and F.B. Matta. 1995. Inheritance of a long juvenile trait in soybean [Glycine max (L.) Merr.]. Proc. Mississippi Acad. Sci. 40(2):9-11.

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NORTH CAROLINA

No publications.

OKLAHOMA

Baker, C.A., J.A. Webster, and D.R. Porter. 1995. Inheritance and mechanisms of Russian wheat aphid (RWA) resistance in wheat PI 245462. Agron. Abstr., Am. Soc. Agron. p. 88.

Mornhinweg, D.W., D.R. Porter, and J.A. Webster. 1995. Registration of STARS-9301B barley germplasm resistant to Russian wheat aphid. Crop Sci. 35:602.

PUERTO RICO

Gonzalez-Velez, A. and I.B. de Caloni. 1995. Evaluacion sensorial de los cultivares de name Binugas y Gunung (Dioscorrea alata) cosechados a distintos meses de la siembra. (Res. note) J. Agric. Univ. PR. 79-85-88.

Martinez, S.L. 1995. Evaluacion de germplasma de guanabana. In Proceedings of Foro sobre el cultivo, produccion y procesamiento de guanabana, nispero y quenepa. University of Puerto Rico at Ponce, June 17, 1994.

Brunner-Fulton, B. 1995. Efecto de la poda de brotes laterales sobre el comportamiento de la papaya. In Proceedings of the 21th Annual Meeting-Sociedad puertorriquena de ciencias agricolas. Guayanilla, P.R. Nov. 10, 1995.

Ortiz, C.E. , A. Gonzalez, L.E. Rivera and R. Velez-Colon. 1995. Comportamiento de repollo en zonas ecologicas y practicas de manejo contrastantes. In Proceedings of the 21th Annual Meeting Sociedad puertorriquena de ciencias agricolas. Guayanilla, P.R. Nov. 10, 1995.

Roman, F., A. Gonzalez, and P. Marquez. 1995. Performance of three citrus cultivars on five rootstocks at Isabela and Corozal. In Proceedings of the 21th Annual Meeting Sociedad puertorriquena de ciencias agricolas. Guayanilla, P.R. Nov. 10, 1995.

SOUTH CAROLINA

Farnham, M.W. and K.D. Elsey. 1995. Recognition of Brassica oleracea L. resistance against the silverleaf whitefly. HortScience 30:343-347.

Rhodes, B. and X. Zhang. 1995. Gene List of Watermelon: 1995 Cucurbit Genetics Cooperative Rept. 18:69-84.

Thomas, C.E. and E.I. Jourdain. 1995. Evaluation of muskmelon plant introductions for resistance to downy mildew, 1988-1989, Part V. Biol. & Cult. Tests for Cont. of Plant Dis. 10:136.

TENNESSEE

No publications.

TEXAS

Burson, B.L. 1995. Genome relationship and reproductive behavior of intraspecific Paspalum dilatatum hybrids: yellow-anthered x uruguaiana. Int. J. Plant Sci. 156:326-331.

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Marshall, D., L.R. Nelson, and B. Tunali. 1993. The occurrence of Acremonium and other endophytic fungi in the indigenous wild cereals of Turkey. In Proceedings of the Second International Symposium on Acremonium/grass interactions. Ed. Hume, D.E., Latch, G.C.M., and Easton, H.S. p. 8-10.

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Ouedrago, M., O.D. Smith, C.E. Simpson, and D.H. Smith. 1995. Leafspot reaction and yield of nineteen interspecific peanut lines. Peanut Science. Accepted.

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Starr, J.L., C.E. Simpson, and T.A. Lee, Jr., 1995. Resistance to Meloidogyne arenaria in advanced breeding lines of peanut. Peanut Science 22:59-61.

Stalker, H.T. and C.E. Simpson. 1995. Germplasm Resources in Arachis. Chapter 2. In H.E. Pattee and H.T. Stalker (eds.) Advances in Peanut Science. pp. 619. Am. Peanut Res. and Educ. Soc., Inc. Stillwater, OK.

Tischler, C.R. and B.L. Burson, 1995. Evaluating different bahiagrass cytotypes for heat tolerance and leaf epicuticular wax content. Euphytica 84:229-235.

Valles, J.F.M., C.E. Simpson, and V.R. Rao. 1994. Case Study-Collection of Wild Species of Arachis. Chapter 30. In Germplasm Collection, Preservation, Evaluation, and Utilization. Published by the International Board for Plant Genetic Resources, FAO, Rome, Italy. IN PRESS.

VIRGINIA

No publications.

PLANT GENETIC RESOURCES CONSERVATION UNIT

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Gillaspie, Jr., A.G., M.S. Hopkins, D.L. Pinnow, and R.O. Hampton. 1995. Seedborne viruses in preintroduction cowpea seed lots and establishment of virus-free accessions. Plant Disease 79:388-391.

Gillaspie, Jr., A.G.and J.M. Wright. 1995. Resistance of Citrullus lanatus germplasm to watermelon mosaic virus 2 strains. Phytopathology Abstr. 85:51.

Wilkinson, R.E. and J.J. Roberts. 1995. Gibberellic acid analogs modification of epicuticular wax. pp. 453-455. In J.C. Kader and P. Mazliak (eds.) Plant Lipid Metabolism, Kluwer Acad. Publ.

Roberts, J.J., B.M. Cunfer, and B. Padgett. 1995. Diseases. In J.L. Day, P.L. Raymer and A.E. Coy (eds.) 1994-95 Small Grains Performance Tests. The Univ. of GA., Agric. Expt. Sta. Res. Rpt. #636.

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Long, D.L., J.J. Roberts, K.J. Leonard, and D.V. McVey. 1995. The role of the USDA-ARS Cereal Rust Laboratory virulence surveys. Phytopathology 85:1168.

NATIONAL GERMPLASM RESOURCES LAB

No publications.

NATIONAL SEED STORAGE LABORATORY

No publications.

NCGR-TROPICAL FRUIT

Zee, F., M. Munekata, and P.J. Ito. 1995. The Rediscovery of 'Noel's Special' Passion Fruit. HortScience 30(5) I098.

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Attachment 16

CLOVER AND SPECIAL-PURPOSE LEGUME CURATORIAL ACTIVITIES

Brad Morris

INTRODUCTION

During the previous two years the PGRCU has undergone the following improvements: Establishing a USDA,ARS sponsored Clover and Special-purpose Legume Curator, Brad Morris. Move of its annual clovers into upright freezers at -20C. Land improvements on the Westbrook Farm include a 9.5 ft.-high chain link deer fence on eight acres, and a trickle irrigation system.

	1992	1993	1994	1995
No. acquisitions (Clover)	10	76	7	
No. acquisitions (Legume)	9	162	1	
No. distributed (Clover)	785	398	209	40
No. distributed (Legume)	793	748	546	553

CURATOR ACTIVITIES

Although all current activities of the PGRCU are essential, maintenance is its prime responsibility.

- -Seed database work continues with 32% of clover complete.
- -Several clovers and legumes performed well in a 1994 growout with the exception of the Egyptian clovers. These were transplanted to the field in late March and most produced flowers and seeds.
- -The use of landscape fabric around all clovers and legumes enhanced their growth and seed production.
- -Plantings will continue around the calender with peaks for the spring-summer items grown in the field and for a large group of species with recalcitrant seeds due to old age in late summer and fall in tissue culture and greenhouses.
- -CGC prescribed descriptors are recorded on all regenerated species.

GOALS:

- -Complete seed database work in 5 years.
- -Regenerate 30 cross-pollinated clovers and 170 self-pollinated clovers and summer legumes per year.
- -Back-up samples prepared and sent to NSSL in 2 years.

NEEDS:

- -2 Wintersteiger threshers to meet demand.
- -50 cages for cross pollinated crops.

Attachment 7

PLANT PATHOLOGY AND VIGNA CURATION PROJECTS

A. Graves Gillaspie

I. Recent Accomplishments:

A. Virus-elimination procedure for cowpea seed of exotic origin and its application for eliminating viruses in seed from Botswana (several hundred accessions)

B. Location of watermelon germplasm with resistance to watermelon mosaic virus

C. Tests of regeneration sites in Arizona, south Georgia, and Griffin for seed production and virus recontamination

D. Development of procedures in the screenhouse for regeneration of *Vigna subterranea* which had not previously been possible in Georgia

E. Detected several unusual viruses in *Vigna* and other legumes and have worked to identify these viruses

F. Inventory of *Vigna* accessions - visual inventory complete on most of the collection and seed weights and counts are available for cowpeas, urd bean, subterranean *Vigna*, and most of the mung beans

II. Work in progress

A. Virus-elimination from 100 additional accessions of Botswana cowpeas

B. Regeneration of an additional 50 accessions of *V. subterranea* in the screenhouse

C. Regeneration of 46 cowpea accessions and 103 accessions of mung beans in the field at Griffin (and a test run of 20 lines of *V. subterranea* in the same field)

D. Tests to identify viruses found in the fields the last two years and testing for the presence of these viruses and new ones in the current field plots at Griffin

1. Tests using double stranded RNA, serology, electron microscopy, and other useful methods for viral characterization

2. Virus detection based mainly on symptomology and serology requiring the use of a larger number of antisera to legume viruses, especially those of cowpeas, clovers, and peanuts, as well as viruses of other crops currently being regenerated by the unit in Georgia

III. Needs

A. Electrical supply to screenhouse. To provide for fans for air circulation and for electrical pesticide applicators.

B. Vehicle. Pick-up truck for carrying personnel and equipment to and from field

C. Grow lights in greenhouse. To improve conditions for winter increase of Botswana cowpeas

Attachment 2

PLANT PATHOLOGY AND THERAPY SERVICES

Mark S. Hopkins, David L. Pinnow, and Stephanie L. Dunn

I. General statement:

Pathology services objective is to assist the curators with their specific needs pertaining to disease diagnosis, incidence (in the collection), and therapy, and the embryo rescue of seeds with low or no germination.

II. Peanut:

A. Clean-up of the seed collection of peanut stripe potyvirus by a nondestructive seed notch DAS-ELISA test and screening of subsequent field increases.
B. Clean-up of the wild and hybrid collection by shoot tip culture and heat therapy.
C. Embryo rescue of old (> 15 years) or low germination seeds via embryo axis culture.
D. Visual and/or serological examination of quarantined material for possible virus infection.

III. Cowpea:

A. Clean-up of germplasm obtained from Botswana, Africa by testing for seven different seed borne viruses in seedlings and ensuring that only virus free plants are grown for seed in the greenhouse.
B. Assay of seedlings for increase for virus infection to ensure only virus free plant are taken to the field.
C. Diagnosis of virus infected plant samples from the field by DAC-ELISA or host plant inoculations.

IV. Sweetpotato:

A. Indexing of sweetpotato clones for sweetpotato feathery mottle potyvirus and sweetpotato virus disease complex via grafting onto indicator plants.

V. Grasses and Miscellaneous:

A. Embryo rescue of zero or low germinating seeds via callus induction and somatic embryogenesis.

VI. Summer Legumes:

A. Visual screeing of seedling for field increase or observation and subsequent field screening for viral, fungal, and bacterial diseases.

VII. Research:

A. Assisting in the evaluation of simple sequence repeats for genetic markers in *Sorghum bicolor*
B. Development of a florescent based diagnostic assay for rapid detection of viral pathogens.

VII. Needs:

A. Grow lights in the greenhouse for improved plant growth during the winter months. This would aid the Botswana cowpea seed increase, sweetpotato indexing, and host range studies.
B. Antisera for ELISA diagnostics; particularly antisera towards viruses commonly found in the southeastern U.S.
C. TaqMan™ LS-50B PCR Detection System for florescent based diagnostic assays consisting of: TaqMan™ PCR reagent kit, GenAmp PCR system 9600 for thermal cycling, and the LS-50B Luminescence spectrophotometer for detection.
D. Additional greenhouse space would be desirable for the proper separation of diseased material to help ensure no cross contamination and for adequate space for curators desiring to increase there respective germplasm to seed either for virus free material or an additional increase during the winter months.

Strategy potyvirus conserved NA sequences. crossbandtics species and

Attachment (9)

GRASSES AND SPECIALTY CROPS

Gil Lovell, Curator
June 13-14, 1995

SORGHUM COLLECTION

This collection is regenerated at the ARS Tropical Agriculture Research Station in Puerto Rico. We currently have 3,000 PI's scheduled for growout at TARS during the next 5 years.

PEARL MILLET

There are 84 PI's scheduled for regeneration at TARS during the Fall and Winter of 1995-96. Negotiations are underway with APHIS to use the ARS unit at St. Croix, V.I. for the growout of some 700 PI's now in quarantine at NSSL.

WARM SEASON GRASSES

This is a complex collection of 106 genera and 537 species. There are 826 PI's that currently need regeneration because of low seed quantity. We are now growing out 272 of these for seed increase. There are another 95 PI's in our embryo rescue program at this time. We are planning to complete the increase of these PI's by the end of 1997.

SESAME

There are 12 PI's unavailable because of low seed quantity and will be grown out in 1996. This season we are growing 7 accessions for NSSL because their seed viability is at a very low level. These accessions will be added to our active collections and duplicate samples will be returned to NSSL.

HIBISCUS

The two primary species, kenaf (H. cannabinus) and roselle (H. sabdariffa), compose 164 PI's of the 297 total PI's in the collection. These two species are subject to short-day responses, cross-pollination, and sensitivity to frost. Plans are to utilize ARS locations at Weslaco, TX and Mayaguez, P.R. to regenerate these PI's over the next 5 years. The remaining 133 PI's are currently being grown out by DR. Doug Wilson, ARS retired at Phoenix, AZ for identification and seed increase.

NSSL OBLIGATIONS

We are growing out 41 PI's (grasses, sesame, and miscellaneous species) for NSSL because of critically low viability levels.

NEEDS

We are submitting requests in our FY-96 Budget for moderately priced seed cleaning equipment items and additional seasonal labor for seed cleaning and inventorying,

attachment (10)

ARACHIS (PEANUT) CURATION PROJECT

Roy Pittman, Chris Jones, and Crystal Saunders

I. General statement:

The *Arachis* project objective is to have peanut germplasm available for distribution to scientists by regenerating and maintaining both cultivated and wild germplasm as needed. Materials distributed may be either vegetative cuttings or seed.

II. Recent Accomplishments:

A. Eight hundred cultivated accessions were planted for increase this year. The 'core collection needs' were given a priority for increase.

B. Approximately 500 different wild accessions (hybrids and species) were maintained in the greenhouses.

C. The curator requested 208 peanut accessions from ICRISAT which had been identified with desirable resistance/tolerance traits. A balance of 48 accessions still need to be re-requested but after a more detailed screening for duplication is done.

D. Cultivated peanuts from India and Africa have been increased under quarantine. Prior to planting in the greenhouse material was evaluated for nematodes and during the grow out material was tested for virus infection.

E. *Arachis marginata* was rescued via tissue culture. The material is being increased in the greenhouse now.

F. The number of *Arachis pintoi* accessions have been increased to address the need for forage legumes.

G. During the 1995 Brazil collection trip, a total of 86 collections were made with 42 being herbarium only.

II. Work in progress:

A. The Cultivated Peanut Descriptor list is in press and at the designer/layout level of publication.

B. Recently requested ICRISAT material will be grown under quarantine restriction in the greenhouse followed by a field increase.

C. Continue the maintenance of the wild germplasm in the greenhouses.

D. Germplasm needs to be backed up at NSSL. The cultivated peanuts are 75% backed up; while the wilds are only 2% backed up based on current GRIN information. Germplasm maintained in the greenhouses needs to be inventoried and properly labelled so that back up can be addressed.

III. Needs:

A. The lack of acceptable levels of back up for the wild peanut collection needs to be addressed. The wild peanut collection needs to increased under controlled pollination and in an area with a very long growing season. Florida would appear to be the most likely spot.

attachment (11)

INTRODUCTION

The Southern Project (S-9) was initiated in 1947 in Griffin, GA and resulted in the establishment of the Southern Regional Plant Introduction Station (SRPIS). The SRPIS is one of four stations nationwide responsible for the acquisition, maintenance and distribution of seed-propagated plant germplasm. These plant materials are being conserved in order to meet the current and future needs of the U.S. agricultural research community and U.S. agricultural industries. The S-9 collection is large and diverse, and currently maintains more than 100,000 accessions. Included in the S-9 are collections of several economically important vegetable crops.

Vegetable production in the U.S. exceeded 1.93×10^6 acres in 1994, with an estimated crop value of 8 billion dollars. In 1994, California, Texas and Arizona ranked 1st, 3rd and 4th, respectively in vegetable crop production in terms of area harvested, total production and crop value. Florida ranked 2nd and Georgia ranked 5th. Harvested areas for the most important crops in the S-9 germplasm collection including bell pepper, watermelon and sweetpotato were 64,000, 208,000 and 77,000 acres respectively. Watermelon was the third largest crop in the U.S. in 1994 in terms of production, with a value of $\$260 \times 10^6$ respectively. In 1994 the fresh market and processing value of bell peppers (excluding chilies, jalapenos and other specialty peppers) was $\$470 \times 10^6$. The principal form of delivery of specific vegetables to the consumer varies with the crop and whereas watermelons and most sweetpotatoes are sold as fresh vegetables, okra and cooked squash are primarily sold frozen. Annual sales of frozen okra and cooked squash exceeded 82×10^6 lbs. in 1993.

Plant introductions from the National Plant Germplasm System have had a significant impact on the development of improved vegetable cultivars in the U.S., and have proven especially valuable as sources of genes for resistance to pests and pathogens. For example, *Capsicum* PIs 159236 and 152225 are used extensively today as sources of virus resistance genes. PI 164835 is a valuable source of cytoplasmic male sterility. PIs 260435 and 439410 are valuable sources of resistance to bacterial leaf spot and Rhizoctonia solani, respectively. Watermelon PI 189225 has been used as a source of resistance to powdery mildew, anthracnose, gummy stem blight

and other diseases. PI 299379 is resistant to anthracnose (race 2) and PI 296341 is being used as a source of resistance to Fusarium wilt (various races). The sweetpotato cultivar Tinian (PI 153655) has been utilized extensively as a source of resistance to various fungal diseases. Other sweetpotato PIs have been utilized as sources of resistance to wireworm, banded cucumber beetle, flea beetle, the grub of Plectris spp., nematodes, other insect pests, and bacterial and fungal pathogens. There are many other examples.

This report was prepared in order to document the current state of the S-9 vegetable crops germplasm collection. For inventory purposes, genera included in the vegetable crops germplasm collections in Griffin have been divided into eight crop groups. Each group contains a principal crop species and occasionally wild related species. The crop groups maintained in the S-9 collection that will be discussed in this report include; pepper (*Capsicum* spp.), eggplant (*Solanum* spp.), okra (*Abelmoschus* spp.), watermelon (*Citrullus* spp.), gourds (various genera and species), sponge gourd (*Luffa* spp.), squash (*Cucurbita* spp.) and sweetpotato (*Ipomoea* spp.).

The report contains some terms and abbreviations with which the reader may not be familiar. Plant introductions (PIs) are accessions in the National Plant Germplasm System (NPGS) that have been assigned a plant identification number. In this report, accessions refers to plant materials that are recognized as being within the inventory of the NPGS but that may have inventory labels other than PI numbers such as GRIF (Griffin inventory number), or National Seed Storage Laboratory (NSSL inventory designations). When seed are initially collected they are considered to be original seed (OS). Original seed are increased in the field. Seed produced from OS are increased seed. Crop Advisory Committees (CACs) are now referred to as Crop Germplasm Committees (CGCs), and these two terms are used interchangeably in this report.

It would be impossible to completely document the condition of each accession in the vegetable crops germplasm collections in a short report such as this, as records prior to data computerization are difficult to access and germination data are rare, or lacking altogether. The data presented are those that are believed to be the most current and relevant available. This report is not intended to be a historical recount of past maintenance practices, although information on previous maintenance practices is included to the extent that this information is relative to future maintenance activities. Many of the comments and recommendations presented have

been expressed in previous CAC, commodity and crop vulnerability reports. For some crops, CAC or other advisory reports were not available, e.g. okra, eggplant, misc. *Solanum* spp., hence in these instances the comments and recommendations expressed are my own. Although this report is incomplete, it is hoped that the information presented provides the reader with a picture of the current status and needs of these plant germplasm collections.

THE S-9 VEGETABLE CROPS GERMPLASM COLLECTIONS

General Information

Total # genera	=	34
Total # of species	=	136
Total # of accessions	=	13,000[1]

[1] Approximately. Includes Plant Introductions (PIs) and other plant materials in the National Plant Germplasm System not assigned PI numbers.

CROP GROUP SPECIFICS

I. Pepper (*Capsicum* spp.)

Previously Utilized Seed Increase Procedure(s). Seed increase of pepper was performed in Griffin until 1988 via open pollination. In 1989, a cooperative agreement was established with New Mexico State University (NMSU), Las Cruces to increase seed stocks, collect characterization data and identify pepper PIs. Subsequently, funds were made available to NMSU for the purchase of pollination cages and technical support. Between 1989 and 1994, 1661 PIs were increased in this fashion. In 1994, fifty cages were moved from Las Cruces to Griffin for increase of pepper locally. Yields of most PIs in Griffin were acceptable. However, 1994 was a season of extreme rainfall in Georgia and seed yields of several PIs were not acceptable. The curator intended to gradually transfer pepper seed increase from Las Cruces to Griffin. However, pepper CAC members raised valid concerns about the lack of irrigated field space and technical and field support available for seed increase in Griffin. Hence, the contract for seed increase with NMSU was extended through 1995. Four hundred PIs are scheduled for increase in 1995 in Las Cruces.

Recommended Seed Increase Procedure(s). This crop is considered to be primarily, but not exclusively, self-pollinated. Some species are self-incompatible. Seed increase is successfully accomplished by caging entire plants or, in certain instances, sib-mating by hand-pollination.

Pepper Collection Specifics

- Total accessions	3783
- Related genera	0
- Total # species	15
-*C. annuum*	2218/2187 [a]
-*C. chinense*	416/403 [a]
-unidentified	240 (7%) [b]
- OS only (*C. annuum*)	45 (2%) [b]
- OS only (crop group)	118 (4%) [b]
- Distributions	3046 [c]
- Ave. # of PIs increased/yr (Griffin)	0 [d]
- # PIs backed up at NSSL	981
- # PIs not backed up at NSSL	2609
- % of collection backed up at NSSL	37
- # held at NSSL only	334

[a] Total #/# available #, Griffin only
[b] % of total, Griffin only
[c] Total since 11/92 / Ave. no./year
[d] Since 1987

Comments. The pepper collection is one of the two most economically important vegetable crop collections maintained in Griffin. Much (approximately 60+%) of the collection is not backed up at NSSL. NSSL holds a collection of 300+ predominately heirloom cultivars, many of which require increase. Pepper was increased in Griffin via open-pollination until 1988 and these seed stocks may be contaminated. Approximately 4% of the collection is maintained only as original seed. Some PIs remain unidentified. A genetic stock collection is maintained at NMSU. The genetic stock collection has recently been duplicated in Italy. The S-9 pepper collection is partially duplicated at the Asian Vegetable Research and Development Center (AVRDC), Taiwan. Seed stocks of pepper were inventoried in 1994.

The pepper collection lacks representative specimens of a number of species. Previous CAC reports have noted the need to acquire additional *Capsicum* spp. and to incorporate, when possible, existing U.S. collections that are threatened, into the NPGS. Much of the material held by Dr. P.G. Smith (University of California) has since been incorporated into the S-9 collection. A collection of *Capsicum* spp. has been assembled by Dr. W. H. Eshbaugh (University of Miami, Ohio). No USDA-funded collecting activities for *Capsicum* have been undertaken in recent years.

Maintenance of *Capsicum* germplasm is, by comparison with other crops in the S-9 collection, relatively easy to accomplish. The environment in Griffin is conducive to flowering and fruit set. Few problems with seed production have been noted previously.

A 1989 Germplasm Advisory Report recommended that "screen isolation be used on all *Capsicum* accessions."

Recommendations. Two to three hundred pepper PIs will require increase every year, for the next 8-10 years. Arguments as to whether or not it is a more prudent use of funds to continue to contract seed increase activities through NMSU or elsewhere, as opposed to conducting these activities in Griffin, requires a more in-depth analysis than this report can provide. However, arguments for seed increase in Griffin are, at this time, a moot point since the equipment and field support for these activities are not currently available in Griffin.

When possible, additional *Capsicum* species should be obtained from existing collections such as that of Dr. W.H. Eshbaugh (University of Miami, Ohio), if arrangements can be made to do so. If arrangements cannot be made to acquire needed materials from existing collections, exploration proposals should be prepared and the materials systematically obtained in cooperation with national programs. The NPGS and the pepper curator are fortunate to have the support of Dr. D. Williams (IPGRI, Cali, Colombia). Dr. Williams will be initiating a *Capsicum* germplasm conservation program in Latin America later this year.

Griffin appears to be an acceptable location for seed increase of pepper. Increase of two to three hundred pepper PIs in Griffin would require the purchase of as many cages, about 3-4 acres of irrigated land and 3 to 5 full time field laborers for a period of about 4 months. Pollination cages for pepper are smaller and less expensive ($50-$100/ea) than those required for vine crops ($500, see section V.). Alternatively, advantage may be taken of the facilities for seed increase, and expertise in identification and characterization available at NMSU (or elsewhere).

II. Eggplant and *Solanum* spp.

Previously Utilized Seed Increase Procedure(s). All eggplant accessions have been previously increased by open-pollination. Seed purity of the these open-pollinated eggplant accessions is not known, but is expected to be high.

Recommended Seed Increase Procedure(s). This crop is self-pollinating and only rarely outcrosses. Conversations with various SAES scientists regarding the degree of cross-pollination that occurs in eggplant were inconclusive. However, the consensus appears to be that open-pollinated seed increase of eggplant may be acceptable if minimal measures are taken to preclude outcrossing, such as the use of border rows between accessions in the field. Alternatively, small cages identical to those used for pepper (see above), may be used.

Eggplant Collection Specifics

- Total *Solanum* accessions	1220
- # Related genera	4
- Total # species	33
- *S. melongena*	824/566 [a]
- *S. gilo*	59/59 [a]
- unidentified	13 (1%) [b]
- OS only (*S. melongena*)	260 (21%) [b]
- OS only (crop group)	266 (22%) [b]
- Distributions	1692 [c]
- Ave. # PIs increased/year (Griffin)	31 [d]
- # PIs backed up NSSL	491
- # PIs not backed up at NSSL	469
- % collection backed up at NSSL	51
- # held at NSSL only	60

[a] Total #/# available
[b] % of total
[c] Total since 11/92
[d] 1988 to 1993

Comments. Of all the vegetable crops in the S-9 collection, seed increase of eggplant requires the least effort in terms of pollination control measures, and overall expense. Seed of most or all of these PIs can be stored at -20°C. No genetic stock collection of eggplant is known to exist. Seed counts of all eggplant PIs were obtained in 1994. Sixty eggplant accessions are maintained at the NSSL that are not backed up in Griffin. The collection contains a large number (266) of PIs as original seed. Original seed of 632 PIs remains available. Approximately 50% of the PIs are currently backed up at NSSL.

Although the collection contains numerous additional *Solanum* species, the collection is lacking in eggplant-related species. A GC for eggplant has not yet been organized.

Morphological characterization data should be acquired during seed increase and entered into the GRIN data base.

Age distribution of original seed samples of eggplant PIs that are more than 20 years old, stored in Griffin.

Date of entry	#PIs	Date of entry	#PIs
1918	2	1962	5
1936	1	1963	17
1941	1	1964	7
1948	5	1965	5
1949	5	1966	16
1951	1	1967	11
1952	1	1968	6
1953	1	1970	19
1954	4	1971	11
1955	5	1972	6
1958	3	1973	64
1959	2	1974	24
1960	12	1975	2
1961	8		
Total			254

Recommendations. All accessions for which seed stocks are low, or are inadequate for NSSL backup, should be grown out for seed increase and NSSL backup. All PIs maintained only as original seed will be grown in the field in 1995 for seed increase. Characterization data will be collected as time and funding permit.

Seed stock inventory data should be used to determine if current stocks are sufficient to provide a backup sample for long-term maintenance at NSSL, and this should be done where possible. Acceptance of samples by the NSSL will depend on germinability of the samples. Data on germinability are not available at this time.

Related species of eggplant should be acquired through cooperative agreements with existing collections, specifically the National Bureau for Plant Genetic Resources, New Delhi.

Funds permitting, research on the extent of natural outcrossing among eggplant PIs would provide data useful in planning future maintenance activities. Griffin offers a suitable environment for seed increase of eggplant. Approximately 150-200 eggplant (*Solanum* spp.) PIs should be gown for increase and/or NSSL backup per year. This effort would require about 2 acres of land and 2 full time field laborers for a period of about 4 months, to assist with fruit harvest, peeling, seed extraction and seed packaging.

III. Okra (*Abelmoschus* spp.)

Previously Utilized Seed Increase Procedure(s). Previous seed increases in Griffin were accomplished predominantly via open-pollination, and these seed lots are likely to be contaminated. Seed increase in 1994 was by controlled-pollination (bagged flowers).

Recommended Seed Increase Procedure(s). Okra is a self-pollinated crop but is readily outcrossed. Flowers must be bagged or plants caged for seed increase.

Okra Collection Specifics

- Total *Abelmoschus* accessions	3125 [a]
- # Related genera	0
- Total # species	7
- *A. esculentus*	1357/1139 [b]
- *A. manihot*	484/435 [b]
- *A. moschatus*	14
- unidentified	1 (<1%) [c]
- OS only *A. esculentus*	117 (6%) [c]
- OS only (crop group)	245 (13%) [c]
- Distributions	1857 [d]
- Ave. # PIs increased/year (Griffin)	30 [e]
- # PIs backed up at NSSL	369
- # accessions not backed up at NSSL	2755 [a]
- % accessions backed up at NSSL	12[a]
- # accessions held at NSSL only	1200 [a]

[a] Includes 1100 accessions introduced from IPGRI (Singapore, 1994) and 100 PIs not duplicated in Griffin.
[b] Total #/# available
[c] % of total, does not include NSSL-only accessions
[d] Total since 11/92
[e] 1988 to 1993

Comments. Most okra PIs maintained in Griffin are available for distribution, although the purity of the seed has not been ascertained. No germination data are available. Approximately 88% of the collection is not backed up at NSSL. No okra genetic stock collection is known. Seed counts of all okra PIs were obtained in 1994. A GC for okra has not yet been organized.

In 1993-94, 1100 accessions of *Abelmoschus* spp. were introduced to the NPGS through the IPGRI office in Singapore. These samples are stored at the NSSL without duplication in Griffin, awaiting increase.

Hand bagging of okra flowers in the field for controlled pollination, is labor intensive. Some PIs flower late in the season, or not at all, under the environmental conditions in Griffin. The flowering and seed-setting characteristics of the related species or specific PIs are not always known. Seed of *A. crinitis* and *A. angulosus* were recently acquired.

Approximately 13% of the *Abelmoschus* collection in Griffin is available as original seed only. Original seed of approximately 700 PIs remain available, although the germinability of these seed is not known. Approximately 1/3 of the original seed samples stored in Griffin are more than 20 years old. Materials obtained through the IPGRI in 1994 (approx. 1100 accessions) are small (<100 seed) samples and are not available for distribution until increased.

Age distribution of original seed samples of okra more than 20 years old, stored in Griffin.

Date of entry	#PIs	Date of entry	#PIs
1934	1	1962	1
1937	1	1963	4
1941	1	1965	2
1948	27	1966	27
1949	64	1967	23
1953	7	1968	3
1954	11	1969	1
1958	3	1970	1
1959	1	1971	5
1960	3	1972	3
1961	16	1974	6
Total			211

Original seed of 678 okra PIs is maintained in Griffin.

Recommendations. All previously open-pollinated PIs require new increase from original seed, when possible.

Samples currently available only as original seed should be increased, and backup samples sent to the NSSL.

Characterization data should be acquired by the crop curator and added to the GRIN database.

A coordinated effort to determine which PIs can be increased under the environmental conditions in Griffin should be initiated, and this information should become a permanent component of the GRIN database. Arrangements for the increase of recalcitrant PIs at an alternative location should be made once these genotypes are identified.

Several okra-related species including; *Abelmoschus ficulneus*, *A. crinitis*, *A. angulosus*, *A. moschatus* var. *betulifolius*, *A. moschatus* var. *biakensis*, *A. moschatus* var. *tuberosus*, *A. manihot* subsp. *tetraphyllus* var. *pungens*, *A. tuberculatus*, should be acquired from existing collections or, if necessary, collected.

Some okra accessions flower too late in the season to be grown for seed increase in Griffin. However, it is believed that Griffin is a suitable location for the increase of 75-90% of the accessions currently in the NPGS.

Arrangements should be made to increase between 200 to 300 accessions (preferably more) of okra per year. To increase three hundred PIs of okra/year would require about 3 to 4 acres of irrigated land and 6 to 8 full time field laborers for a period of about 4 to 5 months to hand bag flowers, label self-pollinations, harvest and clean seed, and package seed. Due to inadequate field support, no okra PIs will be increased in Griffin in 1995.

VINE CROPS

Many (virtually all) vine crops maintained in Griffin have been previously increased by open-pollination over a number of years. Thus, the purity of these seed stocks is not known. However, it is suspected that some cross pollination has occurred. Although recently obtained germination data on watermelon and luffa indicate germination levels within acceptable limits, it is questionable as to whether or not these seed stocks are of sufficient purity for purposes of distribution, further increase or use for long-term storage at NSSL. In some instances (e.g. *Cucurbita moschata*) the majority of the crop exists only as original seed and has never been increased. The viability or germinability of original seed samples is not known.

As with many other crops in the S-9 collection, a priority for the proper maintenance of vine crops germplasm must be the adoption of controlled pollination techniques. As stated by Robinson (1987):

"The genetic integrity of *Cucurbita* plant introductions is seriously threatened by the use of open-pollination to increase introductions at some, but not other, locations. The high rate of natural cross-pollination by insects will contaminate stocks increased by open-pollination."

The need to use controlled pollination techniques for watermelon seed increase was also mentioned in a report by the watermelon sub-committee of the Vine Crops CAC in 1989.

IV. Watermelon (*Citrullus* spp.)

Previously Utilized Seed Increase Procedure(s). Previous seed increases in Griffin were accomplished via open-pollination and the quality of these seed stocks is not known, but is suspect. In rare instances, PIs were increased in isolation.

Between 1988 and 1993 a limited number of PIs were increased through a cooperative agreement with Auburn University. During this 6 year period 371 PIs were increased at a cost of approximately $250/PI.

In 1992 a plan was proposed to increase the watermelon PIs in People's Republic of China. Although the cost/accession to increase seed in PRC was low, the agreement was not formalized for various reasons including concerns for the phytosanitary status of the materials on their return to the U.S..

Recommended Seed Increase Procedure(s). *Citrullus lanatus* (watermelon) is the most economically important vine crop in the S-9 germplasm collection. This crop is self-pollinated, but is readily cross-pollinated by insects. Thus, seed increase requires the use of controlled pollinations, preferably with the use of cages and bees.

Watermelon Collection Specifics

- Total *Citrullus* spp.	1852
- # Related genera	1
- Total # species	3
- *C. lanatus*	1,444/1,274 [a]
-unidentified	28 (<2%) [b]
- OS only (*C. lanatus*)	114 (7.5%) [b]
- OS only (crop group)	141 (9%) [b]
- Distributions	644 [c]
- Ave. # PIs increased/year (Griffin)	1 [d]
- # PIs backed up at NSSL	719
- # PIs not backed up at NSSL	812
- % backed up at NSSL	47
- # held at NSSL only	352

[a] Total #/# available, Griffin only
[b] % of total
[c] Total since 11/92
[d] 1988 to 1993, open-pollination.

Comments. A small watermelon genetic stock collection is maintained at Clemson University. This collection is not duplicated in Griffin. In 1993, 1000 previously open-pollinated *C. lanatus* PIs were tested for germination. Average germination was >85%. More than 50% of the collection requires NSSL backup. NSSL maintains a collection of approximately 300 heirloom varieties, not duplicated in Griffin, many of which require increase.

Nine percent of the *Citrullus* collection exists as original seed only, although viability and germinability of these seed is not known. Approximately 50% (461) of the watermelon original seed samples are more than 20 years old. Original seed of 916 watermelon is maintained in Griffin.

Age distribution of original seed samples of watermelon that are more than 20 years old, stored in Griffin.

Date of entry	#PIs	Date of entry	PIs
1934	1	1963	4
1935	1	1964	14
1948	3	1965	9
1949	1	1966	64
1950	5	1967	1
1951	1	1968	2
1954	1	1969	11
1955	1	1970	90
1958	12	1971	37
1959	12	1972	35
1960	13	1973	40
1961	49	1974	13
1962	91		
Total			461

Recommendations. Although germination levels of previously increased seed are generally acceptable, the purity of this seed is questionable. Where original seed is available, PIs should be increased again using original seed and controlled pollination methods, specifically the use of pollination cages and bees.

Seed of heirloom cultivars stored at NSSL should be increased.

The *Citrullus* collection is lacking in related species and these materials should be acquired from existing collections or collected. A detailed list of species to be targeted for collection can be found in the report by R.W. Robinson (1989). A copy of this report is available on request.

The curator receives frequent requests for pest resistance data on watermelon PIs. The GRIN database contains limited information on the resistance characteristics of most watermelon PIs. Further characterization of *C. lanatus* PIs for their resistance and other characteristics is encouraged.

Given the economic importance of this crop, the collection at Griffin seems disproportionately small. The *Citrullus* collection could be expanded to include a much more diverse array of material. However, in the absence of support for its maintenance, this is not recommended at this time. A study to characterize materials in the *Citrullus* collection, where original seed is available, is planned.

It is recommended that 150-200 PIs of *Citrullus* be increased per year to manage this crop collection on a 10-year turnaround. Land area required for 200 cages (10% of the collection per year) is estimated to be about 4 to 5 acres. Technical support is estimated at 4 to 6 full time field laborers to assist with cage manipulations, weed control, fruit harvest, and seed extraction and packaging. Pollination cages (9' x 20') may be purchased for about $500 each. Apiary services are estimated to cost an additional $50/cage.

In the absence of pollination cages or suitable pollination control measures, further seed increase of *Citrullus* spp. in Griffin has been suspended indefinitely.

V. Gourds

Previously Utilized Seed Increase Procedure(s). Previous gourd seed increases have been accomplished in Griffin via open-pollination.

Recommended Seed Increase Procedure(s). Gourds (*Lagenaria* spp. and *Momordica* spp.) are predominantly self-pollinated but are readily outcrossed. Quality seed increase requires the use of pollination control measures as recommended for *Cucurbita* spp.

Gourd Collection Specifics	
- Gourds (*Lageneria* and *Momordica* spp.)	497
- # Related genera	4
- Total # species	8
- *L. siceraria*	385/242 [a]
- *M. charantia*	19/19 [a]
- unidentified	27 (6%) [b]
- OS only (*L. siceraria*)	121 (28%) [b]
- OS only (crop group)	158 (35%) [b]
- Distributions	68 [c]
- Ave. # PIs increased/year (Griffin)	12 [d]
- # PIs backed up at NSSL	121
- # PIs not backed up at NSSL	321
- % of collection backed up at NSSL	28
- # held at NSSL only	55

[a] Total #/# available
[b] % of total
[c] Total since 11/92
[d] 1988 to 1993, open-pollination

Comments. Self and cross-pollinated (by insects). Seed increase is achieved by use of cages with bees, cages and hand pollinations, or isolation. Should be trellised. Open-pollinated seed increase has been conducted previously.

Recommendations. The gourd collection contains a large number of unidentified PIs. Arrangements should be made to identify these accessions.

Germination data on previously increased seed are not available. Purity of previously increased seed is doubtful. Where possible, PIs should be increased again using original seed and controlled pollination methods.

No cages or field support are currently available in Griffin for seed increase of this crop. In the absence of pollination cages or suitable pollination control measures, further seed increase of gourd germplasm in Griffin has been suspended indefinitely.

VI. *Luffa* spp.

Previously Utilized Seed Increase Procedure(s). Previous seed increases in Griffin have been conducted using open-pollination methods.

Recommended Seed Increase Procedure(s). This crop is predominantly self-pollinated but is readily outcrossed (by insects). Quality seed increase is achieved by use of cages with bees, cages and hand pollinations, in isolation or by the use of overhead trellises and hand-pollinations (sib-mating) in the field.

Luffa Collection Specifics	
- Total *Luffa* accessions	183
- # Related genera	0
- Total # species	3
- *L. acutangula*	67/61 [a]
- *L. aegyptiaca*	87/62 [a]
- unidentified	5 (3%) [b]
- OS only (*L. aegyptiaca*)	22 (14%) [b]
- OS only (crop group)	36 (21%) [b]
- Distributions	183 [c]
- Ave. # PIs increased/year	4 [d]
- # PIs backed up at NSSL	52
- # PIs not backed up at NSSL	116
- % of collection backed up at NSSL	31
- # held at NSSL only	14

[a] Total #/# available
[b] % of total
[c] Total since 11/92
[d] 1988 to 1993

Comments. The average age of previously increased seed is approximately 15 years. A large proportion (21%) of the luffa PIs are maintained as original seed only.

Germination data on a limited number of *Luffa* accessions was obtained in 1995 in anticipation of the need to regenerate seed stocks and increase seed supplies for backup at NSSL. Germination values were within acceptable limits on materials previously increased by open-pollination. However, these samples are likely contaminated. Original seed of those luffa PIs for which original seed remains available is generally more than 30 years old and seed stocks of these are too low for germination testing. A cooperative agreement has been established (1995) between the curator and NC State University for increase of luffa germplasm with priority to be given to original seed only samples. Sufficient seed will be produced for backup samples to be sent to the NSSL. Morphological characterization data, and commercially important sponge characteristics, will be obtained simultaneously during the course of the grow out.

Recommendations. Because this collection is small, the increase of all PIs is readily accomplished through the current cooperative agreement with NC State University, who are currently investigating the commercial aspects of luffa sponge production. Seed stocks from this increase will be sufficient to provide seed for distribution, and NSSL backup. Funding in 1995 has been dedicated for the increase of about 100 luffa PIs. However, additional funding will be necessary in 1996 to complete the seed increase.

Additional accessions of *Luffa*, possessing commercially desirable characteristics, should be acquired as opportunity permits. Additional related species should also be acquired, as funds and CGC support permit.

VII. *Cucurbita* spp.

Previously Utilized Seed Increase Procedure(s). Previous seed increases of *Cucurbita* in Griffin have been achieved by open-pollination only.

Recommended Seed Increase Procedure(s). Members of this crop group are self-pollinating, but are readily cross-pollinated by bees. Seed increase is achieved by use of cages with bees, cages and hand pollinations, by isolation, or by hand-pollination in the field.

Squash Collection Specifics.

- Total *Cucurbita* accessions	1475
- # Related genera	20
- Total # species	45
- *C. moschata*	753/305 [a]
- *C. argyrosperma*	147/104 [a]
- unidentified	162 (12%) [b]
- OS only (*C. moschata*)	597 (82%) [b]
- OS only (crop group)	745 (53%) [b]
- Distributions	325 [c]
- Ave. # PIs increased/year	16 [d]
- # PIs backed up at NSSL	203
- # PIs not backed up at NSSL	1171
- % of collection backed up at NSSL	15
- # held at NSSL only	96

[a] Total #/# available
[b] % of total
[c] Total since 11/92/ave. # year
[d] 1988 to 1993, open pollination.

Comments. Robinson (1987) indicated that genetic vulnerability within *Cucurbita* is probably the greatest for *C. moschata* within the genus, since U.S. butternut squash production is based primarily on 2 cultivars.

A large number of *Cucurbita* PIs (82% of the collection) are currently maintained as original seed only. No cages or field support are currently available in Griffin for increase of this crop. Only 15% of the existing accessions are backed up at the NSSL. The majority of the collection is unavailable for distribution. Twelve percent of the PIs remain unidentified.

As was the case with *Citrullus*, the *Cucurbita* collection is lacking in the representation of related species. Species to be targeted for collection were noted by R.W. Robinson (1987), and it is believed that these priorities have not changed.

As noted earlier, a majority of the *C. moschata* PIs are maintained as original seed only. A distribution of the age of the original seed of *C. moschata* is presented in Table 1. Approximately 80% of the original seed only PIs are more than 10 years old. In the absence of germination data, not taken due to the small number of available seed of most PIs, the germinability of these materials cannot be ascertained.

Table 1. Age distribution of original seed only PIs of *C. moschata*.

Date of entry	#PIs	Date of entry	#PIs
1940	1	1967	1
1941	1	1970	6
1946	1	1971	2
1948	26	1972	1
1949	14	1973	17
1950	2	1977	4
1951	3	1978	4
1952	12	1979	163
1953	6	1980	11
1954	7	1981	97
1957	3	1983	83
1958	2	1984	6
1960	2	1985	17
1961	2	1987	71
1963	4	1988	18
1964	0	1990	1
1965	1	1991	4
1966	3	1993	1
Total			597

Plants of *C. moschata* and other members of the genus are large and must be spaced at wide intervals. Flowers are not profuse. Thus, seed increase requires considerable land area, and also field support to locate flowers for sib-mating. Advice on the most efficient means to increase members in this crop group can be obtained from curators at both NC-7 and NE-9. These plants are generally considered to be too large for caged pollination control.

Large numbers of *Cucurbita moschata* PIs require increase, perhaps the entire collection. In addition, an unusually large percentage of the *Cucurbita* PIs (53%) are available as original seed only. Many of these seed samples are old and in need of increase for distribution and NSSL backup.

Dr. Laura Merrick (University of Maine, Orono) has previously examined PIs in the *Cucurbita* collections at NC-7, NE-9 and S-9 for proper identification. In a recent conversation with Dr. Merrick, it was indicated that, based on new identification data, approximately 100 *Cucurbita* PIs in the S-9 collection would be moved to either the NC-7 or NE-9 collections. Also, plant materials from these stations would likely be moved to the S-9.

Recommendations. Seed of PIs previously increased via open-pollination should be regrown using controlled pollination methods, from original seed. Priority for increase should, however, be given to those PIs currently maintained as original seed only, and that are 20 or more years old. All PIs that have not yet been identified should be grown out for simultaneous increase, identification and characterization.

A coordinated effort by the CAC, crop curator and the Plant Germplasm laboratory in Beltsville to acquire related species, is recommended. Further additions to the collection should however, be delayed until arrangements for maintenance are in place.

Past seed increase activities in Griffin indicate that most of the *Cucurbita* PIs will flower and set fruit under the environmental conditions in Griffin. A suitable alternative location for seed increase of those PIs that flower late or not at all in Griffin should be identified.

As noted earlier, increase of *Cucurbita* accessions is labor intensive and requires considerable land area. It is recommended that 100 *Curcubita* PIs be increased per year in the field using sib-mating techniques with a minimum of 20 plants per accession. This effort is estimated to require 3 to 4 acres of land and 6 to 8 full time laborers to assist with locating and tagging flowers, performing hand pollinations, harvesting fruit, and cleaning and packaging seed. In the absence of adequate field support to ensure proper pollination control, further seed increase of *Cucurbita* spp. in Griffin has been suspended indefinitely.

VIII. Sweetpotato (*Ipomoea*) spp.

Previous and Current Maintenance Practices. The sweetpotato (*Ipomoea batatas*) collection was begun in 1987 at the request of a newly initiated sweetpotato CAC. Clonal material was moved to Griffin from the USDA Vegetable Research Station in Charleston, SC and from various land-grant university breeding programs. All sweetpotato clones are currently maintained in vitro.

Seed propagated *Ipomoea* species are increased via hand pollination. In 1993, ten seed samples (4 species) were grown in isolation in Mayaguez, PR. Relatively good seed yields were obtained. No seed was sent to Mayaguez in 1994 due to constraints on support for these activities at that location. Due to funding and space constraints, only 8 seed samples were sent in 1995.

Sweetpotato Collection Specifics

- Total *Ipomoea* accessions	780
- # Related genera	2
- Total # species	18
- *I. batatas*	672/672 [ab]
- unidentified (*Ipomoea* spp.)	13 (1%) [c]
- OS only (*Ipomoea spp.*)	260 (28%) [c]
- OS only (*Ipomoea spp.*)	266 (28%) [c]
- Distributions	1420 [d]
- Ave. # PIs increased/year (Griffin)	20 [e]
- # PIs backed up at NSSL (*I.* spp.)	50
- # PIs not backed up at NSSL (*I.* spp.)	50
- % of collection backed up at NSSL	50
- # held at NSSL only	0

[a] Total #/# available
[b] In vitro, continuously available
[c] % of total
[d] Propagules since 11/92
[e] 1988 to 1993

Comments. The sweetpotato (*I. batatas*) germplasm collection is currently maintained under reduced growth conditions in vitro. Fresh nodal cultures are established at 9

month to 18 month intervals, as required. Propagules are supplied as in vitro plantlets or vine cuttings. All clonal materials are available continuously.

A collection of related species has been acquired through collaborative exchange of germplasm and plant explorations. The *Ipomoea* spp. collection contains primarily open-pollinated, self- incompatible species that require controlled pollination. Many PIs in this collection are native to littoral, or xerophytic habitats. This collection also contains a number of shrubs and very large perennial vines. These species are difficult or impossible to increase in Griffin due to photoperiod and space requirements. Many species flower in November-Dec. Thus, seed increase is conducted in the greenhouse. A large number of *Ipomoea* spp. seed were received in 1993. These materials are old, poorly documented and generally fewer than 10 seed per accession were provided. Seed viability is not known, however, seed of *Ipomoea* stores well and may remain viable for 50+ years at room temperature. Previous and current seed increases emphasize species of section Batatas.

A large number of *Ipomoea* spp. require seed increase for distribution and NSSL backup. Current greenhouse space permits the increase of about 20 PIs/year.

A plan has been initiated whereby duplicate cultures of all *I. batatas* clones will be maintained at the NSSL. Replacement cultures will be provided to NSSL by Griffin, as necessary in order to maintain viable backup cultures.

A plant exploration trip to Papua New Guinea was conducted in 1994 in order to evaluate the feasibility of collecting *I. batatas* as botanical seed. This study continues. The sweetpotato CAC routinely submits one plant exploration proposal/year for funding.

The International Potato Center (CIP) continuers to actively collect *Ipomoea* spp.. The curator participates in the exploration activities, as funding and acquisition priorities permit.

Recommendations. The cost and effort required to process clonal materials through Plant Quarantine is high, and the number of clones to be introduced on a yearly basis is limited by the funding and facilities available to Plant Quarantine for maintenance and virus indexing. Clonal materials should be obtained, when possible, as previously virus indexed cultures from existing collections. When this is not possible, GC concurrence prior to the introduction of clonal material, is recommended.

Further collection of *Ipomoea* spp. is planned in cooperation with the International Potato Center, Lima, Peru. Areas targeted for collection include most of Central America, Mexico, and parts of the Caribbean.

Ipomoea spp. are difficult to maintain. In order to avoid redundancy, a very conservative collecting strategy should be employed emphasizing maximum

geographic distribution and isolation for each species, as opposed to concentrated collection over a small geographic area.

Plans to maintain a backup in vitro collection at the NSSL should be formalized and actions taken to implement this plan. Increase of *Ipomoea* spp. seed in PR should be expanded to the extent that funding and facilities can be obtained to support this activity in Mayaguez. Emphasis should be placed on increase of section Batatas spp., initially.

GENERAL COMMENTS AND RECOMMENDATIONS

In those instances where seed has been increased via open-pollination PIs should (with the possible exception of *Solanum* spp.), be increased from original seed. Much original seed is old and, if these PIs are to retained, regeneration from original seed should be initiated soon. **An estimate of current regeneration needs indicates that the majority of accessions of all vegetable crops currently maintained in Griffin, perhaps as many as 8,000 - 9,000 require seed increase** for routine maintenance and/or NSSL backup. Data on the flowering and seed setting characteristics of all PIs should be added to the GRIN data base.

Related species are only poorly represented in many collections, although these materials are likely to represent an important future source of disease and insect resistance genes. Crop vulnerability and CAC reports frequently comment on the need to acquire additional wild species germplasm. **CACs, in collaboration with the curator and the Genetic Resources Laboratory (Beltsville) should formulate and initiate plans to acquire crop-related species.**

Many collections contain a large number of unidentified specimens. Botanical expertise for specimen identification is not always readily available, and many CACs do not contain a botanical authority who is competent to unambiguously identify all species within a crop group. **In order to assist curators in the proper identification of plant materials, herbarium voucher specimens of all crop-related species should be prepared and maintained on-site and duplicated at a second site. Alternatively, advantage may be taken of recent advances in computer storage of video images. Storage of images on compact disks (CDS) or diskettes would provide curators with immediate visual access to morphological characteristics.**

Germination data on most vegetable crop PIs are not available. The S-9 has two germination chambers which were purchased in about 1990. The curator has used these chambers to perform germination tests on a portion of the *Citrullus* collection. In other instances (e.g. *Luffa*) germination tests were subcontracted to a seed testing laboratory. **In view of the large number of samples in the S-9 collection that require germination testing (including non-vegetable crops germplasm) consideration should be given to expanding the S-9's in-house germination**

testing capabilities over time (in terms of equipment and/or personnel), in support of NSSL's efforts in this area. A portion of an existing metal storage building could, with some modifications, be used for this purpose.

Pollination cages are fundamental pieces of equipment in a gene bank. Curators at Griffin obtained estimates of approximately $500/cage (9' x 20') in 1994. Estimates of apiary services run from $30 to $40/unit. **The S-9 should purchase 200 cages for the 1996 growing season and allocate funding for apiary services.** Pollination cages are required for numerous other crops in the S-9 collection, but these needs will not be discussed in this report.

At the present time the S-9 has approximately 10 to 12 acres of fenced, irrigated farmland dedicated specifically for seed increase in Griffin. For comparative purposes, the NE-9 collection (Geneva, New York) leases 25 acres of land for maintenance of their 10,000 accessions of vegetable crops germplasm.

The CACs of the crops discussed in this report (pepper, sweetpotato and watermelon) have not yet designated core collections. Although generally considered a strategy to enhance germplasm characterization (and thus utilization), core collections may also be utilized as a management technique. Use of core collections can result in a significant dollar savings to the NPGS as a result of a reduction in the frequency of required regeneration. Under one such scenario, core collections are maintained under medium-term storage conditions (4°C) in Griffin and under long-term storage conditions at Fort Collins. All other materials (non-core accessions) are maintained only under long-term storage conditions at NSSL (-20°C or preferably liquid N). Long-term storage reduces the frequency (and thus the cost) of regeneration for those accessions that are infrequently requested. However, before non-core collection accessions can be moved to long-term storage, sufficient quantities of seed of high germinability and vigor must be available. As this report indicates, seed of only a relatively small percentage of the S-9 vegetable crop accessions meet these qualifications. Nevertheless, movement of infrequently requested accessions to long-term storage should, when possible, be accomplished. The in vitro sweetpotato collection does not currently lend itself to the adoption of a long-term maintenance strategy since cryopreservation techniques are not yet well defined for this crop. Thus all accessions must be periodically recultured to maintain viability.

A large proportion of the S-9 vegetable crops collections is composed of vine crops (*Citrullus* spp., *Luffu* spp., *Cucurbita* spp., *Lagenaria* spp. and other gourds). Maintenance of these types of crops is labor intensive and requires either the use of pollination cages and bees or a large amount of field area and technical support for hand pollinations in the field. **An exact estimate of the cost of field support required for the maintenance of vine crops is difficult to ascertain, since this location has little expertise in these practices.** However, comparisons can be drawn between the S-9 collection in Griffin and the NC-7 Plant Introduction Station in Ames, Iowa. For comparative purposes, Griffins' collections of *Cucurbita* and *Citrullus* may be compared to Ames's collections of *Cucurbita* and *Cucumis*, due to the similar maintenance

requirements of these genera. The vine crops curator in Ames, Iowa has approximately 10 full time field laborers and, in times of intense activity, as many as 30. The Ames station has purchased and is utilizing approximately 600 pollination cages for vine crops seed increase. NC-7 routinely increases between 600 and 900 accessions of vine crops per year. In contrast, Griffin has not increased vine crops germplasm since 1988. No pollination cages are available in Griffin for increase of these genera. No further increase of vine crops germplasm in Griffin is planned until funding for adequate facilities and field support are made available to the curator.

A -20°C seed storage chamber was constructed in 1994-5. This unit is complete and is currently operational. It is the vegetable crop curators intention to utilize this storage chamber for maintenance of infrequently requested PIs, emphasizing those accessions that are most likely to benefit from -20°C, as opposed to 4°C, storage. **Advice and assistance pertaining to the implementation of -20°C seed storage should be sought from the NSSL, and utilization of this storage unit should begin as soon as possible.**

In previous years, seed increase of pepper and watermelon was contracted to various SAES scientists. In 1995, the S-9 contracted the increase of seed of *Capsicum* and *Luffa*. In both instances, this permitted the curator to take advantage of facilities and expertise not available in Griffin. Although Griffin offers a suitable environment for seed increase of pepper, crop expertise at NMSU has proven invaluable in that identification, characterization and increase can be accomplished simultaneously. It is recommended that the increase of pepper seed at NMSU be continued, while the Griffin site is utilized to increase seed stocks of other genera. A modest amount of funding should also be made available to complete the increase of *Luffa* germplasm at NCSU.

The number of vegetable crops PIs being regenerated in Griffin has decreased continuously since 1987. This is due both to the recognition that proper pollination controls were not being taken and the establishment of alternative funding priorities within the unit. A major portion of the S-9 budget has, since 1986, been used for the construction of physical facilities for laboratory-based research, plant health (quarantine) and plant pathology-related research activities, construction and upgrading of seed storage facilities, and more recently, construction of a DNA sequencing laboratory with additional related laboratory facilities, and staffing for those laboratories. Increasingly less funding emphasis is being placed on germplasm maintenance. **The NPGS and the S-9 TAC should clearly define the priority for seed increase activities at Griffin. The priority for seed increase should be reflected in curator budgets.**

Funding allocated for maintenance of S-9 vegetable crops germplasm in the 1995 CWU was as follows; sweetpotato ($160,000), melons ($106,000) and peppers ($106,000) for a total of $372,000. No funding was allocated in the 1995 CWU specifically for maintenance of eggplant, okra or squash. For comparative purposes, support for the NE-9 vegetable crops germplasm collections (total of 10,000 PIs) is

approximately $451,000. Seed of pepper and watermelon has not been increased in Griffin since 1988, although a total of $212,000/yr is currently allocated for maintenance of these two crops. Since 1988, expenditures for contracted seed increase have averaged $15,000/yr and $16,000/yr for watermelon and pepper, respectively. At the current time, there is no CWU specifically for the maintenance of vegetable crops germplasm in Griffin. This has a deleterious effect on efforts to manage these genetic resources. **A CWU should be established for the management of the S-9 vegetable crop germplasm collections in Griffin.** This CWU should include funding for maintenance of squash, okra and eggplant germplasm.

These comments and suggestions are based on the assumption that Griffin continues to serve as a site for germplasm regeneration, and that the S-9 continues to assume the responsibility for the proper maintenance of these crops. The environmental conditions in Griffin are suitable for seed increase of most of the vegetable crops germplasm in the S-9 collection. However, alternative locations may be equally as suitable.

SUMMARY

The Griffin Genetic Resources Unit is responsible for the maintenance of approximately 13,000 accessions of vegetable crop germplasm. Cultivation and sale of pepper, watermelon, sweetpotato, okra, eggplant and squash represents a multi-billion dollar industry in the U.S. The collections of germplasm of these crops maintained in Griffin are the only ones publicly available, and are the genetic basis for the future improvement of these crops, and the continued viability of the vegetable industry. Current funding support for maintenance of the S-9 vegetable crop collections in Griffin is inadequate. No CWU currently exists for maintenance of vegetable crop germplasm. Seed increase that has been conducted in Griffin has previously been accomplished primarily by open-pollination methods. The extent to which this practice has compromised the purity of the original genotypes, is not known. Collections of several crops contain large numbers of seed that has not yet been increased, and PIs that have not yet been identified. Germination data on most PIs are lacking. Herbarium vouchers for related species in the various crop groups have not been taken in most instances. For most collections, less than 25% of the PIs are currently backed up at the NSSL. Approximately 10 acres of fenced irrigated farmland are available for seed increase in Griffin. Fewer than 100 PIs of vegetable crop germplasm were increased in Griffin in 1993. Funding for the purchase of pollination cages and apiary services was not available for the 1995 growing season.

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