

CALIFORNIA ANNUAL REPORT TO THE W-6 TECHNICAL COMMITTEE FOR DISTRIBUTION YEAR 2013

by Dan E. Parfitt

June 19, 2014

452 requests for plant introductions from California users were filled by the NPGS in 2013, representing 437 different users, an increase over 2012. Figure 1 shows the usage of germplasm in California from the National Plant Germplasm System expressed by the number of requests for California from 1993 to the present. The request level for this year was about average for recent years.

Collection of germplasm information:

The collection methodology was similar to that used last year. All requests were sent via e-mail. I did not attempt to query those requesters that did not have or provide an e-mail address (almost all requesters are providing e-mail addresses at this time). 437 queries were sent. Some requesters had multiple requests and these were consolidated into a single query. 6% of the e-mail addresses bounced back (either the addresses were no longer valid or the recipients filters blocked them), a remarkably low number compared to prior years. 71 responses were received, about the same as last year, but a lower 16.2 % response rate, almost identical to last year (Fig. 2). Comments from the respondents are provided below. 83% of recipients that received delivered e-mails did not respond, similar to last year's value of 80%.

The distributed germplasm was used in a wide variety of applications, from basic research to home gardening. No single crop or crop group was especially requested. Much of the germplasm continues to be used for commercial breeding or research (University or USDA), but a significant number of the requests were for materials to be used in molecular/biochemical studies, but less than last year. Fewer respondents reported on clonal (fruit/nut) germplasm than in the past. Several members of the California Rare Fruit Growers requested materials for distribution to their members as in past years. A summary of the replies is provided below to show the nature of germplasm use. In addition to the responses received, a list of expected uses provided by some of the recipients is provided below.

The Viticulture Department at UC Davis continues to maintain grape collections for teaching and research.

Figure 1. Requests for NPGS Germplasm from California

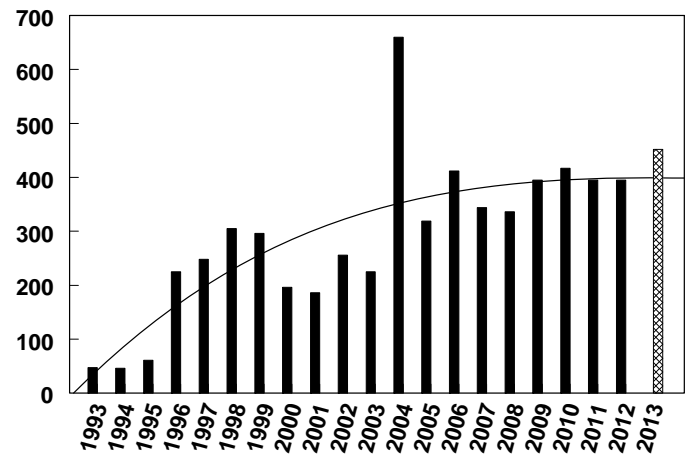
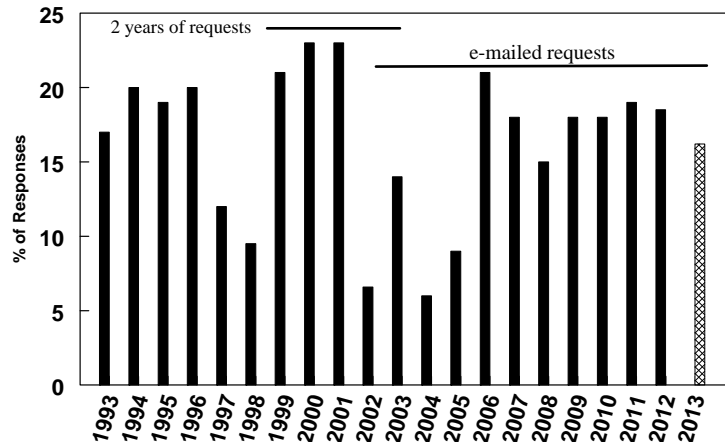


Figure 2: % of Responses



Several Plant Science fruit and nut germplasm collections continue to be maintained (almond, walnut, pistachio, pecan, cherry, peach, apricot, persimmon), but as noted previously there is no formal mechanism for ensuring their continued protection and the Pis that assembled and/or used them are retired, retiring soon, or have left the University. The Department of Botany and Plant Science at UC Riverside maintains several collections of *Citrus* germplasm for cultivar evaluation and disease related research, as well as collections of avocado, cherimoya, and persimmon. Annual reports from the UC Davis Seed Biotechnology program may be accessed at http://sbc.ucdavis.edu/publications/annual_reports.html and other SBC information at <http://sbc.ucdavis.edu/>. The Tomato Genetic Resources Center activities complete report can be accessed at <http://tgrc.ucdavis.edu/reports.aspx> and TGRC activities at <http://tgrc.ucdavis.edu/index.aspx>.

Narratives and publications are provided below. Italics highlight items that may be of particular interest to the reader. Items in red are my comments. A set of more extensive reports is provided at the end of the report.

Publications:

- Angus, A.A., et al. 2014. Plant-Associated Symbiotic Burkholderia Species Lack Hallmark Strategies Required in Mammalian Pathogenesis. PLOS One 9(1):e83779
- UCLA Newroom. 2014. UCLA life scientists, colleagues differentiate microbial good and evil. Jan. 23, 2014.
- Rwahnih, Al, M., Daubert, S., Sudarshana, M., and Rowhani, A. 2013. Gene from a novel plant virus satellite from grapevine identifies a viral satellite lineage. Virus genes, 47: 114-118.
- Rwahnih, Al, M., S. Daubert, C. Islas, D.A. Golino, and A. Rowhani. 2014. Characterization of a fifth Vitivirus in grapevine. Journal of Plant Pathology. 96 (1), 219-222
- Osman, F., Al Rwahnih, M., and Rowhani, A. 2014. Improved Detection of Ilarviruses And Nepoviruses Affecting Fruit Trees Using Quantitative RTqPCR. Journal of Plant Pathology. In press
- Nitcher R., A. Distelfeld, C.T. Tan, L. Yan, J. Dubcovsky. 2013. Increased copy number at the FT-H1 locus is associated with accelerated flowering time in barley. Molecular Genetics and Genomics. 288:261-275.
- Saintenac, C., W. Zhang, A. Salcedo, M. Rouse, H. Trick, E. Akhunov, and J. Dubcovsky. 2013. Identification of wheat gene Sr35 that confers resistance to Ug99 stem rust race group. Science 341:783-786.
- LoPresti, E.F. 2014 Chenopod salt bladders deter insect herbivores. Oecologia. 174: 921-930 doi: 10.1007/s00442-013-2827-0
- Stover, E., T.G. McCollum, R. Driggers, R. Lee, R. Shatters, Jr., Y.P. Duan, M. Ritenour, J.X. Chaparro and D.G. Hall. 2013. Resistance and tolerance to huanglongbing in Citrus. Acta Hort. (in press).
- Stover, E.W., M.L. Richardson, R. Driggers, D.G. Hall, Y.P. Duan and R.F. Lee. 2013. Incidence and severity of asiatic citrus canker on Citrus and Citrus-related germplasm in a Florida field planting. Hortscience 49:4-9.

Narratives from recipients concerning germplasm observations and the value of NPGS.

Ann M. Hirsch, UCLA	See attached report
Emma Fong, Bio-Rad, Hercules	See attached report.
Benjamin Winslow, Ball FloraPlant Arroyo Grande	See attached report.
Suraj Gurung, Salinas	See attached report.
Kevin Solis, Panorama City	Grin Report Date: 6/2/14 Grain Accessions: PI 518452 TR00ID SD Came true to type, spring habit observed, crosses will be made in Fall/Winter this year, highest yielding PI 428166 Came true to type. spring habit also observed, grew an average of

4 ft., crosses with previous accession will be made
PI 119435 Came true to type, spring habit observed, will also be used in mass cross
Rest: Multiplication will take place in 1-3 years due to limited space
Other Accessions: "Marshall" Strawberry: In grow-out, will be crossed with other cultivars still to be determined!

Clara Litman, Sacramento

The germplasm that we received for our garden unfortunately, did not survive even though we took really good care of it by planting it in the shades and in a good pot of soil.

Joseph Kepiro, Glenn

I have requested rice germplasm; mostly Japanese short and medium grain with high cooking quality (sushi type). I want to introgress Japanese sushi cooking quality into the California type genetic background. I am particularly interested in intermediate amylose varieties for that purpose. I am also very interested in Japanese sake type rice and want introgress those characteristics into the California type genetic background.

Brian Dykstra, Willow Creek

I have asked and received germplasm from the USDA. This has enriched my life greatly. Local farmers and permaculturalists tell me what I am doing is awesome, as I will be able to judge the ability of these plants to grow in this climate. I have grown the Lycium spp. from seed - planted in 2012 and again this year 2014 (second round of same seeds). Germination has been good both years. The first round of plants is in the ground, and I am waiting for those plants to become old enough to judge fruit production (in 3 years from now - i.e. at 5 years of age they should be producing well). The seedlings are growing now, and look successful so far. The pomegranates did not survive - unfortunately. I intend to request more seeds for additional experimentation this year. I am happy to make good use of this amazing resource to enhance the diversity and productivity of our local permacultural activities and home gardening.

Jenny Smith, NCGR-Davis

In 2013, I collected and used 140 accessions of olives for an olive knot screening project with CPGRU and CDFA. I collected and used 13 accessions for an olive dwarfing rootstock trial with UC Davis. I also received 46 accessions of kiwifruit from Corvallis for propagation as we move the collection from Corvallis to Davis. The Davis Repository also received seeds of three Prunus accessions from NC7. We also received a lot of Prunus material from PGQP.

Scott Heath, Tilted Shed Ciderworks,
Sonoma

I received 25 specimens of malus from ARS in 2013, as in 2012. I specifically chose varieties that are said to be good for making hard cider. I have been attempting to propagate 4 of each type for trialing on our small farm. Due to graft or rootstock failure, however, I sometimes end up with only one or two of each type. I intend to grow these out to bearing age to evaluate their compatibility with the local climate and suitability for hard cider making. It is still too early to have any concrete info on the varieties I've gotten. Info about our farm: We've been planting what should be about 2 acres total of cider apple orchard. We started in Jan 2011 with 84 trees from a nursery. I started

grafting that spring and have to this point put about 570 sticks in the ground, with a couple hundred more in pots or nursery rows.

Bianca Larson, Orange

Unfortunately the subjects I received did not make it, I am very said to say. *I believe it had to do with not having enough moisture (the thin layer of paper towel that was wrapped around the ends)in the package when they arrived.*

Junda Jiang, Lundberg Family Farms,
Richvale

In 2013, Lundberg Family Farms introduced 77 accessions of heirloom bean from NPGS for our new crop research project use. We received an average of 15 seeds per line and grew them out under organic management conditions. The objective was to screen heirloom bean lines which may be suitable for organic production in North California. We found some accessions having unique visual interest, texture and flavor and desired agronomic traits. We will be planting some selected lines again this year for further observation and testing. The NPGS bean germplasm is no doubt invaluable genetic resource for our organic food research program, and we really appreciate NPGS staff's generous support and service. *We have observed that some accessions seeds were not uniform with mixed seed color or size.* If needed, we will be glad to provide related information.

Oliver Colmenar, Fremont

I received germplasm material in the spring of 2013. I am a horticulture student at Merritt College in Oakland. My plant propagation course started me on a journey grafting the fruit trees in the backyard of the home I bought with my partner. I had wanted to revitalize the fruit trees. So, in addition to the scions I got from the annual scion exchange at a local CRFG, I ordered some from the repository. They were all fig scions. The selected varieties were based on my favorites at the annual fig tasting Wolfskill Experimental Orchards had the year before. All of these scions took, six in all. One I had to do over again. I could not remember which one it was. Of the original six, only four remained. One broke during a windstorm in Fremont, where I live. One got broken during the winter pruning I did on the stock plant. The successful scions were just starting to fruit this year. This spring I received several seedless table grape material from the repository and grafted them onto the existing *Vitis californicus* I had planted in my front yard last year. Some of them have already taken. Some of them that have taken were already blooming. My main objective for grafting the table grapes was for personal consumption. I plan to request other material, stone fruits more particularly. I have already made my choices. *In fact, I was at the Wolfskill Orchards this morning for the stone fruit and mulberry tasting.*

Shung-Yee Kung, UC Riverside

I am very grateful to receive the *Cuphea* seeds from (NPGS) to do my research project. I requested the seeds twice, 2012 and 2013. I haven't had a chance to use the batch from 2013 (they are well kept in our dry box in the lab) but the seeds received from 2012 help me a lot on my research (I planted them and collect the seeds to analyze the TAG composition and extract gDNA and RNA from different plant organs). I am still in the middle of the project and hasn't produced any research paper yet. The germinate rate of the seeds either in 15 °C and 25 °C, and the germination pattern is like this:

Cuphea hookeriana: 15 °C: 10 %; 25°C:20 %

Cuphea hyssopifolia:15 °C: 30 %; 25°C:0 % (this is wired)

Cuphea decandra: 15 °C: 90 %; 25°C:100 %

Cuphea racemosa: 15 °C: 20 %; 25°C:30%

I didn't test the others but do observed that Cuphea lanceolata grows perfectly in north california but somehow its stem become sticky and and hard to grow when transfered indoor with approximately same day/night condition and more humidity.

Yasha Magarik, Leichtag Foundation,
Encinitas

Thanks so much for soliciting our feedback and descriptions. First, I believe the NPGS is an absolutely essential institution for the purposes of agricultural research, maintained biodiversity, and resilience to disturbances in our agricultural system. The NPGS is thus necessary for preserving not merely economic and environmental but also national security. I think the program should be funded at not just current levels but, in the face of further consolidation of industrially monocropped land and increasingly turbulent and unpredictable climate conditions, should be expanded to more institutions, with an even more diverse set of crops (particularly perennials), teams of researchers, and outreach to farmers and gardeners who could greatly benefit from the services you provide.

Second, our specific situation. On behalf of the Leichtag Foundation, I requested a few dozen cultivars from 2 repositories---Corvallis and Davis---last fall. Leichtag has just started to transform a 67.5-acre poinsettia-growing ranch in Encinitas into a hub for sustainable agriculture; as part of that vision, we hope to implement a wide variety of edible landscaping on site, including within a food forest and trail system on the northern edge of the ranch, which will be built and maintained in cooperation with the city of Encinitas. The edible perennials on site will provide a food source and teaching tool for community residents, a showcase and clonal resource for local farmers and growers' institutions (such as the San Diego Botanic Garden, California Rare Fruit Growers, the Farm Bureau, San Diego's extension station, and others), a forum for community programming around themes of food access and food justice, and, perhaps most relevantly for your purposes, a resource for agricultural research, including both identifying cultivars resistant to climate change in our region and breeding new varieties that may be of use to local growers.

A quick note on the ordering. Each repository's web site is slightly different; standardizing them in a more useable format might be a valuable project. The best site I encountered was Corvallis's, which listed info on each variety in a long-form format, so that it was easy to research the right varieties without navigating between too many pages; Corvallis also provided essential data on the results of cold-hardiness and disease tests. I received 2 different shipments from Corvallis, one of various berries in December, one of quinces and pears in January or February. I used them all for propagation rather than grafting; for now there isn't any rootstock on-site and my first priority is building a diverse and healthy population of perennials that could only later be harvested for scions. I never received any shipments from Davis---although the species I am most excited about growing here (figs, pomegranates, etc.) are based there; this was one of my frustrations with the NPGS system. Instead, I've had to rely on 2 CRFG scion exchanges and a number of informal arrangements with local growers, many of whom do not yet have the biodiversity I'm seeking to create here. But my experience with the Corvallis cuttings was very positive; many

of them have succeeded to root, and as they grow into trees we will expand the collection and begin to experiment with growing clones of each under different controlled conditions.

Please consider this an open invitation---to you and to any other agricultural researchers you know of who might be interested in seeing our facility. In case you're interested in learning more about the organization, here is our website: <http://www.leichtag.org/>

Jeremy Le Roque, Redding

I received about 30 budwood apples. Through my own error they all died.

Roy Wiersma, Alta Loma

In 2013 I received seeds of Cucurbita digitata (PI 240879) from the USDA-ARS-Plant Genetic Resources Conservation Unit at Griffin, Georgia. Some of these seeds were given to Jim Henrich Senior Biologist at the Los Angeles Arboretum where they were subsequently grown and readied for planting on the grounds for public display. At the time I gave him these seeds I also gave him seeds of Cucurbita palmata (PARL 115) which I received earlier from NALPGRU at Parlier, Calif. These too have grown. I also have plants of both species which are growing well.

A shipment of mostly hybrid blackberries of historical interest was sent to me from NCGR-Corvallis, Oregon, in late 2013. These were mostly unrooted cuttings and almost all have survived to present. One of my research goals is to identify the old varieties of blackberries dating to the first one ('Dorchester') of the late 1840s (please see photocopies to be mailed separately due to hard disk drive failure). This project is probably better suited for someone based in the eastern United States but I am figuring out as much of the puzzle as I can here in southern California.

In January 2014 I donated extra potato plants of the varieties Burbank and Early Rose to Gold Ridge Farm at Sebastopol, Calif. These varieties were acquired from the USDA-ARS-Potato Introduction Project at Sturgeon Bay, Wisconsin, also in 2013.

The USDA-ARS-Crop Germplasm Research facility at College Station, Texas, sent me seeds of eight strains of mostly colored cotton in 2008. In addition to growing and evaluating these cottons myself, I gave seeds to NALPGRU at Parlier and to Ed Read Greenhouse Manager at Cal State Fullerton where they were grown for demonstration purposes for the students.

Cladodes received from NALPGRU at Parlier resulted in the publication of my book, Luther Burbank Spineless Cactus Identification Project (2008).

Presently I am evaluating past and current (2014) Prunus material sent to me from USDA-ARS-National Germplasm Repository at Davis, Calif. Sadly, someone has been poisoning my plants and so I have had to re-request scionwood and re-do many grafts.

The NCGR system is important. Please don't allow tragedies like the national rubber tree collection destruction at Turrialba, Costa Rica, of 1954-1955 (collected by Richard Evans Schultes - see One River: Explorations and Discoveries in the Amazon Rain Forest (1996) by Wade Davis) and the loss of so many Opuntia species collected by USDA scientist David Griffiths from 1906 to 1916 happen again (Taxon 37(1): 76-107. February 1988). Both men spent over a decade each collecting germplasm. Secondly, many NCGR holdings are not available commercially. Also, see scanned report.

Eric Hovakimian, Walnut Creek

I received one cutting (Madeira black fig), but it is still struggling to produce more leaves, I hoped to receive more than one cutting, but according to the administrator, that fig was very popular and demand for it was great so I could get only one cutting.

Laurie G. Smith, UC San Diego

This email describes the use of maize germplasm I received from NPGS in 2012, which I used in 2012 and again in 2014. I received an email requesting information for the report last year but was on sabbatical at that time and was not able to respond. So I apologize if this info is not exactly what you need for the 2013 report but feel free to modify the dates below as needed to suit your purposes.

In early 2012 I received 27 inbred lines of maize (generally 100 seeds/line) that constitute the founders of the nested association mapping (NAM) population. These were used for two studies as follows:

1. In the summer of 2012 they were used for an experiment analyzing genetic diversity in the allocation of biomass to shoots vs. roots, how this differs under well watered vs. water deficit conditions, and how it relates to yield under water deficit conditions. The results of this study, which was conducted in collaboration with Elsa Cleland at UCSD, are in preparation for publication.

2. In the winter of 2014, they were used for another experiment investigating genetic diversity in leaf cuticle function as a barrier to water loss. Adult leaves from each line were analyzed to determine the rate at which water is lost from detached leaves under conditions where stomata are closed. Selected lines were also assayed for leaf cuticular wax composition to investigate how water loss rates relate to wax composition. Results were used as preliminary data for a proposal to the NSF Plant Genome Research Program submitted in April 2014. These experiments are being repeated in the field in summer 2014 with the intention to publish the findings.

Specific comments and observations: All the lines I received in 2012 germinated but some germinated rather slowly and/or had low germination rates. Over the past two years in my seed storage room, these lines almost completely lost the capacity to germinate while others were still fine: CML333, Tzi8, CML322 and NC350.

When grown in the field, I noted that both Tzi8 and CML52 were segregating for anther color (some plants had colored anthers, some did not) indicating that at least in this respect they exhibit genetic variation within the line and thus are not fully inbred.

Thanks again to NPGS for sending these lines!

Paul Brookhouzen, Patriot Seed Co.,
Sacramento

We have been evaluating and crossing a number of introductions from the USDA germplasm collections. We work in 3rd world environments developing useful germplasm for agricultural improvement. We also do some development for the USA markets. Without access to this germplasm it would be very difficult for us to make these improvements. We being a

smaller company without tremendous assets , access to this germplasm is critical.

We sincerely appreciate all the efforts accomplished to maintain and distribute this germplasm. We are getting close to releasing some materials and will at that time be more able to provide specifics about the germplasm that was useful. Thanking you always.

Jerome Bernier, Monsanto,
Woodland

We have received several lines (watermelon) that have been shown (in public literature) to carry disease resistance to diseases where no resistance is present in the elite germplasm. Once we receive the germplasm, the first step is to cross it with an improved line, self once and then evaluate the level of resistance and segregation ratio of the F2/BC1. If the level of resistance is large enough and the inheritance not too complex, we will then proceed to attempt to use this source in breeding.

Chuck Chan, San Francisco

I mostly got several things from the Corvallis Germplasm in particular *Fragaria* sp. in the last two years for breeding purposes so I have the following species:

Several *F. vesca*. crosses were made. Sowing F2 generation for variation

Several *F. virginiana* species. Sowed F1 generation this year

F. ananassa white fruiting species and selected red fruiting species (Crosses made)

Synthetic octaploid *Fragaria* species. One of them just bloomed

It takes about 1 year to get plants obtained from the germplasm to bloom.

Synthetic octaploid species just bloomed this year and we were able to cross one of them.

So far I do not have results. But I have submitted 21 plants total to corvallis germplasm http://www.ars-grin.gov/cgi-bin/npgs/html/cno_acc.pl?144535

Carole Ambauen, Petaluma

I'm not a researcher and planted the red currant in our garden. two plants are alive, still tiny but I am keeping them going.

Douglas Heath, Bejo Seeds,
Oceano

This year the only accessions I am using are listed as sources of potato virus y [pvy]. They are PI 247087 which is was already familiar with and PI 365904. Right now I am just growing them out for observation and for seed increase so I can make some breeding crosses this fall to start the process to introgress the resistance. I am now in a smaller company and we will need to do in-house development of a molecular marker so this may take some time. Luckily, it is not a disease common to many tomato growing areas so it is not a high priority project. Nevertheless it is something for me to add to my new program and continue with on a limited scale into the future.

Jeff Dahlberg, UC-ANR,
Kearney Ag. Res. Ext. Center,
Parlier

I got the seed too late last year to plant in 2013, so the sorghum I got was planted yesterday. I was asked to evaluate them, since the NPGS had no information on them in terms of race, agronomic information, etc... So I should be seeing something to evaluate sometime this fall. I will probably not have the opportunity to publish on them, but I will provide NPGS with

up to 39 descriptors, which I developed as the Sorghum Curator for the ARS years ago, along with potentially photos of the panicles, if they produce a panicle. I'm not sure how much of this is photoperiod sensitive, so they might not even flower.

If you want a chance to see some diversity in sorghum, which it looks like there will be a lot, I would venture to guess late September or early October would be a good time to see it.

Waldemar Juschin,
Amaranth Bio Company,
San Diego

The use of the received amaranth grain was to grow it at the urban garden "SMARTS FARM" in downtown San Diego.

Due to the weather condition, it hasn't started to grow until recently.

Attached is a picture for your review.

We are growing amaranth to demonstrate to the general public how it looks like and also want to create educational programs about the health benefit of it.



There are no studies or publications intended to be done in the future.

Adib Rowhani, Foundation Plant Services,
UC Davis

We have used dormant cuttings collected from 166 grapevine and tree accessions in the National Grapevine and Tree Germplasm Repository in our research to evaluate the molecular methods developed for the detection of some of the viruses in these crops and also to conduct a survey for the new viruses found and characterized. The publications resulted from these works are listed below. Thank you

Susan Alvarez, US Agriseeds,
San Luis Obispo

I am the manager of a commercial seed company testing laboratory. My requests for seed samples have been the result of a quest to obtain reference specimens for a seed herbarium. We use these reference samples when identifying contaminants in the samples we receive for physical purity analysis.

I have particularly been searching for specimens which are listed on recommended seed herbarium lists provided by the International Seed Testing Association, the Association of Official Seed Analysts, and the Canadian Food Inspection Agency.

Thank you for the opportunity to obtain these valuable samples.

By the way, I am a graduate of the M.S. program in Horticulture at UC Davis. Best regards,

Linda Gray, Ukiah

On April 22, 2013 I started soaking about half (approx. 70) the Iroquois corn seed. Sixty of them sprouted. On April 26th I planted the sprouted seeds into separate containers in my greenhouse. *When the seedlings emerged I put them outside, bringing them in at night if the weather required. Unfortunately, birds ate more than half of them.* On May 6th I transplanted the remaining 25 seedlings to the garden and also started soaking 40 more seeds. On May 14th I planted the 30 corn sprouts in the garden that I had started soaking on May 6th. The stalks were a little spindly. I think the place I planted them may have been too wet, so I'll plant them in a drier part of the garden next year. June 26th the corn began to show tassels. Altogether I grew 55 plants which averaged about 7' tall to the tip of their tassels. They produced 62 ears. I Harvested the corn September 6th. Dried it and shucked 45 ears to use for seed for next year. There were a significant number of white seeds on 4 ears. I intend to plant some of the seed I saved from last year June 3rd of this year.

Xiahua He, USDA-ARS, Albany

Yes, I did receive some castor seeds from NPGS in Griffin, Georgia. *The seeds I requested were used by Dick Auld as parents for castor low ricin cultivar, Brigham. We try to examine the ricin contents in these seeds and compare them with their offspring.* But we have not done the experiment yet because of the funding problem. These may be valuable materials for development of low ricin castor plants.

Franklin Borkat, La Mesa

In 2012 I requested cutting from the National Plant Germplasm System (NPGS) (request made to the National Clonal Germplasm Repository {NCGR}) to be received in 2013. The cuttings were for fig, pomegranate and mulberry, all to be rooted. *I requested three varieties of figs, three of pomegranates and one mulberry. Not all the cuttings rooted, but at least one of all the fig and pomegranate varieties rooted successfully. The mulberry (Gerald Dwarf) did not.* *I am a home hobbyist who is a UCCE Master Gardener and also member of the California Rare Fruit Growers. I have a small collection of pomegranates, mostly growing in 15 gallon nursery pots. The majority are soft seeded. I also have a small collection of figs that have been recommended by others in the two organizations I belong to.* As a method, some of the longer cuttings were cut in half so that at least two leaf nodes were below the rooting medium with only one or two above. The cuttings were dipped in rooting hormone. All were rooted in 1 gallon nursery pots with commercial potting soil and kept outside. The soil was kept moist. This year (2014) all that successfully rooted were transferred to 5 gallon pots for further maturing. On removal from the 1 gallon pot the roots on those that grew looked extremely well developed and healthy. Other than being successful at rooting most of the cuttings, I have not done anything else with them. They are still too young to bear fruit or to report on how successful they are in the climate and growing conditions of La Mesa, CA, 91942. I appreciate very much the availability of the germplasm and made a similar request in 2013 for material received in 2014. Should there be future

request to me about the material received when the plant material matures, I will be happy to provide.

Shifeng Pan, Gilroy

First of all, thanks to USDA to provide the free samples on sunflower species I requested in 2013. The following lines from the request, have been sown in a current greenhouse trial. They are still in an early none flower stage at the moment. *What we are looking for from the genetic germplasm are mainly for disease resistances and other ornamental traits.* Since those lines are much later to flower than other Lines we have in trial. We are not sure if we will infuse these germplasm to our program or not. I will give you a further report after the plants have flowered, and decisions have been made.

The following are the lines, and germination information included in our trials:

PI468638	seed sow 30	seed germinated 0
PI664638	seed sow 30	seed germinated 0
PI649860(AN0-2346)	seed sow 30	seed germinated 1
PI539893 (AN0-1509-2)	seed sow 30	seed germinated 28
PI539894 (AN0-1509-1)	seed sow 30	seed germinate 29

Graham, Savio, Butembo, DRC

I received 10 lines of cowpea (Vigna unguiculata) which I planted in early 2013 using greenhouse space borrowed from the UC Davis student farm. The purpose of these growth trials was to evaluate the lines I'd selected for their potential use as a vegetable crop - i.e. the tenderness, taste and productivity of the leaves and the green beans. This research was carried out in collaboration with NaSARRI (the National Semi-Arid Resources Research Institute) in eastern Uganda, with funding from the Trellis Project, a USAID-funded program run through HortCRSP (now the Horticultural Innovations Lab). I reported the results of my trials in meetings with partners in Uganda in June 2013, and they will source those lines and use those recommendations in their future work on the subject of horticultural uses of Vigna unguiculata.

Suja George, Dept. Plant Mol. Bio.
Swaminathan Res. Foundation,
Taramani, Chennai, India

Thank you very much for your mail. first of all, I would like to tell you how grateful I am at for the timely delivery of the germplasm i requested which enabled me to complete my work.

I was a visiting Research Scholar at the plant sciences department of UC Davis when i received the germ plasm. my studies were in the field of molecular and bioinformatics analysis of abiotic stress induced plant transcriptome. i had obtained different accessions of the following species form the USDA GRIN

1. Sorghuum bicolor
2. Pennisetum typhoides
3. Macrotyloma uniflorum
4. Chrysopogon zizanioides
5. Prosopis juliflora

Experiment methodology

I have subjected all the accessions to salt/drought stress and leaf and root tissue were harvested at 0th (control) and 36 hour of salt/drought stress. from each accession, i have created 6 RNAseq library each (control leaf, control root, salt stressed leaf, salt stressed root, drought stressed root, drought stressed leaf). those libraries were pooled and sequenced by Next Generation Sequencing methods.

Currently, I am analyzing the sequence data to gain insights into the molecular mechanisms of plant abiotic stress tolerance. The studies have not been completed yet, and we haven't published any papers. I would mail you when I do.

Christopher Craven, San Diego

I received ficus carica cuttings last year and distributed some of them to the San Diego chapter of the California Rare Fruit Growers (CRFG). I am evaluating the current trees for their quality in coastal areas of southern California and will be reporting my findings to the CRFG. If you have any other questions, please let me know.

Thomas Curry, Temecula

The olive cuttings that we received were not viable for the second year in a row.

Julia Inestroza, Springfield

I believe you're asking about the assads seeds we received. We have not propagated them yet. We also received some medjool date seeds. Also, have not propagated them.

Chuck Fleck, Fowler Nurseries,
Newcastle

Fowler Nurseries received several Pyrus communis samples from NPGS. These were obtained with the intention of fruit evaluation for possible cultivar development. *There is no alternative reliable source for these pear cultivars, therefor the NPGS is invaluable to scion variety development.*

Jorge Dubcovsky, UC Davis

We have rallied heavily on the NSGC for all our germplasm requests. *We used barley accessions in our mapping of flowering genes (1). In wheat the requested germplasm was essential for our project to clone the Sr35 resistance gene to stem rust race Ug99 (2). We also used Triticum sphaerococcum germplasm to validate the cloning of the VRN4 vernalization gene (unpublished)*

The NSGC is a critical resource for our wheat breeding and genetics program

Kishor Bhattarai, Syngenta,
Woodland

We have received seeds for all the requests we have made. Currently we are in the planning stage of experiments and have not been able to put those materials in ay experiments yet. But, certainly we would like to evaluate them for resistance to different pathogens. We will continue requesting some new materials as we start building our germplasm base. And I look forward to getting your support in the future as well.

Xiaohua He, USDA-ARS, Albany	<i>I have never received any plant germplasm from the National Plant Germplasm System. (Obviously, she/he did, but didn't recognize the source of his/her research materials - highlights a PR issue for NPGS.)</i>
Matthew Gilbert, UC Davis	My laboratory has used diverse germplasm from the Phaseolus genus (spp. lunatus, vulgaris and acutifolius) to conduct a series of experiments to dissect the nature of the drought responses. This work is underway and will be used to understand the physiological diversity available to bean breeders in California, the US and at CIAT for improvement of bean production under drought. The data will be published upon completion of the extensive experiments.
Richard Lee, NCGR Riverside	<i>The citrus germplasm has been used largely for evaluation of diverse germplasm for tolerance to huanglongbing and Asian citrus psyllid with cooperators in Florida where the disease/insect is now endemic. The date palm DNA is being used for genotype by sequencing research in cooperation with USDA ARS in Ft. Collins, CO. The results will be summarized and located in GRIN-Global once the studies are complete.</i>
Mark Lessner, Mountain House	I was hoping to see if I could grow the plant, citron/etron and the seeds I received unfortunately did not germinate.
David Mckill, UC Davis	<i>In 2013 I received 36 samples of rice in 2013. All of them were screened for grain quality traits, mainly amylose content. The information was useful but we have not found any of the entries that would be used for further breeding.</i>
Rebecca Newburn, Richmond	<i>The Domasna fava beans (order 252051) that we received are still in the ground and the pods aren't quite ripe for seed harvest. Once the seeds are mature, they will be added to the Richmond Grows Seed Lending Library where they can be borrowed by members of the public. Our service is located in the Richmond Public Library and we provide seed for free to the community along with free education on seed saving. Since they are a special seed, we'll make a note to borrowers that they should only be borrowed if the borrower knows how to save them properly and intends to do so.</i>
Gregory Redmond, Oakland	They are in flower pots trying to grow. I do not know what their value is. Send more.
Helen Mira, Littlerock	I, personally, am a very novice gardener. Meaning my success comes from the most child levels of seed germination to maturity with excess care not natural to actual growing conditions here. Then there's the ol' stand by of just sticking, roots and all into some fresh water and see what happens. I am fortunate though, in the amount of land I have and the intelligent gardeners and farmers in the surrounding area. My neighbors and I just wanted to expand "possibilities" and variety to this very arid place. I and

one other had the room for this project. However, I am the only one tasked with requesting seeds last year.

Well, I regret having to reply to you without any useful information about the seeds that I received last year. My neighbors/colleagues and my self, were so excited in trying to start and nurture a more diverse "draught hardy" fruit, root, and nut type crops, here in the California High Desert. Which was why I applied to the seed bank.

However, the one sample of seeds I was able to receive, also came with a warning that it was possibly "contaminated" and what extra measures to take before planting. Well. I followed the directions and nothing came of it. I made sure, by consulting with those around me, that my soil conditions were fine and environmental temps were satisfactory....but nothing. The seeds are dead. The instructions did warn of this but....Arrrg!

I'm not alone though. What little that got planted, so far, isn't showing anything to make note of. Elevation levels and alternative water tests are being discussed.

Again. I am really sorry about not having a better report about the seeds we tried here, from the seed bank. I do hope that you have more positive reports returned to you.

This year, I'm taking a chance on Heirloom Japanese Peaches! Wish me luck for next couple of years!

Eric LoPresti, UC Davis

I received many accessions of various species of Chenopodiaceae over the past year and a bit. I am investigating plant defenses in the family, specifically in relation to the salt bladder system, an osmoregulatory system in halophytic species and seemingly defensive in all. I am able to look at phylogenetic hypotheses because of the number of species available, which would be prohibitive otherwise. They have been absolutely invaluable to my dissertation thus far (2 years in) and will continue to be, as many of them cannot be collected easily (foreign or would require travelling all over the country). Additionally, David Brenner, at ARS in Iowa, has given me a huge amount of information, advice and references in addition to the seeds. I used 20+ accessions in this paper and have this sort of data for a good number more: (see publications). I should have another paper next year in the same vein using the same (and more) accessions.

David O'Donnell, UC Davis

In the calendar year 2013 I made several requests for Zea mays seed from the National Plant Germplasm System. This seed was vital for my studies as a continuing Genetics graduate student. My home laboratory at UC Davis studies multiple populations of maize derived from Mesoamerica, and we are interested to compare their growth patterns relative to ancestral teosinte subspecies, commercial inbred and conventional hybrid varieties of maize. I requested for several different lines of teosinte as well as the aforementioned commercial/conventional varieties, and these accessions allowed me to obtain invaluable results as I work toward completion of my thesis. On a larger scale, I am certain that this work will aid in maize research across the globe, as this data should provide valuable insight into the conservation of distinct traits passed down from ancestral teosinte species, as well as information pertaining to artificial selection during modern maize improvement. Again I must state that without the National

Plant Germplasm resource, my research would not be accelerating at its current rate. I am certain that a great many other scientists will agree that this resource is absolutely invaluable, and it is imperative to continue funding for this tremendous system.

Cristian Saldana, Porterville

The germplasm we received was in good condition. We are using the germplasm for research.

Johnathan Sinclair, San Juan Batista

Unfortunately none of the material I requested germinated, so I don't have any information for you.

Paul Sober, Sacramento

We received 10 seeds for *Hippophae rhamnoides* from the National Plant Germplasm System. Our processes have been improving and we are currently achieving around 80% success rate with commercially obtained germplasm material. It is being used to develop a strain without thorns for commercial production. It is hoped that the additional genetic variation provided by the program will increase our chances of successfully developing a strain that is more useful than what is currently available.

Matthew Powers, O'Neals CA

Germplasm has been utilized in Horticulture, the school garden & in small groups of students focused on an AG related career path. We have a greenhouse & an outdoor garden featuring many of the germ plasm received. Sadly, much of what we received did not root, germinate or survive in our drastic climate. In our area we experienced a severe drought this year (Zone 9B foothills of Central Valley CA). Some plants bolted and then burned before they could properly seed. Many didn't germinate. The trees and cuttings might have been ordered at the wrong times: the first batch didn't work and the more recent ones sent are still nascent in development. (The strawberry plant looked like it was in the fridge in a bag forever!) Most of the seed genes ARE valuable, but I think they are not resilient enough for our area sadly. In my own experience I've had to nurse many plants for a few years, saving seed, until they adapt properly to the area. The value has two sides: learning from mistakes is always valuable for developing minds & skill sets, but it may be an uphill battle if the seeds are being treated on GRIN's end with sprays & chemical supports. I think it may be a combination of our environmental stresses and the lack of resilience in the germ plasm genetically, but despite having a stellar record with plants, *I have found the GRIN germplasm to be finicky and not resilient.*

If I'm mistaken about the chemical supports (pesticide, herbicide, fertilizer, etc), please correct me. Many of the accessions were from areas with hardier plants than my region. I only theorized that a dependency might have developed weakening this resilience.

I would appreciate ANY advice you have to help our program. Helping the next generation of farmers, horticulturalists & GRIN workers develop is our aim!

Terry Radi, Nuseed Americas,
Woodland

Dan, we requested some PI germplasm from the State of CA, but never received it.

Xuemei Zhang, United Genetics Seeds,
Bakersfield

PI 414723	USDA	10 plants	Piel De Sapo type?
PI618838	USDA	10 plants	Hami type?
Ames 22802	USDA	10 plants	France Puy-de-Dome Ananas type
Ames 21656	USDA	10 plants	Egypt Ananas type El-Dokki
Ames 13331	USDA	10 plants	Spain Alicante Piel De Sapo type
Ames 13319	USDA	10 plants	Spain Murcia Piel De Sapo type

Six accessions were planted in the greenhouse in 2013 at Bakersfield research station. 10 plants are planted of each accession for observation. Ames22802 and Ames 21656 are the Ananas type melon and Ames13331 and Ames 13319 are the Piel De Sapo type melon. Because of the small fruit size and not very strong plant growth of the above four accessions, no seeds were harvested and are not used in the melon breeding program at United Genetics Seeds. Co. . PI618838 had fruits that splitted bad before the fruits got mature. No seeds were harvested.

PI414723 was used as the resistant check for ZYMV and PRSPV inoculation on melon virus breeding program. Four single fruit were harvested and inoculated with ZYMV and PRSPV separately. There were different degrees of virus resistance among the four single fruit selections. We are continuing with the selection on strong growth plants with higher degree of resistance. The breeding plan is to transfer the virus resistance into different type melon inbred lines.

Ed Stover, USDA-ARS,
Ft. Pierce, FL

We have extensively tested accessions from the National Clonal Germplasm Repository for Citrus and Dates in Riverside, CA. We have received seed of over 300 accessions in the last 5 years. The repository staff are critical collaborators in these experiments which primarily focus on resistance/tolerance to the disease huanglongbing, but also include evaluations of accession response to Asian Citrus Canker, Asian Citrus Psyllid, Citrus Leaf Miner and cold hardiness. The publications which include results from these tests are listed below: (see publications) Work with these materials has revealed substantial resistance to huanglongbing in distant citrus relatives. It is my expectation that this work will provide yet more compelling evidence of the value of the National Plant Germplasm System in sustaining US agriculture.

Thomas Valentine, Hollister

The pumpkin/squash " Nigarian Local" received in 2013 had rather poor germination and was impossible to set seed due to the female and male flowers bloomed at different times. I have a few remaining seed but have given up hope for this P.I. source for a source for zucchini yellows mosaic resistance.

Michael Towne, Sacramento

I attempted to germinate black currant seeds that I received from the germplasm. I was unsuccessful 2 years in a row. I tried potting soil, native soil, and a mix of the two. I didn't do a wonderful job of stratification, and perhaps that was the largest issue. At any rate, I struck out, but sure would love to get some of those black currant varieties one of these days. Best of luck with your report.

Sven Merten, Jamul CA

The plant material was in great shape when it arrived and the cuttings were doing well and some were showing growth. Unfortunately, I am very sad to report, our misting timer had a problem while we were out of town and the person we had watching the place didn't notice and we lost all of the cuttings. Sorry for any wasted effort.

They were summer (green) Mulberry cuttings. They might have grafted, but I was fairly confident they would root so I didn't even think to try. I'll see if I can order some hard wood cuttings this winter. They'll root easier anyway, and I'll graft some on to my bigger trees just in case.

We are a small farm and sell our produce and products at local farmers markets and had hoped to bring some of the fruit to market as well as share some of the plants with other growers as well as other CRFG members. Please let me know if you need any more information.

Colleen Doherty

I received germplasm in previous years (not 2012) which I used in 2012. I requested and received Nipponbare seeds. They arrived in excellent shape, a sufficient quantity, and germinated well. This allowed me to perform experiments in rice to determine if the observations I had made concerning the circadian clock and salt stress responses in Arabidopsis were preserved in a 'real crop'. Although I do not have publications on this germplasm yet, the preliminary data I have did allow me to apply to faculty positions last year and I will be starting my own lab, with the intent of working in rice, this year. There is one point I would especially like to make about this germplasm, the people running the program and the overall usefulness of it. In my particular situation, I was having a terrible time "bulking up" on the rice, I could not find facilities with the proper light and humidity here to grow rice and I was not able to generate many viable seeds. Then my PI left and I was left without any chambers for growing the rice, even as poorly as I was (I only had small chambers for growing seedlings and doing experiments). I was quite panicked about being able to keep doing experiments. I contacted y'all about this problem and the response I received was wonderful, supportive, and basically said, if I need more seeds, just ask. This was a huge relief, and even though I have not needed more seeds yet (I will when I start my lab) it helped tremendously to know I did not have to spend the precious little time and resources I had trying to grow rice and I could focus on finishing up the experiments (and applying for jobs and grants). The generous attitude has been very useful in helping me make the transition away from Arabidopsis and more confident in applying some of my expertise into a crop plant. If y'all were not as supportive, I might have "chickened out" and gone back to Arabidopsis. Now I am excited about the possibilities of working in rice and can't wait to start working with other varieties. It helps me to stay excited about research to hope that there will be a direct connection between what I do

and feeding people, so I'm glad this resource is available so that I can work in rice, even if I'm lousy at growing it.

Ann M. Hirsch, UCLA

See report on following pages.

W6 report-2014

Vigna unguiculata subsp. *unguiculata* (cowpea) have been tested for their response to inoculation with the South African nitrogen-fixing bacterium, *Burkholderia tuberum* STM678. This strain was originally isolated from the South African legume *Aspalathus carnosus* L., but did not re-nodulate this host. We have been testing both bean (*Phaseolus vulgaris* L.) and cowpea inoculation with *B. tuberum* STM678 because the literature indicated that these two legumes were alternative hosts. We determined that *Phaseolus vulgaris* L. cv. Negro Jamapa (received from the late J. Cabellero-Mellado of Cuernavaca, MX) was an excellent host for this South African bacterial strain (1), after testing some of the bean accessions from the National Germplasm System. We also reported in that previous report that two different cowpea accessions (PI313544 from Mexico and PI339603 from South Africa) nodulated with *B. tuberum* STM678. We had difficulty bulking up on seeds of PI313544, but continued our inoculation studies with *B. tuberum* STM678 using PI339603 because it was easy to bulk up seed under our growing conditions. The other two accessions we received in 2009, PI312203 (was not nodulated) and PI583255 (did not germinate) were dropped from the study.

We requested more cowpea seeds and we increased the number of *B. tuberum* isolates (see Table 1) in the next set of experiments. **Our ultimate goal is to understand the host ranges of these *B. tuberum* strains better** because unlike the better-known *Rhizobiaceae* (alpha-rhizobia), the nodulating *Burkholderiaceae* (beta-rhizobia) do not have obvious host specific nodulation genes (unpubl. data). Due to time constraints, we tested only one Botswanan accession (PI583255), which had not germinated earlier, in addition to re-testing PI339603 with the strains listed in Table 1.

Table 1. *B. tuberum* strains (based on 16S RNA analysis) tested on cowpea accessions.

Strain number	Origin	Nodulation genes	Location	Plants nodulated
STM678	South Africa	nodUSCBA***CJI	chromosome-borne	Species of <i>Cyclopeia</i> (2), <i>P. vulgaris</i> L. (1), <i>V. unguiculata</i> (this work)
DUS833	South Africa	unknown	probably chromosome-borne	<i>Cyclopeia</i> spp. (2), <i>V. unguiculata</i> (this work)
CCGE1002	Mexico	nodDBCJIHASUQ (3)	plasmid-borne	<i>V. unguiculata</i> (this work)

*** an insertion of *nif* genes separates the *nod* genes.

In a series of experiments, we re-evaluated our methods for growing the cowpeas. We found that if the cowpea roots were exposed to light, the plants would not nodulate. Red/far red light perception via phytochrome is known to interfere with nodule development (4). We changed to a system of growing the plants in black boxes containing Seramis®, a type of clay, watered with a nitrogen-free nutrient medium. Under these conditions, PI339603 plants did not grow as well as they did in the Magenta jars, which

used 1:1 vermiculite:perlite as a substrate. Nevertheless, the PI339603 cowpeas nodulated, and developed nodules, but after 4 weeks, they were few and small (Fig. 1A).



The surprising result, however, was that *Burkholderia* sp. CGGE1002 also induced nodules on roots of PI339603 cowpea (Fig. 1B). This was unexpected, because this strain was

isolated from a nodule of *Mimosa occidentalis* growing in Mexico (3), and *Burkholderia* strains with their nodulation genes on a plasmid are believed to nodulate Mimosoid legumes preferentially (5). The uninoculated control did not nodulate and after a month of growth, the plants were yellowing (Fig. 1C). Another surprising result was that the *B. tuberum* STM678 strain induced highly effective (large and red-colored) nodules on one of the Botswana cowpeas (PI; Fig. 1D). Finally, the last surprising result was that PI339603 did not nodulate in response to inoculation with *B. tuberum* DUS833, which effectively nodulated the same *Cyclopeia* species as did STM678 (2) (Fig. 2). Because the *B. tuberum* DUS833 genome has not been sequenced yet, we do not know how it differs from the STM678 strain.



Fig. 2. Cowpea PI339603 was nodulated one month earlier with *B. tuberum* DUS833. None of the inoculated plants formed nodules. However, the roots were highly branched and not as elongated as the roots of the uninoculated control (cf. Fig. 1C).

Dry weight measurements were obtained for the shoots and roots. For the shoots, the DUS833 inoculated plants exhibited the greatest increase in biomass whereas for the roots both CCGE1002 and DUS833 inoculated resulted in the greatest increase in dry weight over the other treatments. Because these were pilot experiments, we do not have enough data to generated final conclusions about the results. These experiments need to be repeated and we also need to test the other Botswanan accessions to see whether the same results are obtained for the nodulation phenotype.

We are still concerned about using the clay substrate by itself so future experiments will use a 1:1 mixture of Seramis® with either perlite or vermiculite. This mixture retains more moisture than the clay substrate.

Respectfully submitted

A handwritten signature in blue ink that reads "Ann M. Hirsch".

Ann M. Hirsch

Literature Cited

1. Angus, A.A., Lee, A.S., Lum, M.R., Shehayeb, M., Hessabi, R., Fujishige, N.A., Yerrapragada S., Kano, S., Song, N., Yang, P., Estrada de Los Santos, P., Faria, S.M., Dakora, F.D., Weinstock, G., and Hirsch, A.M. 2013. Nodulation and effective nitrogen fixation of *Macroptilium atropurpureum* (siratro) by *Burkholderia tuberum*, a beta-proteobacterium, are influenced by environmental factors. *Plant Soil*. 362: 543-562.
2. Elliott GN, Chen, W-M, Bontemps C, Chou, J-H, Young, JPW, Sprent JI, James EK. 2007. Nodulation of *Cyclopia* spp. (Leguminosae, Papilionoideae) by *Burkholderia tuberum*. *Ann. Bot.* 100: 1403-1411.
3. Ormeño-Orillo E, Rjogel MA, Chueire LMO, Tiedje JM, Martínez-Romero E, Hungria M. 2012. Genome sequences of *Burkholderia* sp. Strains CCGE1002 and H160, isolated from legume nodules in Mexico and Brazil. *J. Bacteriol.* 194: 6927.
4. Suzuki A, Suriyagoda L, Shigeyama T, Tominaga A, Sasaki M, Hiratsuka Y, Yoshinaga A, Arima S, Agarie S, Sakai T, Inada S, Jikumaru Y, Kamiya Y, Uchiumi T, Abe M, Hashiguchi M, Akashi T, Sato S, Kaneko T, Tabata S, Hirsch AM. 2011. *Lotus japonicus* nodulation is photomorphogenetically controlled by sensing the R/FR ratio through JA signaling. *Proc. Natl. Acad. Sci. USA*. 108:16837-16842.
5. Elliott GN, Chou J-H, Chen W-M, Bloemberg GV, Bontemps C, Martínez-Romero E, Velázquez, Young, JPW, Sprent JI, James EK. 2009. *Burkholderia* spp. are the most competitive symbionts of *Mimosa*, particularly under N-limited conditions. *Environ. Microbiol.* 11:762-778.

Emma Fong, Bio-Rad, Hercules See report on following pages.

2013 NPGS W-6 Report

By: Emma Fong

Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547

Phone: 510-741-3940, Email: Emma_Fong@Bio-Rad.com

Background and project goals:

We requested corn kernels from NPGS for each of the following 4 inbred corn strains:

- B73 (PI 550473)
- Mo17 (PI 558532)
- B97 (PI 564682)
- Mo18W (PI 550441)

We used the corn kernels and leaves grown from the seeds for quantitative PCR genotyping experiments. These experiments were used to validate a new Bio-Rad qPCR SNP (Single Nucleotide Polymorphism) genotyping Supermix reagent product, for its compatibility with crude DNA lysate samples from plant seeds and leaves.

Our goal is to develop a high performance qPCR SNP genotyping Supermix that is easy to use and highly compatible with variety of sample types and purities. It is, therefore, very essential for the new product to be validated with multiple plant samples of known SNP information. The inbred corn kernels provided by NPGS were the perfect material for our corn sample compatibility validation testing.

Our new SNP genotyping Supermix product will be extremely useful to the agricultural research community for plant genetics, crop breeding, and GMO strain identification. It can also be used for disease mutant identification and drug discovery researches. Overall, Bio-Rad's goal is to develop products that would benefit society through science discoveries.

Methods:

1. We prepared purified DNA from corn leaves, and prepared crude DNA extracts from corn kernels and leaves.
2. We designed TaqMan SNP genotyping assays for SNPs between the 4 different corn strains, using the SNP and sequence information obtained from the Panzea database (<http://www.panzea.org/>).
3. We performed SNP genotyping tests for all of the assays designed, using the Bio-Rad qPCR Supermix for SNP Genotyping. The tests were run on Bio-Rad CFX384 real-time PCR instrument and data was analyzed with genotyping software from Bio-Rad CFX Manager.
4. We were able to evaluate the performance of the Bio-Rad qPCR SNP genotyping Supermix for its compatibility with crude plant DNA samples and purified DNA samples through testing of these samples with different SNP genotyping assays.

Results:

1. Three TaqMan SNP genotyping assays were designed and validated with pure DNA samples from four inbred corn strains. The SNP genotyping results agree with known SNP genotypes of the strains. (Fig. 1-a)
2. Crude DNA lysate samples from corn seeds (Fig.1-b) and leaves (Fig.1-c) were successfully genotyped with the Bio-Rad qPCR Genotyping Supermix for all three SNP assays, with 100% genotype call accuracy. An artificial 'heterozygous' mixture of lysates from inbred corn seeds with different homozygous alleles correctly genotyped as "heterozygous", just as we expected (Fig.1-b).

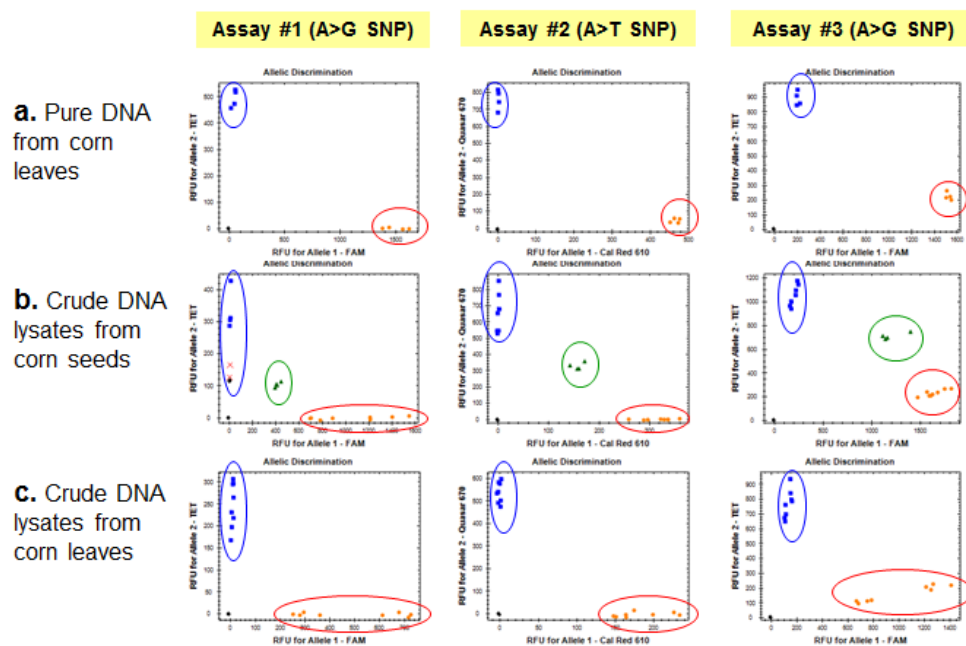


Figure 1. SNP genotyping with corn pure and crude DNA samples. a. Three SNP assays validated with pure DNA from different corn strains; b. and c. SNP genotyping with crude DNA lysates from corn seeds and leaves, respectively. ○ Hom1; ○ Hom2; ○ Het.

Conclusion:

Our validation test using NPGS corn kernel material clearly demonstrated that our new Bio-Rad qPCR SNP Genotyping Supermix is compatible with crude DNA lysate samples from corn seeds and leaves. This test, along with many other validation tests, assures us that our new qPCR genotyping product will be a high performance and easy to use tool for genotyping of plant samples in agricultural genetics and biotechnology research.

Benjamin Winslow, Ball FloraPlant, Arroyo Grande See report on following pages.

W6 2013 Responses

Ball Floraplant- Ball Horticultural
Benjamin Winslow
Plant Breeder
725 Xenon Way
Arroyo Grande, CA 93420

10 June 2014

All material received from the USDA is entered into a new crops trial. I conduct the trial at our research station in Arroyo Grande, CA and replicate it in Texas and Illinois. We are looking for plants with ornamental potential that can be produced from cuttings. If I see something with potential, I either transfer it to another breeder in the company or begin exploratory breeding with the crop.

Genus	Spp	Var	Acc no	Unit	Trial Yr	Notes
Alternanthera	bettzickiana		Ames 26264	AMES	2013	Trialed- invasive potential
Alternanthera	pungens		Ames 29375	AMES	2013	Trialed- invasive potential
Bauhinia	galpinii		94- 0031	DLEP	2013	In trials- slow to bloom, difficult from cuttings
Bauhinia	galpinii		99- 0022	DLEP	2013	In trials- slow to bloom, difficult from cuttings
Bauhinia	macranthera		90- 0455	DLEP	2013	In trials- slow to bloom, difficult from cuttings
Brunfelsia	spp		PI 442168	GRIF	2013	Too tall w/ very small flowers
Dombeya	burgessiae		MIA 35556	GRIN- MIA	2013	Very slow to bloom, future trial in tropical areas to evaluate flowering from cuttings
Dombeya	cymosa		MIA 35570	GRIN- MIA	2013	Very slow to bloom, future trial in tropical areas to evaluate flowering from cuttings

Dombeya	kirkii		MIA 36447	GRIN- MIA	2013	Very slow to bloom, future trial in tropical areas to evaluate flowering from cuttings
Dombeya	rotundifolia		MIA 36448	GRIN- MIA	2013	Very slow to bloom, future trial in tropical areas to evaluate flowering from cuttings
Erythrina	flabelliformis		89- 0309	DLEP	2013	Does not bloom in first year, spines too sharp
Erythrina	flabelliformis		89- 0401	DLEP	2013	Does not bloom in first year, spines too sharp
Erythrina	lysistemom		91- 0011	DLEP	2013	Does not bloom in first year, spines too sharp
Erythrina	lysistemom		91- 0127	DLEP	2013	Does not bloom in first year, spines too sharp
Erythrina	lysistemom		99- 0035	DLEP	2013	Does not bloom in first year, spines too sharp
Jacaranda	caerulea		PI 138505	GRIN- MIA	2013	Cuttings received too large and woody, did not root
Leonotis	nepetifolia		PI 304978	OPGC	2013	Too large, menthifolia is better for ornamental use
Lobelia	siphilitica		OPGC 1573	OPGC	2013	No germination
Ocimum	gratissimum	var. gratissimum	PI 652063	AMES	2013	Attractive flowers, purplish leaves. Sent to vegetable breeding group internal to Ball
Ocimum	x africanum		PI 500954	AMES	2013	Attractive flowers, purplish leaves. Sent to vegetable breeding group internal to Ball
Podalyria	biflora		99- 0056	DLEP	2013	Woody shrubs, slow to bloom, but attractive pink pea shaped flowers
Podalyria	calyptрата		99- 0057	DLEP	2013	Woody shrubs, slow to bloom, but attractive pink pea shaped flowers
Podalyria	canescens		99- 0058	DLEP	2013	Woody shrubs, slow to bloom, but attractive pink pea shaped flowers
Podalyria	sericea		99-	DLEP	2013	Woody shrubs, slow to bloom, but attractive pink

				0059				pea shaped flowers
Sesamum	indicum		PI 248971	GRIF	2013	Interesting potential for home gardener - Sent to vegetable breeding group internal to Ball		
Sesamum	indicum		PI 250748	GRIF	2013	Interesting potential for home gardener - Sent to vegetable breeding group internal to Ball		
Sesamum	indicum		PI 490205	GRIF	2013	Interesting potential for home gardener - Sent to vegetable breeding group internal to Ball		
Sphaeralcea	ambigua		41396	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		
Sphaeralcea	ambigua		42320	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		
Sphaeralcea	ambigua		43628	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		
Sphaeralcea	coccinea		43905	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		
Sphaeralcea	coccinea	ssp coccinea	34224	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		
Sphaeralcea	emoryi		42321	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		
Sphaeralcea	grossularifolia		32701	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		
Sphaeralcea	laxa		42322	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial		

Sphaeralcea	munroana	var munroana	32635	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial
Sphaeralcea	parviflora		40886	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial
Sphaeralcea	rusbyi		35189	USDA W6	2013	Poor performance under humid conditions. Discontinued but has excellent potential for a perennial
Glandularia	aristigera		OPGC 332	OPGC	2013	Incorporated in breeding and trialing of commercial verbena cultivars- awaiting results
Olea	europaea		Dole 31	DAV	2013	Cuttings did not root
Olea	europaea		Dole 39	DAV	2013	Cuttings did not root
Olea	europaea		Dole 186	DAV	2013	Cuttings did not root
Mimulus	alsinoides		W6 30921	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder
Mimulus	aurantiacus		W6 30259	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder
Mimulus	cusickii		W6 40826	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder
Mimulus	guttatus		PI 232583	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder

Mimulus	guttatus		W6 37491	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder
Mimulus	guttatus		W6 39122	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder
Mimulus	lewisii		W6 27313	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder
Mimulus	nannus		W6 39119	W6	2013	Used for interspecific cultivar development- F1 population developed with other species and commercial varieties, seed sent to internal Pan American seed breeder
Lobularia	maritima		PI 304977	NCGR P	2013	Used in the development of heat toelrant Lobularia varieties, awaiting trial results
Arachis	hypogaea	var. aequatorian a	PI 497615	GRIF	2013	In trial this year, looking for ornamental potential, mutation for yellow foliage ideal
Arachis	hypogaea	var. hirsuta	PI 576615	GRIF	2013	No germination
Arachis	hypogaea	var. hypogaea	PI 501272	GRIF	2013	In trial this year, looking for ornamental potential, mutation for yellow foliage ideal
Arachis	pintoi		PI 604805	GRIF	2013	In trial this year, looking for ornamental potential, mutation for yellow foliage ideal
Arachis	pintoi		PI 604812	GRIF	2013	No germination

Suraj Gurung, Salinas

See report on following pages.

Identification of novel sources of *Capsicum annuum* accessions resistance to multiple

***Verticillium dahliae* isolates:** Verticillium wilt of peppers (*Capsicum annuum* L.), caused by the fungus *Verticillium dahliae* Kleb., is a serious threat to production of peppers worldwide (4-7, 11-12, 18). Verticillium wilt in peppers was first reported in the United States from pepper fields in California, where the disease resulted in more than 20% of yield loss (15). Since then, Verticillium wilt in pepper had been observed and described from different parts of the world (2, 4, 7-8, 18). *Verticillium dahliae* is pathogenic to several agronomically important crops grown in coastal California (1) and a number of weed species (16, 19), all of which add to the survival and increase in population density of the pathogen in the soil (11).

Principally, the Verticillium wilt in any crop is hard to manage because *V. dahliae* can easily survive and remain viable as microsclerotia in soil up to 14 years (11, 20), thus, limiting the efficacy of crop rotation. Hitherto, Methyl Bromide (MeBr) is the most effective soil fumigants that is currently being used to control several soil borne pathogens including *V. dahliae*. However, MeBr has now phased out as part of the United States of America's adherence to the Montreal Protocol to eliminate ozone depleting compounds for industrial and agricultural uses (17). At present, there are no other alternative soil fumigants or chemicals as effective as MeBr (14), and more importantly, using chemicals are also not economically and environmentally safe. Soil fumigation for Verticillium wilt of pepper also seems very cost-prohibitive, thus, host resistance to Verticillium wilt represents the best long-term management strategies to control this disease (9).

Few partial resistance sources against Verticillium wilt of pepper had been identified under both field under natural inoculum and greenhouse under artificial inoculum conditions (8, 10, 21). Although field testing seems practical for selecting Verticillium wilt resistant pepper germplasm and breeding resistance for Verticillium wilt, the resistance observed under field conditions were reported to be susceptible with artificial inoculation under greenhouse conditions (10). In addition, the field testing requires lots of time and space and there is always a high chance of disease escape due to the nature of heterogeneous disease pressure of Verticillium wilt in the field due to the variation in pathogen distribution and several other environment conditions (9). It is also very difficult to find a field with a consistent history of Verticillium wilt. Farmers often decide to fumigate their fields as soon as they observe Verticillium wilt problem

and plant strawberry in an endeavor to bound the dissemination of this pathogen, and continue other crops production afterward. In this scenario, identification of potential sources of resistance in the greenhouse condition is the best feasible option.

There are very limited sources of resistance available against *Verticillium* wilt in peppers, and finding additional sources is very difficult and challenging (21). Previously, most of the 456 *Capsicum annuum* accessions tested with *V. dahliae* were susceptible and only few lines showed low level of resistance (21). Similarly, González-Salán and Bosland (8) screened 125 *Capsicum* accessions (*C. baccatum* and *C. annuum*) and failed to find the accessions completely resistance to *Verticillium* wilt disease, however, three accessions showed partial resistance based on lowest disease severity ratings. Resistance to *Verticillium* wilt in pepper seems very complex and resistance is governed by several minor genes or polygenes (10). Relative to cultivars of chili pepper, greater resistance to *Verticillium* wilt has been found in bell pepper cultivars. This might also be one of the reason why the level of microsclerotia is always lower in jalapeno and bell pepper (mean of 6 microsclerotia/gm of soil) in comparison to chili pepper cultivars (mean of 6 microsclerotia/gm of soil) (2). Although, there are reports of tolerant pepper cultivars (Padrón and Yolo Wonder), resistance genes have not been detected and well characterized for *C. annuum-V. dahliae* interaction (13). At present, there are no any commercial pepper cultivars showing complete resistance to *Verticillium* wilt (12). Thus, searching for novel sources of high resistance pepper cultivars are highly imperative.

The United States Department of Agriculture, National Plant Germplasm System has the large collection of worldwide pepper accessions and recently, Byron et al. (3) and Yanar and Miller (22) identified several *C. annuum* accessions resistance to multiple isolates of *Phytophthora capsici* and *Sclerotinia sclerotiorum*. It appears that the pepper accessions at NPGS might have the possible sources for *V. dahliae*. The objective of this study was to evaluate resistance to *Verticillium dahliae* in 388 diverse pepper accessions from the NPGS collection center. Such genetic resources could be used in pepper breeding programs.

In total, 388 *Capsicum* species accessions with different improvement status from the NSGC is being evaluated with *V. dahliae* isolates (VdCf45 and VdCa59) in the greenhouse condition at USDA-ARS, Salinas, CA. *Verticillium dahliae* isolate VdCf45 was isolated from Anaheim pepper from the southern Salinas Valley, and *Verticillium dahliae* isolate VdCa59 was isolated from bell pepper from the Santa Clara Valley. Of the 388 *Capsicum* species accessions

evaluated, 70 were resistant to at least one of the *V. dahliae* isolates tested. At present, we are further confirming the resistance of these 70 *Capsicum* species accessions in the greenhouse condition.

References:

1. Bhat, R., and Subbarao, K. 1999. Host range specificity in *Verticillium dahliae*. *Phytopathology* 89:1218-1225.
2. Bhat, R., Smith, R., Koike, S., Wu, B., and Subbarao, K. 2003. Characterization of *Verticillium dahliae* isolates and wilt epidemics of pepper. *Plant Dis.* 87:789-797.
3. Candole, B. L., Conner, P. J., and Ji, P. 2010. Screening *Capsicum annuum* accessions for resistance to six isolates of *Phytophthora capsici*. *Hortscience* 45:254-259.
4. Douira, A., Ben Kirane, R., Ouazzani Touhami, A., Okeke, B., and Elhaloui, N. 1995. *Verticillium* wilt of pepper (*Capsicum annuum*) in Morocco. *Journal of Phytopathology* 143:467-470.
5. Evans, G., and McKeen, C. 1975. A strain of *Verticillium dahliae* pathogenic to sweet pepper in southwestern Ontario. *Can. J. Plant Sci.* 55:857-859.
6. García-Mina, J., Jordana, R., Aguirreolea, J., and Hernández, M. 1996. The effect of a special organic amendment on the development of pepper plants cultivated in a soil infested with *Verticillium dahliae*. Pages 301-303. in: *Fertilizers and environment*. Springer.
7. Goicoechea, N., Aguirreolea, J., Cenoz, S., and Garcia-Mina, J. 2000. *Verticillium dahliae* modifies the concentrations of proline, soluble sugars, starch, soluble protein and abscisic acid in pepper plants. *Eur. J. Plant Pathol.* 106:19-25.
8. González-Salán, M., and Bosland, P. 1992. Sources of resistance to *Verticillium* wilt in *Capsicum*. *Euphytica* 59:49-53.
9. Hayes, R. J., Vallad, G. E., Qin, Q.-M., Grube, R. C., and Subbarao, K. V. 2007. Variation for resistance to *Verticillium* wilt in lettuce (*Lactuca sativa* L.). *Plant Dis.* 91:439-445.
10. Palloix, A., Pochard, E., Phaly, T., and Daubèze, A. 1990. Recurrent selection for resistance to *Verticillium dahliae* in pepper. *Euphytica* 47:79-89.
11. Pegg, G. F., and Brady, B. L. 2002. *Verticillium* Wilts. CABI Publishing, New York, NY, USA.
12. Pernezny, K., Roberts, P. D., Murphy, J. F., and Goldberg, N. P. 2003. *Compendium of pepper diseases*. American Phytopathological Society (APS Press).
13. Pomar, F., Novo, M., Bernal, M. A., Merino, F., and Barceló, A. R. 2004. Changes in stem lignins (monomer composition and crosslinking) and peroxidase are related with the maintenance of leaf photosynthetic integrity during *Verticillium* wilt in *Capsicum annuum*. *New Phytol.* 163:111-123.
14. Rekanovic, E., Milijasevic, S., Todorovic, B., and Potocnik, I. 2007. Possibilities of biological and chemical control of *Verticillium* wilt in pepper. *Phytoparasitica* 35:436-441.
15. Rudolph, B. A., and Snyder, W. C. 1937. *Verticillium* wilt of pepper. *Plant Dis.* 21:404.
16. Sanogo, S., and Clary, M. 2003. Pathogenicity on chile pepper of *Verticillium dahliae* recovered from three weed hosts in New Mexico. *Plant Dis.* 87:450-450.

17. Subbarao, K. V. 2002. Introduction. *Phytopathology* 92:1334-1336.
18. Tsrer, L., Erlich, O., Amitai, S., and Hazanovsky, M. 1998. Verticillium wilt of paprika caused by a highly virulent isolate of *Verticillium dahliae*. *Plant Dis.* 82:437-439.
19. Vallad, G. E., Bhat, R. G., Koike, S. T., Ryder, E. J., and Subbarao, K. V. 2005. Weedborne reservoirs and seed transmission of *Verticillium dahliae* in lettuce. *Plant Dis.* 89:317-324.
20. Wilhelm, S. 1955. Longevity of the Verticillium wilt fungus in the laboratory and field. *Phytopathology* 45:180-181.
21. Woolliet, G., Denby, L., and Hanson, A. 1962. Screening sweet and hot peppers for Verticillium wilt resistance. *Can. J. Plant Sci.* 42:515-520.
22. Yanar, Y., and Miller, S. 2003. Resistance of pepper cultivars and accessions of *Capsicum* spp. to *Sclerotinia sclerotiorum*. *Plant Dis.* 87:303-307.