**USDA National Clonal Germplasm Repository for Citrus and Dates,**

**(NCGRCD), Riverside, CA**

**Annual Report to the Citrus Crop Germplasm Committee and Date** **Crop Germplasm Committee**

**CY 2017**

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| --- |
| **Mission** |

The mission of the National Clonal Germplasm Repository for Citrus and Dates is to collect, maintain, evaluate, preserve, and distribute germplasm of citrus, dates, related Aurantioideae genera, and date palms and other *Phoenix* species. The achievement of this goal involves: 1) acquisition of the widest possible genetic diversity within citrus and dates to reduce genetic vulnerability in the future, 2) testing and treatment of accessions for pathogenic organisms, 3) maintenance of accessions in a protected, pest-free environment, 4) genetic, horticultural, and physiological characterization and evaluation of accessions, 5) establishment of an informational record for each accession covering acquisition, inventory, evaluation, and gene descriptor data, 6) distribution of germplasm to qualified researchers throughout the world, and 7) research into improved methods of collection, evaluation, propagation, preservation, and distribution.

**Permanent/Term Federal Staff**

MaryLou Polek, Research Leader/Plant Pathologist (Category 1)

Robert Krueger, Curator/Horticulturist (Category 4)

Manjunath Keremane, Plant Pathologist (Category 3)

Vicki Newman, Biological Science Technician

Brittany Moreland, Biological Science Technician

Esteban Rodriguez, Biological Science Technician (Lab) (Term Position)

Patricia Moore, Secretary

Lee Gross, Agricultural Science Research Technician (Half time)

**University grant funded laboratory technician**

Amanda Rawstern (MAC Funding)

**Student workers**

Brooke Gómez

Benedict Mamaril

Andres Márquez

Chidera Mbonu

Ngoc Tran

Gerardo Uribe

**Germplasm Holdings**

NCGRCD germplasm accessions and inventory as of 2017-12-31 are shown in Appendices 1, 2, 3, and 4. Overall there was little change in either accessions or inventory in CY 2017.

**Germplasm Backup**

Citrus germplasm maintained in Riverside exists as both protected plants and field plantings. The University of California- Riverside (UCR) Citrus Variety Collection (CVC) maintains all Rutaceous genotypes in a traditional field planting. About one third of the genotypes exist in the protected, pathogen-tested collection (USDA APHIS-certified screenhouse). In 2008, due to the presence of the Asian citrus psyllid (ACP) in Southern California, the entire CVC was propagated and is maintained as potted plants in greenhouses. Thus, all genotypes without regard to pathogen status are backed up as a protected propagation.

Beginning in 2012, efforts were made to further secure citrus genetic resources by establishing them in cryopreservation at the USDA-ARS National Laboratory for Genetic Resource Preservation (NLGRP) in Fort Collins, Colorado. Initial efforts were funded by the California Citrus Research Board (CRB) and were aimed at developing and optimizing protocols to preserve the valuable commercial cultivars maintained by the UC Citrus Clonal Protection Program (CCPP). More recently, National Programs and the Pacific West and High Plains Area Offices allocated additional resources in CY 2016 to expand efforts to include all sanitized genotypes maintained by NCGRCD. In CY 2016, 219 accessions (approximately 38,325 buds) were collected from the protected, pathogen-tested collection and sent to NLGRP for processing and preservation. In addition, Repository technicians were trained in cryo-technology and an additional 7 accessions (approximately 1,225 buds) were processed in Riverside and will be sent to Fort Collins in May 2018.

In CY 2017, 17 additional accessions were cryoprocessed in Riverside. However, due to the Hold Order placed on the repository when the HLB quarantine was implemented (see section on Distributions), these have not been sent to Ft Collins at the time of this writing. It is expected that they will be sent in June or July, 2018. (See Appendix 7 for summary.)

**Table 1: Accessions cryopreserved in CY 2017 include:**

|  |  |  |
| --- | --- | --- |
| CULTIVARS CRYOPROCESSED @ RIVERSIDE | ACCESION NUMBER | MERISTEMS CRYOPRESERVED |
| Rusk #1 | RRUT 44 | 180 |
| Fukushu | RRUT 199 | 175 |
| Bergamotto Fantastico | RRUT 387 | 180 |
| China S-20 | RCRC 4198 | 180 |
| Ain Taoujdate | RCRC 4212 | 180 |
| Hiryu | PI 433262 | 175 |
| S-1 | PI 539441 | 180 |
| Chinotto | PI 539452 | 175 |
| Tahoe Gold (TM) | PI 539539 | 180 |
| Madam Vinous | PI 539625 | 180 |
| Anseikan | PI 539673 | 180 |
| C-32 | PI 539820 | 175 |
| Citrus macrophylla | PI 600628 | 175 |
| Arizona 861 | PI 600651 | 175 |
| Hamlin + Flying Dragon | PI 600676 | 175 |
| C&M sweet lime | 654899 | 180 |
| Amoa 8 | RRUT 455 | 180 |

With the bulk of the sanitized accessions secured for the long-term in liquid nitrogen, future citrus cryopreservation efforts will be prioritized as follows: the remaining accessions from the protected screenhouse (SH), accessions newly released from quarantine status, sanitized commercial varieties from the CCPP, pre-tested with negative results (using multiplex assay developed by CCPP) accessions from the secure backup CVC, and sanitized accessions imported from the Florida certification program (through USDA MAC funding). Funding was sought but not received through the Citrus Research Board for expanding the cryopreservation work to include seeds and pollen. The NCGRCD and the NLGRP agreed to initiate this work using base funds, but the HLB Quarantine has prevented the NCGRCD from sending germplasm to the NLGRP. National Programs has offered additional funding for this purpose and plans are being formulated to carry out this project for CY 2018.

The NCGRCD has expanded its collaboration with the NLGRP and a private entity, Phoenix Agrotech, to develop and optimize a cryopreservation protocol for date palms. This crop presents some considerable hurdles in that it is a monocot and mature plants are 80 to 100 feet in height and therefore, different cryopreservation protocols from those used for woody dicots (citrus) are necessary. To overcome these hurdles, the NCGRCD has provided date palm germplasm to Phoenix Agrotech who are using their expertise to put accessions into tissue culture. Date palms will then be cryoprocessed in this very young stage. The first batch of samples in tissue culture has been sent to the NLGRP and the development of appropriate cryoprocessing regimes has commenced. Four date palm cultivars (2 male and 2 female) were received from Phoenix Agrotech as in vitro cultures. Research is currently focused on improving the proliferation and shoot tip regrowth media so that ample numbers of shoot tips are available for cryoprotectant and liquid nitrogen (LN) exposure tests. A Materials Transfer Research Agreement (MTRA) was executed in September 2016 to legally allow for this 3-way exchange.

**Germplasm Acquisitions**

NCGRCD acquired 3 new citrus accessions in CY 2017: Okitsu wase X Maltese ovale (RSD 2017002), Micromelum minutum (RSD 2017001), and TI (Rush) (RRUT 545). RSD 2017002 was a controlled pollination specifically intended for use at NLGRP and will not be maintained as an accession. RSD 2017001 did not establish. RRUT 545 was received as sanitized budwood from the Citrus Clonal Protection Program (CCPP).

**Germplasm Sanitation & Quarantine**

**Table 2: In CY 2017, NCGRCD completed sanitation of 17 accessions that were released from State and Federal quarantine in November of 2017.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| INDEX NUMBER | CULTIVAR | GROUP | ACCESSION NUMBER | INVENTORY NUMBER | PROP NUMBER | ORIGIN | BINOMIAL |
| I2016003 | Mesero | lemon | PI 209862 | 10325 | STG2014-25-05 | California (nd) | Citrus limon |
| I2016004 | M'guerqueb | citron | PI 265834 | 10326 | STG2014-23-03 | Florida (1997) | Citrus medica |
| I2016001 | Caoshixiangju (1-11-12) | mandarin | RRUT 150 | 10322 | STG2014-20-01 | China (1985) | Citrus reticulata |
| I2016002 | Beibeiyou (15-3) | pummelo | RRUT 153 | 10323 | STG2014-17-03 | China (1985) | Citrus maxima |
| I2016012 | un-named sweet orange (?) ex-Nepal | sweet orange | RRUT 157 | 10334 | STG2014-05-02 | Nepal (1987) | Citrus sinensis |
| I2016006 | Shekwasha X Koethen (C61-252) | mandarin | RRUT 168 | 10328 | STG2014-28-04 | Texas (1997) | Citrus reticulata |
| I2016008 | Rico #6 | sweet orange | RRUT 169 | 10330 | STG2014-32-02 | Texas (1997) | Citrus reticulata |
| I2016007 | Tomango | sweet orange | RRUT 175 | 10329 | STG2014-29-03 | Texas (1997) | Citrus reticulata |
| I2016009 | Long huang kat | mandarin | RRUT 177 | 10331 | STG2014-34-08 | Florida (1997) | Citrus reticulata |
| I2016016 | USDA 1-42-70 | mandarin hybrid | RRUT 372 | 10313 | TH2014-01-07 | Florida (2007) | Citrus hybrid |
| I2016014 | USDA 1-25-1 | mandarin hybrid | RRUT 375 | 10345 | TH2014-02-06 | Florida (2007) | Citrus hybrid |
| I2016013 | USDA 1-49-105 | mandarin hybrid | RRUT 377 | 10342 | TH2013-02-07 | Florida (2007) | Citrus hybrid |
| I2016015 | USDA 1-22-32 | mandarin hybrid | RRUT 382 | 10314 | TH2014-03-07 | Florida (2008) | Citrus hybrid |
| I2016017 | USDA Navel 1-N | navel orange | RRUT 390 | 10347 | TH2014-06-09 | Florida (2008) | Citrus hybrid |
| I2016005 | Valencia SPB-1-14-19 | valencia orange | RRUT 440 | 10327 | STG2014-27-04 | Florida (2009) | Citrus sinensis |
| I2016011 | Bearss | lemon | RRUT 443 | 10333 | STG2014-44-01 | Florida (2009) | Citrus limon |
| I2016010 | Italian pink-fleshed variegated | lemon | RRUT 452 | 10332 | STG2014-36-03 | Italy (2009) | Citrus limon |

**Laboratory testing of Germplasm accessions**

During the period 2016 – 2017, the following sixteen accessions were laboratory tested and indexed. Release is pending dsRNA analysis on 10 of the 16. Note: The dsRNA analysis has become difficult to carry out because the resin (CF-11) used in the classical protocol has been discontinued. Identification of an alternative or modification of the current dsRNA assay is in progress in collaboration with the Vidalakis lab.

**Table 3: 16 accessions were pathogen tested and biologically indexed during 2016-2017, release pending.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| INDEX NUMBER | CULTIVAR | GROUP | ACCESSION NUMBER | INVENTORY NUMBER | PROP NUMBER | ORIGIN | BINOMIAL |
| I2017001 | USDA 5-51-2 | mandarin hybrid | RRUT 448 | 10365 | TH2014-09-09 | Florida (2009) | Citrus hybrid |
| I2017002 | Iwaikan | pummelo | RRUT 173 | 10363 | TH2014-08-04 | Texas (1997) | Citrus maxima |
| I2017003 | Etonia citrange nucellar | citrange | PI 29160 | 10362 | TH2014-10-09 | Texas (1997) | XCitroncirus webberii |
| I2017004 | Fumin Evergreen trifoliate (OPS) | trifoliate | RSD 1998001 | 10412 | STG2015-06-01 | China (1998) | Poncirus polyandra |
| I2017005 | Gou Tou Xiang Yuan (B) | sour orange | RRUT 321 | 10413 | STG2015-50-02 | Florida (2004) | Citrus aurantium |
| I2017006 | Nin Kat | mandarin | PI 433265 | 10414 | STG2015-12-03 | Florida (2009) | Citrus reticulata |
| I2017007 | pummelo #2 ex-Sichuan | pummelo | RRUT 197 | 10415 | STG2014-52-04 | China (2002) | Citrus maxima |
| I2017009 | US Early Pride | mandarin hybrid | RRUT 389 | 10417 | STG2014-46-01 | Florida (2008) | Citrus hybrid |
| I2017010 | Old Indian | lemon | RCRC 4211 | 10418 | STG2014-42-04 | California (2007) | Citrus limon |
| I2017011 | Canaliculata di Palermo | lemon | RRUT 222 | 10419 | STG2014-39-04 | Italy (2007) | Citrus limon |
| I2017012 | Dona Adelina | sweet lime | RRUT 451 | 10424 | STG2015-38-05 | Arizona (2009) | Citrus aurantiifolia |
| I2017013 | Orange | lemon | RRUT 487 | 10423 | STG2015-48-01 | California (2011) | Citrus limon |
| I2017014 | Shatianyou (2-1) | pummelo | RRUT 154 | 10425 | STG2015-19-03 | China (1980's) | Citrus maxima |
| I2017015 | Cariappa-pummelo3 | pummelo | RSD 2013003 | 10420 | STG2015-24-04 | India (2013) | Citrus maxima |
| I2017016 | Toronja ex-Paso Hondo | grapefruit | RRUT 539 | 10421 | STG2015-60-02 | Mexico (2011) | Citrus paradisi |
| I2017017 | USDA Navel 3-S | navel orange | RRUT 392 | 10422 | STG2015-63-05 | Florida (2008) | Citrus sinensis |

The following seventeen accessions were tested in the laboratory in CY 2017 and are in the process of being biologically indexed. Pending tests for quarantine release include sPAGE, stubborn culture, and dsRNA. Both the proceeding and the following should be released in CY 2018.

**Table 4: Seventeen accessions are currently in biological index, release pending.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| INDEX NUMBER | CULTIVAR | GROUP | ACCESSION NUMBER | IVENTORY NUMBER | PROP NUMBER | ORIGIN | BINOMIAL |
| I2018001 | Kuharske | citrange | RRUT 439 | 10389 | TH2015-02-05 | California (2009) | X Citroncirus webberii |
| I2018002 | H-56 | tangor | PI 539241 | 10395 | TH2015-05-07 | California (1957) | Citrus nobilis |
| I2018003 | Temecula Sweet | mandarin | RRUT 463 | 10394 | TH2015-04-07 | California (2008) | Citrus reticulata |
| I2018004 | USDA Navel 2-C | navel orange | RRUT 391 | 10572 | STG2015-39-02 | Florida (2008) | Citrus sinensis |
| I2018005 | Seville | sour orange | PI 539169 | 10387 | TH2015-01-02 | California (1961) | Citrus aurantium |
| I2018006 | USDA 5-8-122 | mandarin hybrid | RRUT 447 | 10570 | STG2015-14-01 | Florida (2009) | Citrus hybrid |
| I2018007 | Goutoucheng (4-1) | sour orange | RRUT 149 | 10571 | STG2015-42-03 | China (1980's) | Citrus aurantium |
| I2018008 | USDA 1-46-30 | mandarin hybrid | RRUT 371 | 10552 | STG2015-47-03 | Florida (2007) | Citrus hybrid |
| I2018009 | Red ling mung | rangpur | RRUT 176 | 10406 | TH2015-10-08 | Texas (1997) | Citrus limonia |
| I2018010 | Pomo d'Adamo nucellar | lemon | RRUT 138 | 10411 | TH2015-13-10 | EU (2000) | Citrus aurata |
| I2018011 | Consolei (OPS) | sour orange | RSD 2000003 | 10409 | TH2015-12-03 | EU (2000) | Citrus aurantium |
| I2018012 | Hyuganatsu-mikan | pummelo | PI 280540 | 10391 | TH2015-03-02 | texas (1997) | Citrus tamurana |
| I2018013 | Hudson #1 (4N) | grapefruit | RRUT 385 | 10407 | TH2015-11-06 | California (1989) | Citrus paradisi |
| I2018014 | Swingle | tangelo | RRUT 188 | 10397 | TH2015-06-07 | Florida (2001) | Citrus x tangelo |
| I2018015 | Valencia seedless | valencia orange | RRUT 394 | 10404 | TH2015-09-06 | Argentina (2009) | Citrus sinensis |
| I2018016 | Hawaiian | pummelo | RRUT 143 | 10402 | TH2015-08-06 | California (2000) | Citrus maxima |
| I2018017 | Bahman Persian #2 nucellar | Lime/sweet lime | PI 658374 | 10400 | TH2015-07-02 | Iran (1998) | Citrus limettioides |

**Figure 1: During CY 2017, a total of 7988 laboratory tests were performed for the purposes shown below**:

As per the California Citrus Tristeza Virus Interior Quarantine, all plants maintained within the protective screenhouse were tested for CTV using ELISA (1,100 plants were assayed). This was conducted in June after all accessions had been pruned back in April. The Category 3 Support Scientist has begun collecting plant tissue from Field 12B of the Citrus Variety Collection and is assaying for HLB-associated pathogens; to date all samples have been negative. The RNR primer set is being used, 20 qPCR reactions per tree are conducted.

NCGRCD currently holds 99 accessions in quarantine (Appendix 5); this number includes those accessions currently in the 2017 therapy and testing process and those termed “self-quarantine”. Self-quarantine means existing germplasm accessions that are not importations. Nucleic acids have been extracted and lab tests have been conducted to determine which approach is necessary to clean up each accession. The long-term goal is to complete sanitation of these and other un-sanitized trees. This will be done in conjunction with collection rationalization. The NCGRCD seeks commentary regarding prioritization of these accessions for sanitation.

Therapy procedures at NCGRCD include shoot-tip grafting (STG) and thermotherapy. A total of 37 accessions were subjected to therapeutic procedures: 27 as STG (261 plants total) and 10 as thermotherapy (100 plants total). It should be noted that a low percentage of STG’s survive the process.

**Table 5: Accessions therapied during 2017.**

|  |  |  |
| --- | --- | --- |
| CULTIVAR | ACCESSION NUMBER | THERAPY METHOD |
| Campeona | RRUT 393 | STG |
| Caoju (1-18-47) | RRUT 151 | STG |
| Cariappa-pummelo2 | RRUT 523 | STG |
| Clementine X Murcott (C54-4-2) | RRUT 167 | STG |
| Clementine X Orlando (F-6-9-10) | RRUT 166 | STG |
| Dweet | PI 539240 | STG |
| Fumin Evergreen trifoliate (OPS) | RSD 1998001 | STG |
| Fuming evergreen trifoliate | RRUT 178 | STG |
| Gainesville 71 | RRUT 388 | STG |
| Garcia-Yanez | RRUT 449 | STG |
| Gunlong Hill wild (OPS) | RSD 2012002 | STG |
| Hamlin 1-4-1 | RRUT 442 | STG |
| Horned | RRUT 454 | STG |
| Limequat | RRUT 146 | STG |
| Little Sweetie | RRUT 113 | STG |
| Man Ju (OPS) | RRUT 104 | STG |
| Nan Feng Mi Ju (OPS) | RSD 2002003 | STG |
| Pummelo #1 ex-Sichuan | RRUT 196 | STG |
| Pummelo #3 ex-Sichuan | RRUT 198 | STG |
| Reina | RSD 2010004 | STG |
| Sunki | RRUT 444 | STG |
| Sweet orange ex-San Isidro | RSD 2011002 | STG |
| Thimmaiah1 | RSD 2013001 | STG |
| US- 942 | RRUT 532 | STG |
| USDA 6-2-53 | RRUT 446 | STG |
| Xizhang smooth round (OPS) | RSD 2012015 | STG |
| Yuzuquat | RRUT 49 | STG |
| Limon Pummelo' Reili County nucellar | RRUT 84 | Thermo |
| Mediterranean nucellar | RRUT 90 | Thermo |
| Suanju nucellar | RRUT 81 | Thermo |
| Hongju nucellar | RRUT 74 | Thermo |
| Ruby 4N | RRUT 31 | Thermo |
| Sarah #2 | RRUT 172 | Thermo |
| Temple 4N | RRUT 146 | Thermo |
| Lemon pummelo ex. Rancho Santa Rosa | RSD 201009 | Thermo |
| Local Lime 2-8 ex-Reili County | RRUT 85 | Thermo |
| Pummelo ex. Huerta Nicanor | RSD 201010 | Thermo |

**Distributions**

Distribution of citrus germplasm was greatly reduced in CY 2017 due to phytosanitary restrictions implemented by USDA-APHIS and CDFA after HLB was detected approximately 3 miles from the UCR Agricultural Operations area in July. This resulted in the NCGRCD, CVC, and CCPP being within the 5-mile radius quarantine zone (See map, Appendix 6). Within 24 hours of confirmation, APHIS instilled a Hold Order on the repository. As a result, material from the SH can not be distributed until sampled twice at 6 month intervals by an certified laboratory (specifically, the CDFA diagnostic laboratory). Repository staff petitioned the regulators to accept the in-house assay results of each individual tree in lieu of one of the two required tests, but was denied.The first sampling was done in September, 2017 and the second in March, 2018; the results of both testing dates were negative as expected. Because of this, domestic distributions of budwood were lower this year and there is a backlog of requests to catch up on when the new compliance agreement is in place. The restriction on movement of vegetative materials from the SH included materials destined for cryopreservation at NLGRP, Fort Collins.

The quarantine and other associated regulations have also limited the types of seeds that can be moved domestically. USDA-APHIS formerly restricted all seeds of Aurantioideae. The restriction on *Citrus* spp and *Poncirus* spp was removed in 2017, but not restrictions on other types, including *Citrus* X *Poncirus* hybrids, the most common rootstocks. Interstate movement of pollen, leaves, and other germplasm is also restricted; but most of these can be moved if the appropriate permits are in place.

In CY 2017, NCGRCD distributed 262 items to 99 requestors. All but 6 items were citrus or related taxa, the remaining 6 being date palm materials. In recent years there was a high number of requests for date palm accessions from scientists conducting genomic research. This project is nearing completion and therefore, the number of date palm distributions has decreased.

Regarding Citrus, the breakdown of requestor category and for distributed items is shown in the following graphics (Figures 2, 3, and 4):

UARS = ARS; UFED = Federal, non-ARS; STA = State entities, including Universities; UCOM = Domesti commercial entity; UPRU = Domestic, non-profit; UIND = domestic, no affiliation; UAID = US AID; INT = CGIAR; FGEN = international genebank; FCOM = international commercial entity; FPRU = international non-profit; FIND = international, no affiliation

The distribution form of the 262 items is shown here:

BD = budwood

SD = seed

PO = pollen

CT = cutting

FR = fruit

LV = leaf

MI = male inflorescence

The number of requests for citrus fruit was significantly higher in 2017; this is unusual. This can be attributed to a commercial company requesting a large array of citrus types to investigate their pharmaceutical properties.

Another type of NPGS distribution is termed “backup”; materials sent to the NLGRP for cryopreservation fall into this category. In CY 2017, 106 citrus items were sent to the NLGRP. In addition to budwood and meristems sent to NLGRP for routine cryopreservation, seeds and pollen were sent to develop protocols to expand cryopreservation of the citrus resources. As described above in the “Germplasm Back Up” section, a cryopreservation effort was also initiated with date palm in tissue culture. The NLGRP has received 4 date palm cultivars (2 male and 2 female) as in vitro cultures from Phoenix Agrotech.

The types of items of citrus sent to NLGRP for cryopreservation are as shown here:

SD = seed

BD = budwood

MS = meristem

PO = pollen

**Collection Rationalization**

NCGRCD and UC-CVC personnel cooperate in the rationalization of the citrus and date palm collections to identify and eliminate the redundancies and to attempt to fill the gaps of genetic diversity. The goal is to more efficiently manage and utilize these valuable resources. In making decisions, we take into account molecular markers, morphological observations, passport data, and other documentation. Elimination of redundancies is of utmost importance now due to the increased threat of pests and diseases to the field collection. Having to maintain the repository accessions under a protected screen structure strains financial resources and therefore, there is an immediate need to prioritize accessions for backup via cryopreservation. Cryopreservation is an efficient and economical means to conserve the genetic diversity of germplasm and specific genotypes for the long-term.

**Propagations**

**Table 6: CY 2017 Propagations: there were 283 total propagations made from 170 genotypes**

|  |  |
| --- | --- |
| **PROPAGATION TYPE** | **NUMBER** |
| Citrus Relatives | 70 |
| New Accessions | 17 |
| CVC – new releases | 54 |
| CVC - backup  | 5 |
| SH repropagation | 73 |
| Release Trees | 47 |
| New Phoenix accessions | 10 |
| Miscellaneous | 7 |

**Permits**

Various Federal and State permits are needed for NCGRCD program delivery. They are:

* USDA-APHIS PCIP-16-00438 (import citrus germplasm)
* USDA-APHIS PCIP 16-00116 (import tissue culture date palms)
* USDA-APHIS P526-16-04047 (pathogens)
* USDA-APHIS P526-16-04084 (pathogens)
* CDFA 2778 (pathogens)
* CDFA 33-ACPQ-00488 (ACP compliance agreement; pending renewal after HLB testing of SH)
* USDA APHIS PPQ P526P-17-01725 (import rootstock seeds from Florida)
* CDFA 3330 Research permit to assay the CVC for HLB-associated pathogens
* CDFA 3221 Amended (receive psyllids from UCD CRF for assay)

**Databases**

After a lengthy period in development, the GRIN-Global (GG) system went live in November 2015, replacing GRIN Classic, which is offline to the public and is not being updated. The Curator has received training in the GRIN-Global system and is now using it. However, this new system presents many challenges due to its unfamiliarity, weightiness, and lack of specific features as compared to GRIN Classic.

NCGRCD maintains a local database in MS Access in addition to using the GRIN system. Both databases are up to date as far as accessions. Inventory is current and up to date in the local database however, it is currently not maintained in the GRIN database. The local database contains information including;

1. management data used in day-to-day operations,
2. quarantine and pathogen testing data,
3. documentation of propagations, and
4. therapy records.

It is not clear at this time whether or not these observations can be maintained effectively in GRIN, or if it even has a place there. Although inventory will probably be loaded into GRIN Global in the medium term, the local database will have to be maintained as well.

Currently, the Curator is the only person with training in the GRIN system, but the Technicians (V Newman & B Moreland) assist in maintaining the local database. If the NCGRCD had to rely solely on GRIN-Global, it would likely necessitate additional training for them while taking time away from their other job responsibilities. The level of human resources devoted to database management is inadequate at NCGRCD; additional funding would be necessary to continuously update the GRIN system.

**Citrus Taxonomy Committee**

As is well known, the state of citrus taxonomy is confusing. In addition to the traditional conflict between the Swingle and Tanaka systems, recent molecular work has called into question some long-held beliefs or concepts in citrus taxonomy. This affects more than citrus germplasm conservation. For instance, it has regulatory and hence economic (trade) implications. With this in mind, Dr Melanie Schori, the new NPGS taxonomist, assembled an ad hoc committee with the task of cleaning up and updating the citrus taxonomy used in the GRIN system. This is meant to facilitate germplasm conservation and exchange, not to be a definitive rewriting of citrus taxonomy. The committee has international participation from many well regarded workers in breeding, phylogenetics, etc. Polek and Krueger are participating in this effort.

**Facilities**

The NCGRCD maintains Federal facilities on land located on the University of California, Riverside campus and leased from the University of California (UC). The lease expired in 2011 and is expected to be renewed shortly. Although still in negotiation, the University of California intends to increase the lease rate from USD 1 per year to USD 2,250. In addition, mandatory municipal fees totaling USD 20,000 will be charged for fire and police services. These municipal fees are based on square footage of facility space with an annual increase of 3% for inflation. Therefore looking to the future, any expansion of the protective screenhouse will be financially problematic. These increases will have a significant negative impact on the operational budget. Discussions with the Pacific West Area Office regarding long term options are ongoing.

Federal facilities include approximately 16,000 sq. ft. of APHIS-certified screenhouse (used for maintenance of the protected, pathogen-tested collection); 6,050 sq. ft. of greenhouse space (used for propagations, maintenance of pathogen controls, and pathogen testing); 1,372 sq. ft. of headhouse space (work and storage); 850 sq. ft. of lab space; 88 sq. ft. of office/storage space; and 480 sq. ft. office trailer (houses the PCR equipment). In addition, approximately 7,500 sq. ft. of greenhouse space is rented from the University which is used to maintain accessions that have not been sanitized and tested, and therefore are held under APHIS/CDFA quarantine. In CY 2016, the renovation of two UCR greenhouses commenced using funds from the National Clean Plant Network (NCPN) that were awarded to Georgios Vidalakis specifically for this purpose. The renovations of GH 16-50 were completed and the plants transferred in the summer of 2017, whereas we anticipate the renovation of GH 16-46 to be completed in 2018. The NCPN funds have been essential to meet the repository’s needs since Federal funds cannot be used for the improvement of State facilities and current Federal facilities are inadequate in size.

A mission critical need withstanding is the expansion of the protected screenhouse which is near capacity. It is absolutely imperative that this structure be expanded in the next few years as new accessions are cleaned, tested, and released. A pad is available for up to a 60-foot (7,200 sq ft) addition to the existing structure. There are two obstacles preventing this construction from going forward; first, without a signed lease agreement with the university, the land footprint cannot be changed; and second, insufficient funds. Currently, each accession is maintained in duplicate, one tree in each compartment. While this strategy is vital, it does restrict the number of accessions maintained in the protective structure.

Several improvements were made to NCGRCD facilities in CY 2017. An upgraded work counter area and additional sink were installed in the headhouse. In addition to providing increased storage and improved aesthetics, it has partially alleviated a safety concern regarding separation of work and eating areas. Pending in CY 2018 is Phase 2 of the headhouse remodel; a defined seed extraction and processing area will be installed. In addition, the facility windows will be replaced for increased energy efficiency.

**Personnel**

In addition to the permanent Federal staff of 5.5 FTE, NCGRCD employs 2.0 FTE of temporary staff. Esteban Rodriguez, who worked at NCGRCD for a number of years starting as an undergraduate intern, is a temporary ARS Biological Technician assisting with laboratory activities. This position was extended prior to the hiring freeze and will expire in 2022. A technician position (UC Riverside Laboratory Assistant) funded by a USDA Multi-Agency Coordinated (MAC) Response grant (G. Vidalakis, PI) provides additional laboratory, cryoprocessing, and greenhouse support. This position is currently held by Amanda Rawstern and funding will expire in 2 to 3 years. Six Student Assistants were employed through the Research Support Agreement with UCR. Repository staff is lacking genetic expertise however, current resources prohibit this gap from being filled.

**Support**

NCGRCD is located at the Riverside Location of the USDA-ARS Pacific West Area (PWA). Federal administrative support is primarily provided by the staff in Riverside and at the Pacific West Area Office. The Riverside administrative staff include Nancy Knap, Location Administrative Officer, Patricia Gonzalez, Financial Analyst and Daniel Kain, IT Specialist.

NCGRCD maintains five agreements with UC Riverside: two Research Support Agreements (RSA) and three Non-Assistance Cooperative Agreements (NACA). The RSA’s are with the Agricultural Operations Department (P Mauk, PI) to provide infrastructure support (utilities, communications, facilities maintenance, cultural care for field plantings, etc). The NACA’s are with T Kahn (Botany and Plant Science), M Roose (Botany and Plant Science), and G Vidalakis (Plant Pathology & Microbiology). Cooperation with Kahn is aimed at characterizing horticultural traits, documenting, and maintaining the field genebank (Citrus Variety Collection). The NACA with Roose focuses on genetic characterization of citrus accessions and is currently the only means of dealing with genetic questions as NCGRCD staff lacks a geneticist position. The NACA’s with Kahn and Roose expire in 2018 and will not be renewed due to a lack of funds. The NCGRCD works closely with Vidalakis to improve diagnostic protocols, conserve genetic resources for the long-term, import and evaluate germplasm developed in Florida, and other phytosanitary issues. This NACA will expire August 31, 2021.

**Health, Safety, Environmental Management**

Biological Science Technician Brittany Moreland is the Collateral Duty Safety Officer (CDSO) for the Riverside Location (including the US Salinity Laboratory) and participates on USDA and UC safety committees as appropriate. NCGRCD also takes part in various USDA and UC safety activities and initiatives such as mock fire and earthquake drills, hands-on fire extinguisher training, and review of shut-off valves. The lab is certified as Biological Safety level II laboratory by UCR. The chemical inventory was reviewed and old and unused chemicals were properly disposed of through the University Environmental Health and Safety Department.

In CY 2017, the Riverside Location had a second party safety and environmental management audit conducted by Ken Cushman, Safety Manager Western Business Service Center. A total of 43 safety deficiencies/ recommendations for NCGRCD as well as USSL came out of this audit and appropriate remedial actions are being taken. All but 3 deficiencies were corrected within a 30 day time limit. These 3 deficiencies require additional time and resources to correct. One outstanding deficiency in particular; mandates a separation of a work space and an employee break area in the headhouse building. Construction to further separate out these two areas started in late 2017. Construction included an installation of a food-use only sink and replacement of cabinets and countertops. Further funds are required to completely separate out a break area or create another space for an employee break area. All actions were documented in a corrective action plan and communicated with the WBSC.

**Research Activities**

**HLB resistance:** An USDA NIFA SREP grant was awarded in late 2016 titled: Selection, molecular and genetic analysis of HLB tolerant/resistant variant citrus plants. Naturally occurring mutant citrus plants or bud sports of commercially grown cultivars will be identified. The nature of the genetic variation will be determined by genome sequencing. Using CRISPR technology, HLB tolerant/resistant cultivars with desirable fruit traits will be delivered to the industry. NCGRCD involvement includes the establishment of field evaluation plots, development of public outreach and extension materials, the organization of grower education and the annual progress report meetings.

**Early Detection Technologies**: The Citrus Research Board (CRB) has funded several research groups to develop early detection technologies (EDTs) to detect CLas in mature citrus trees prior to symptom development. In February 2017 and in cooperation with UCR (Mauk, Kahn, Roose, Vidalakis, Ma), ARS (Gottwald & Polek) arranged to bring a team of HLB detection canines to the UCR Ag Research Center. As the first extensive field evaluation of the canine technology in California, several objectives were accomplished; the most important being the field exposure of the canines to trees known to be infected with *Spiroplasma citri*. Canines were also exposed to citrus trees infected with viroids, psorosis and multiple pathogens, and tomato plants infected with *Ca* Liberibacter solanacearum. The repository continues to participate in the evaluation of EDTs by conducting qPCR assays and monitoring dog-alert trees enclosed in insect-proof cages. The CRB has funded a project in 2018 to further evaluate EDT’s at UC Riverside and also on the UC Lindcove Research and Extension Center in Exeter, CA. The NCGRCD Category 3 Support Scientist is a member of the CRB EDT Task Force. In addition, diagnostic assistance was provided to the Contained Research Facility at UC Davis to analyze research samples for CLas.

**Effect of Endemic Pathogens on HLB Development:** Funding was provided for one year only by the Citrus Research Board for a project titled: Interactions of endemic plant pathogens with *Candidatus* Liberibacter asiaticus (CLas) in citrus. Citrus trees infected with a single, double and triple pathogen(s) (Citrus tristeza virus, *Spiroplasma citri* and *Ca* Liberibacter asiaticus) were evaluated for pathogen interaction within the host and the ability of Asian citrus psyllids to vector CLas. Research was conducted within the UC Davis Contained Research Facility. Polek cooperated with R Yokomi, USDA ARS, K Godfrey, UCD, S Hajieri, CCTEA, G Douhan, UCCE.

**Use of CTV as a vector for antimicrobial peptides:** Polek in cooperation with James Ng, UCR, secured funding from the Citrus Research Board for a project titled, ‘High performance, California-derived CTV-based vecors for the control of HLB and other applications’. Similar to the CTV-vector developed in Florida, mild California CTV isolates collected by the Central California Tristeza Eradication Agency are being cloned and transformed by the Ng lab. The NCGRCD are providing healthy citrus plants, inoculating them with purified virus produced by the Ng lab, and assaying for CTV. The ultimate goal of this research is to have ready a virus to use as a delivery mechanism for therapeutic antimicrobial peptides against pathogens such as HLB-associated bacteria.

**Puffy Skin in Date Palms:** Production of soft cultivars of dates (fruit consistency) suffers from a problem involving skin separation, called “puffy skin” by the growers. This is a fruit quality defect in which excessive portions of the skin of the date pull away from the flesh, causing a blistered or puffed appearance. This decreases the market value of the date. This problem appears to be influenced by temperature and humidity during the khalal period of date fruit maturation. A cooperative project with Tom Perring, Entomology, UC Riverside is investigating bunch and irrigation management as tools to mitigate this problem. Research conducted in 2017 suggested that specific types of fruit thinning or irrigation management can reduce the incidence of the problem. The plan for CY 2018 is to move the experiment to a different location that has more standard management and further refine bunch management techniques in addition to regulated deficit irrigation. The irrigation program will also provide an estimate of actual water use by date palms under current growing conditions. Initial results indicate that date palms can grow with substantially less water applied than standard industry practice.

**Date Palm Water Use:** To supplement the above experiment, an SCRI grant was applied for and the pre-proposal accepted. The PI on the proposal is Ali Montazar, UCCE Farm Advisor for Irrigation in Riverside and Imperial Counties. R Krueger is a co-PI along with other UCCR personnel. If accepted, experimental plots will be established in Coachella and Bard Valleys, actual irrigation application measured, and measurements of stress, yield, and fruit quality made.

**Date Palm Decline in Sky Valley:** The Sky Valley area is experiencing a large amount of date palm decline and death. Initial symptoms appear in the fruit bunches, then lower leaves necrose, and finally the terminal bud dies and the tree with it. In many cases, the root system is weak or nearly non-existant. Initial sampling was inconclusive, with various usually non-pathogenic fungi being isolated. This problem is under current investigation. R Krueger is working with Don Hodel and José Aguiar, UCCE, and with Akif Eskalen, Peggy Mauk, and Philippe Rohlshausen, UC Riverside.

**Miscellaneous Date Palm Activities**

In 2016, NCGRCD received 5 genotypes as tissue cultured plantlets under a PCIP. The conditions of the permit include quarantine and specific testing for phytoplasmas and Coconut Cadang-Cadang viroid. Positive controls and a protocol for detection were obtained from Brian Bahder, University of Florida. The viroid assay was successfully implemented, although the TC plantlets have not yet been tested due to their small size. A potential source for CCCVd positive controls has been identified but yet obtained. NCGRCD will be participating with Dr Bahder and other domestic and international collaborators in an SCRI proposal for phytoplasmas in palms during CY 2018.

**Committee Service and Meeting Attendance (M Polek)**

UC Citrus Day, Invited Speaker, tour of NCGRCD facilities

International Research Conference on Huanglongbing (IRCHLB) V: member of Steering Committee, Moderator for session on the Pathogen

Oxnard, California Pest Control District, Invited Speaker

Presentation to Citrus Research Board: “Securing Vulnerable Citrus Germplasm”.

Poster: XIX International Botanical Congress, Shenzhen, China: “The Citrus Variety Collection As a Key Resource For Conserving Citrus Biodiversity in the USA”.

Plant Germplasm Operations Committee

Central California Tristeza Eradication Agency Technical Advisory Committee, Vice Chair

California Citrus Nursery Society (Annual Meeting, Member of panel to discuss ramifications of the HLB Quarantine, tour of NCGRCD facilities; Variety Committee meeting)

American Phytopathological Society (Pacific Division Meeting, tour of NCGRCD facilities)

Indio Date Festival (educational booth co-sponsored with UCR)

UCR Date Field Day (presentation – Freezing your asssets)

Advisory Committee for USDA-NIFA project “Developing an Infrastructure and Product Test Pipeline to Deliver Novel Therapies for Citrus Greening Disease”, (S Brown PI)

Advisory Committee: Bt toxin-based strategies for management of Diaphorina citri and citrus greening (B Bonning PI)

Advisory Committee: USDA NIFA SCRI CAP – Project No. 2015-10483: Design and Delivery of Therapeutic Proteins for HLB Protection, (G Gupta PI)

International Research Conference on Huanglongbing (IRCHLB) VI and IOCV: member of Steering Committee, Organizing Committee, Scientific Program Committee

**Committee Service and Meeting Attendance (R Krueger)**

Plant Germplasm Operations Committee

Riverside Location Environmental Management System Committee (meet quarterly)

PWA Workforce Diversity Committee (conference calls)

ARS Citrus Research Conference Calls (regular calls)

Central California Tristeza Eradication Agency Technical Advisory Committee (1 meeting)

California Citrus Nursery Society (Annual Meeting, tour of NCGRCD facilities; Variety Committee meeting)

California Date Commission (co-present research presentation with T Perring)

American Phytopathological Society (Pacific Division Meeting, tour of NCGRCD facilities)

Indio Date Festival (educational booth co-sponsored with UCR)

UCR Date Field Day (presentation)

Various UCR and RCC classes, Career Center, etc (Botany, Plant Pathology, Pesticide Training, etc)

Invited Speaker, International Symposium on Citrus Production (Hermosillo, April 2017)

**Grants CY 2017**

Several grants were applied for and received in CY 2017 that benefitted NCGRCD activities:

National Clean Plant Network, FY 2017: PI G Vidalakis, ARS PI M Polek, R Krueger. Of approximately USD 1.3 M, NCGRCD received USD $50 K used for facilities renovations (see Facilities section above), electrostatic sprayer, and student salaries (greenhouse care).

Multi-Agency Coordinated Response, FY 2016 - 2017: G Vidalakis, PI, ARS PI M Polek, R Krueger. NCGRCD will receive approximately USD $70 K for 2 years to fund a position supporting greenhouse operations (see Personnel section above).

Citrus Research Board, FY 2017: K Godfrey, R Yokomi S Hajieri, ARS PI M Polek. “Interaction of endemic plant pathogens with Candidatus Liberibacter asiaticus in citrus”. NCGRCD will receive $1000 to investigate the effects of single, double, and triple infections of citrus with CLas, CTV, and *S. citri*. In addition, the effects these pathogens have on psyllid phenology will be examined.

Citrus Research Board, FY 2017-2019: PI J Ng, UCR, ARS PI M Polek. NCGRCD will receive $6,547 for plant propagation, maintenance, inoculation, and assay. Project will develop clones of CTV to be used as a delivery system for antimicrobial peptides and RNAi systems.

Citrus Research Board, FY 2017-2019: “Ensuring Security and Integrity of Valuable Breeding, Research, and Germplasm Collections”. PI P Mauk, UCR ARS PI M Polek. NCGRCD will receive $4000 to conduct qPCR assays and assist with other Early Detection Technologies.

USDA NIFA SREP 2016 to 2022: Selection, molecular and genetic analysis of HLB tolerant/resistant variant citrus plants. F Luo, F Gmitter, Y Duan, M Polek, Z Deng, L Cano. NCGRCD will receive approximately $90 K/year for public/extension outreach and the organization of the annual progress report meetings.

California Date Commission, FY 2017: R Krueger (ARS PI), T Perring (UCR, PI). Skin separation in ‘Medjool’ dates. USD 0 net to NCGRCD, consulting role only.

 **Publications CY 2017**

Volk G, Samarina L, Kulyan R, Gorshkov V, Malyarovskaya V, Ryndin A, Polek M, Krueger R, Stover E. 2017. Citrus genebank collections: international collaboration opportunities between the US and Russia. Genetic Resources Crop Evolution DOI 10.1007/s10722-017-0543-z.

Rios S, Krueger R. 2017. Current statewide updates regarding the battle of the Asian Citrus Psyllid and Huanglongbing. CAPCA Advisor 20(2):36-39.

Kahn T, Siebert Wooldridge T, Krueger R, Greer G, Polek ML, Vidalakis G. 2017. Looking for something new? Check out background, results of potential new cultivars for California. Citrograph (ns) 8(2):72-89.

Chin, E., Godfrey, K., Polek, M., Slupsky, C. 2017. H NMR analyses of Citrus macrophylla subjected to Asian citrus psyllid (Diaphorina citri Kuwayama) feeding. Arthropod-Plant Interactions. doi:10.1007/s11829-017-9546-.

Wu, F., Kumagai, L.B., Cen, Y., Chen, J., Wallis, C.M., Polek, M., Jiang, H., Liang, G., Deng, X. 2017. Analyses of mitogenome sequences revealed that Asian citrus psyllid (Diaphorina citri) from California was related to those from Florida but different from those in Southern China. Scientific Reports. 7:10154/doi:10.1038/s41598-017-10713-3.

**Critical Issues**

The Crop Germplasm Committee needs to be made aware of critical issues threatening the future of the NCGRCD. These include:

* Budgetary shortfalls: The annual NCGRCD budget allotment has remained stagnant for the past several years while the cost of operations has significantly increased. In particular these include but are not limited to; increased lease assessment ($2,250/yr), the new levy of municipal fees ($20,000/yr), UC greenhouse bench rent (15% increase for 3 consecutive years), janitorial services (almost doubled in 2017).
* Laboratory equipment is aging and and will be needing replacement soon; the cost of service agreements are unsustainable.
* Protective Screenhouse is almost at capacity; expansion is vital.
* Impact of HLB Quarantine
* Threat of palm weevils to the CVARS field collection
* Personnel Issues: the unit lacks genetic, data management, and tissue culture expertise; anticipated retirements within the next 5 years, term (temporary) positions need to be converted to permanent.

**Appendix 1. Accessions and inventory maintained at NCGRCD (2017-12-31)**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | ACCESSIONS | INVENTORY |
| **Total** |  | **1846** | **6419** |
|  | *Citrus* & related taxa | 1617 | 5582 |
|  | *Phoenix* spp | 147 | 802 |
|  | Citrus pathogens | 102 | 136 |

**Appendix 2. Aurantioideae accessions maintained at NCGRCD**

|  |  |  |
| --- | --- | --- |
| SPECIES | GROUP | ACCESSIONS |
| **Total** |  | **1618** |
| *Citrus spp* |  | 1227 |
| *Citrus sinensis* | Total | 245 |
|  | Blood orange | 22 |
|  | Navel orange | 81 |
|  | Sweet orange and hybrids | 105 |
|  | Valencia orange | 37 |
| *Citrus aurantium* and hybrids | Sour orange and hybrids | 64 |
| *Citrus limon* and hybrids | Lemon and hybrids | 174 |
| *Citrus aurantiifolia* and hybrids | Lime and hybrids | 65 |
| *Citrus medica* and hybrids | Citron and hybrids | 107 |
| *Citrus maxima* and hybrids | Pummelo and hybrids | 127 |
| *Citrus paradisi* and hybrids | Grapefruit and hybrids | 59 |
| *Citrus madurensis* and hybrids | Calamondin and hybrids | 3 |
| *Citrus reticulata* and hybrids | Mandarins and hybrids | 240 |
| *Citrus* spp and hybrids | Papedas and hybrids | 31 |
| *Fortunella* spp and hybrids | Kumquats and hybrids | 30 |
| *Microcitrus* spp | Total | 30 |
|  | *M australasica* | 10 |
|  | *M australis* | 7 |
|  | Other *Microcitrus* spp |  21 |
| *Poncirus* spp and hybrids | Trifoliates and hybrids | 143 |
|  | *Poncirus* spp | 83 |
|  | Citrange | 24 |
|  | Other trifoliate hybrids | 36 |
| Other Aurantioideae taxa |  | 71 |
|  | *Aegle marmelos* | 1 |
|  | *Aeglopsis chevalieri* | 1 |
|  | *Afraegle* spp (2) | 2 |
|  | *Atalantia* spp (4 spp) | 6 |
|  | *Balsamocitrus daweii* | 1 |
|  | *Bergera koenigii* | 4 |
|  | *Citropsis* spp (4 spp) | 4 |
|  | *Clausena* spp (4 spp) | 7 |
|  | *Clymenia polyandra* | 1 |
|  | *Eremocitrus glauca* and hybrid | 2 |
|  | *Feroniella oblata* | 1 |
|  | *Glycosmis* spp (4 spp) | 5 |
|  | *Limnocitrus littoralis* | 1 |
|  | *Merrillia caloxylon* | 1 |
|  | *Micromelum minutum* | 1 |
|  | *Murraya paniculata* | 4 |
|  | *Naringi crenulata* | 1 |
|  | *Oxanthera neo-caledonica* | 1 |
|  | *Pamburus missionis* | 2 |
|  | *Paramygnia* spp (2 spp) | 2 |
|  | *Pleiospermium* spp (2 spp) | 2 |
|  | *Severinia buxifolia* | 10 |
|  | *Severinia disticha* | 2 |
|  | *Swinglea glutinosa* | 2 |
|  | *Triphasia trifolia* | 2 |
|  | *Wenzelia dolichoophylla* | 1 |
| Rutaceae (not Aurantioideae) | *Esenbeckia, Ruta, Vepris, Zanthoxylum* | 6 |

**Appendix 3. *Phoenix* accessions maintained at NCGRCD**

|  |  |  |
| --- | --- | --- |
|  |  | ACCESSIONS |
| **Total** |  | **147** |
| *P dactylifera* |  | 128 |
|  | Named Old World female cv | 28 |
|  | Named New World female cv | 9 |
|  | Superior male selections | 5 |
|  | Backcrossed male accessions | 30 |
|  | Hybrid “Breeding Lines” | 17 |
|  | Baja California Sur seedlings | 13 |
|  | Spanish seedlings | 7 |
|  | Miscellaneous unverified | 19 |
| *P acaulis* | seed source | 1 |
| *P canariensis* OPS | seed source | 1 |
| *P hanceana* OPS | seed source | 2 |
| *P loureiroi* | seed source | 1 |
| *P loureiroi* var  *loureiroi* | seed source | 1 |
| *P paludosa* OPS | seed source | 1 |
| *P reclinata* | 1 clonal, 1 seed source | 2 |
| *P roebelinii* |  | 3 |
| *P slyvestris* |  | 5 |

**Appendix 4. Citrus accessions and inventory maintained at different sites**

|  |  |  |
| --- | --- | --- |
|  | ACCESSIONS | INVENTORY |
| **Total** | **1617** | **5582** |
| Citrus Variety Collection (Riverside – field planting) | 1053 | 1915 |
| Protected (pathogen tested – APHIS-certified screenhouse) | 546 | 962 |
| GH (Riverside) | 701 | 1534 |
| Quarantine | 99 | 127 |
| Coachella Valley Agricultural Research Station (CVARS) | 79 | 237 |
| South Coast Field Station (SCFS) | 51 | 90 |

**Appendix 5. Accessions held in quarantine (GH 16-50).**

|  |  |  |  |
| --- | --- | --- | --- |
| CULTIVAR | GROUP | ACCESSION NUMBER | QTYPE |
| 'lemon pummelo' ex-western Yunnan (OPS) | lemon | RRUT 107 | INTL |
| 'Limon pummelo' ex-Reili County nucellar | lemon hybrid | RRUT 84 | INTL |
| Bahianinha Monte Parnazo | navel orange | RRUT 184 | STATE |
| Bahianinha Piracicaba | navel orange | PI 133261 | STATE |
| Bahman Persian #2 nucellar | lime - sweet lime | PI 658374 | SELF |
| Baiju nucellar | mandarin | RRUT 76 | SELF |
| Cami | mandarin hybrid | RRUT 386 | INTL |
| Campeona | mandarin | RRUT 393 | INTL |
| Canaliculata di Palermo | lemon | RRUT 222 | INTL |
| Caoju (1-18-47) | mandarin | RRUT 151 | INTL |
| Cariappa-CM3 | mandarin | RRUT 524 | INTL |
| Cariappa-pummelo2 | pummelo | RRUT 523 | INTL |
| Cariappa-pummelo2 | pummelo | RSD 2013002 | INTL |
| Cariappa-pummelo3 | pummelo | RSD 2013003 | INTL |
| Clementine X Murcott (C54-4-2) | mandarin | RRUT 167 | STATE |
| Clementine X Orlando (F-6-9-10) | mandarin | RRUT 166 | STATE |
| Consolei (OPS) | sour orange | RSD 2000003 | INTL |
| Corniculata (OPS) | sour orange | RRUT 119 | INTL |
| Corsican | citron | PI 539421 | SELF |
| Corsican | citron | PI 539422 | SELF |
| Dayap | lime | RRUT 35 | SELF |
| Diamante | citron | PI 539423 | STATE |
| Dona Adelina | sweet lime | RRUT 451 | STATE |
| Etonia citrange nucellar | citrange | PI 29160 | SELF |
| Etrog | citron | PI 539425 | SELF |
| Foerster "mutant"' nucellar | sour orange | RRUT 89 | STATE |
| Fuju nucellar | mandarin | RRUT 82 | INTL |
| Fumin Evergreen trifoliate (OPS) | trifoliate | RSD 1998001 | INTL |
| Fuming evergreen trifoliate | trifoliate | RRUT 178 | INTL |
| Gainesville 71 | trifoliate | RRUT 388 | STATE |
| Gaojiantou Mitong nucellar | mandarin | RRUT 77 | INTL |
| Garcia-Yanez | sweet lime | RRUT 449 | STATE |
| Gou Tou Xiang Yuan (B) | sour orange | RRUT 321 | INTL |
| Goutoucheng (4-1) | sour orange | RRUT 149 | INTL |
| H-56 | tangor | PI 539241 | SELF |
| Hamlin 1-4-1 | sweet orange | RRUT 442 | STATE |
| Hawaiian | pummelo | RRUT 143 | SELF |
| Hongju nucellar | mandarin | RRUT 74 | INTL |
| Horned | Sour orange | RRUT 454 | INTL |
| Huangguogan #1 nucellar | tangor | RRUT 106 | INTL |
| Huangguogan nucellar | tangor | RRUT 83 | INTL |
| Hudson #1 (4N) | grapefruit | RRUT 385 | STATE |
| Hyuganatsu-mikan | pummelo | PI 280540 | STATE |
| Iwaikan | pummelo | RRUT 173 | STATE |
| Kuharske | citrange | RRUT 439 | STATE |
| Limequat | lime hybrid | RRUT 146 | SELF |
| Ling Mung | rangpur | RRUT 174 | STATE |
| Little Sweetie | sweet lemon | RRUT 113 | SELF |
| Local lime 2-8 ex-Reili County (OPS) | lime | RRUT 85 | INTL |
| Local lime ex-Xiaochenhang nucellar | lime hybrid | RRUT 86 | INTL |
| Man Ju (OPS) | mandarin | RRUT 104 | INTL |
| Marmalade tree |  | RSD 2015001 | SELF |
| Mediterranean nucellar | blood orange | RRUT 90 | STATE |
| Melanesian (PNG) | papeda | RRUT 533 | INTL |
| Nan Feng Mi Ju (OPS) | mandarin | RSD 2002003 | INTL |
| New Zealand Grapefruit | grapefruit | PI 539464 | SELF |
| Nin Kat | mandarin | PI 433265 | STATE |
| Nine Pound | lemon | RRUT 485 | STATE |
| Old Indian | lemon | RCRC 4211 | SELF |
| Orange | lemon | RRUT 487 | STATE |
| Pomo d'Adamo nucellar | lemon | RRUT 138 | INTL |
| Pon tri DPI-50-7-6 | trifoliate | RRUT 441 | STATE |
| pummelo #1 ex-Sichuan | pummelo | RRUT 196 | INTL |
| pummelo #2 ex-Sichuan | pummelo | RRUT 197 | INTL |
| pummelo #3 ex-Sichuan | pummelo | RRUT 198 | INTL |
| Red ling mung | rangpur | RRUT 176 | STATE |
| Red Mexican | grapefruit | RRUT 170 | STATE |
| Reina | mandarin | RRUT 536 | SELF |
| Ruby 4N | blood orange | RRUT 31 | STATE |
| Sarah #2 | sweet orange | RRUT 172 | STATE |
| Seville | sour orange | PI 539169 | SELF |
| Shamel | tangor | RRUT 148 | STATE |
| Shatianyou (2-1) | pummelo | RRUT 154 | INTL |
| Suanju nucellar | mandarin | RRUT 81 | INTL |
| Sunki | mandarin | RRUT 444 | STATE |
| Swingle | tangelo | RRUT 188 | STATE |
| Temecula Sweet | mandarin | RRUT 463 | STATE |
| Temple 4N | tangor | RRUT 32 | STATE |
| Thimmaiah1 | pummelo | RSD 2013001 | INTL |
| Tonkan nucellar | tangor | RRUT 91 | STATE |
| Toronja ex-Paso Hondo | grapefruit | RRUT 539 | SELF |
| Tung kum | mandarin | RRUT 185 | STATE |
| Umatilla | tangor | RRUT 445 | STATE |
| un-named trifoliate ex-Beibei | trifoliate | RRUT 155 | INTL |
| US Early Pride | mandarin hybrid | RRUT 389 | STATE |
| US-897 | trifoliate hybrid | RRUT 384 | STATE |
| US-942 | citrandarin | RRUT 532 | STATE |
| USDA 1-46-30 | mandarin hybrid | RRUT 371 | STATE |
| USDA 5-51-2 | mandarin hybrid | RRUT 448 | STATE |
| USDA 5-8-122 | mandarin hybrid | RRUT 447 | STATE |
| USDA 6-2-53 | mandarin hybrid | RRUT 446 | STATE |
| USDA Navel 2-C | navel orange | RRUT 391 | STATE |
| USDA Navel 3-S | navel orange | RRUT 392 | STATE |
| Valencia seedless | valencia orange | RRUT 394 | INTL |
| Variant citradia | trifoliate hybrid | RRUT 171 | STATE |
| Vecino | sweet lime | RRUT 450 | STATE |
| Xiangchen (OPS) | papeda hybrid | RRUT 100 | INTL |
| Yemen | citron | PI 539434 | STATE |
| Yuzuquat | kumquat hybrid | RRUT 49 | STATE |

**Appendix 6: HLB Quarantine Map of Riverside, CA**



**Appendix 7. Progress made towards securing accessions maintained in the protected screenhouse in liquid nitrogen for long term storage.**

