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T G R C

Tomato Genetics Resource Center

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ANNUAL PROGRESS REPORT

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Fruit of a novel delayed ripening trait discovered in *Solanum sitiens*. Research by the TGRC has led to the development of breeding lines containing genetic material from *S. sitiens*, a wild nightshade of the Atacama Desert, in the genetic background of cultivated tomato. These breeding lines are the first of their kind and express many novel traits. One line, shown above, exhibits extremely delayed fruit ripening and failure of petals and anthers to abscise after fruit set. The trait has been provisionally named *nor-2* (*nonripening-2*)

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SUMMARY

Acquisitions. The TGRC acquired two new accessions this year, both long storage, delayed ripening varieties from Spain. In addition, we rescued three accessions of *S. ochranthum* that had never been successfully grown for seed increase. Obsolete or redundant accessions were dropped. The current total of number of accessions maintained by the TGRC is 4,344.

Maintenance and Evaluation. Over 1,050 cultures were grown for various purposes, of which 561 were for seed increase, including 92 wild species accessions. Germination tests were run on 707 seed lots. Progeny tests were performed on 122 stocks of male-steriles, trisomics, and other segregating lines or accessions with unexpected phenotypes. 197 stocks were grown for introgression of the *S. sitiens* genome. Other stocks were grown for research on interspecific reproductive barriers. All plants grown for seed regeneration were tested for PSTVd; no positive plants were found. Newly regenerated seed lots were split, with one sample stored at 5° C to use for filling seed requests, the other stored in sealed pouches at -18° C to better maintain long term seed viability. 86 seed samples were sent to the USDA and 25 to the Svalbard Global Seed Vault for long term backup storage.

Distribution and Utilization. A total of 7,154 seed samples representing 2,045 different accessions were distributed in response to 339 requests from 263 researchers and breeders in 32 countries; at least 32 purely informational requests were also answered. The overall utilization rate (i.e. the number of samples distributed relative to the number of accessions available) was 165%. Information provided by recipients indicates our stocks continue to be used to support a wide variety of research and breeding projects. Our annual literature search uncovered 90 publications that mention use of TGRC stocks.

Documentation. New images of mutants and wild species were uploaded. Passport data on new accessions was added. Revised guidelines for seed germination, pollen collecting, and maintenance of wild species were posted on our website. Seed request records and passport information on seed samples submitted for off-site back up were provided to the USDA for uploading to their GRIN-Global database.

Research. The TGRC continued research on the mechanisms of interspecific reproductive barriers and on introgression of the *S. sitiens* genome. We published a paper on a previously unknown mechanism for pollen recognition and rejection by flowers of the wild species *S. pennellii*. We completed development of a set of introgression lines that capture ca. 95% of the genome of *S. sitiens* in the background of cultivated tomato.

ACQUISITIONS

Germinating seed on ½ MS medium.

The TGRC acquired two new accessions in 2018, LA5241 and LA5242. They are traditional Spanish cultivars with the capacity for long shelf life, known as 'tomate de colgar' (i.e. delayed ripening). They were donated by Dr. Maria Jose Diez at the University of Valencia. In addition, the TGRC reactivated three accessions of *S. ochranthum* (LA2118, LA2161 and LA2203) which had not been previously multiplied. The seed were quite old, but we were able to germinate a few of each by strong bleach treatment, removal of the seed coat, and plating of ovules on half strength MS tissue culture medium without sucrose. We find this method of seed germination often works

well for rescuing weak or very old seed. Details are available at https://tgrc.ucdavis.edu/seed_germ.aspx .

More information on the recently acquired accessions can be found on our website at http://tgrc.ucdavis.edu/acq.aspx. Obsolete or redundant accessions were dropped. The current total of number of accessions maintained by the TGRC is 4,344.

Table 1. Number of accessions of each species maintained by the TGRC. The figures include accessions that are temporarily unavailable for distribution. Interspecific hybrids and recombinant inbred lines are listed under "Other".

***Solanum* spp.**	**# Accessions**
S. lycopersicum	3,212
S. pimpinellifolium	332
S. cheesmaniae	42
S. galapagense	28
S. chmielewskii	16
S. neorickii	47
S. arcanum	45
S. peruvianum	70
S. huaylasense	16
S. corneliomulleri	53

***Solanum* spp.**	**# Accessions**
S. chilense	116
S. habrochaites	120
S. pennellii	47
S. lycopersicoides	23
S. sitiens	13
S. juglandifolium	5
S. ochranthum	7
Other	152
Total	**4,344**

MAINTENANCE

Scott Peacock, Adryanna Corral and their many dedicated undergraduate student assistants again carefully regenerated seed from a large number of accessions this year. A total of 1,050 families were grown for various purposes: 561 were for seed increases, including 92 wild species accessions, most grown in the greenhouse; 197 were for introgression and analysis of the *S. sitiens* genome; 122 were for progeny tests to verify the presence of segregating genes (e.g. male-sterility loci) or to confirm phenotypes; 707 were for germination tests.

Identifying accessions in need of regeneration begins with seed germination testing. We test all seed lots after 10 years of storage. Seed samples that do not meet our threshold of 80% germination after two weeks are normally regenerated in the same year. Seed lots that meet this threshold are retested again in two years. Other factors, such as available greenhouse space, age of seed and supply on hand, are also taken into account. Newly acquired accessions are typically regenerated in the first year or so after acquisition because seed supplies are limited and of uncertain viability. This year, 707 seed lots from 2008 or earlier were tested for germination rates. Average germination values continued to be relatively high for most species (Table 2), however we obtained relatively low germination rates from some seed samples of cultivated tomato and *S. chilense,* and the *S. peruvianum* complex species. The lower than usual germination rate of *S. peuruvianum* seed was due in part to the fact that seeds were not sufficiently bleached prior to sowing with sodium hypochlorite.

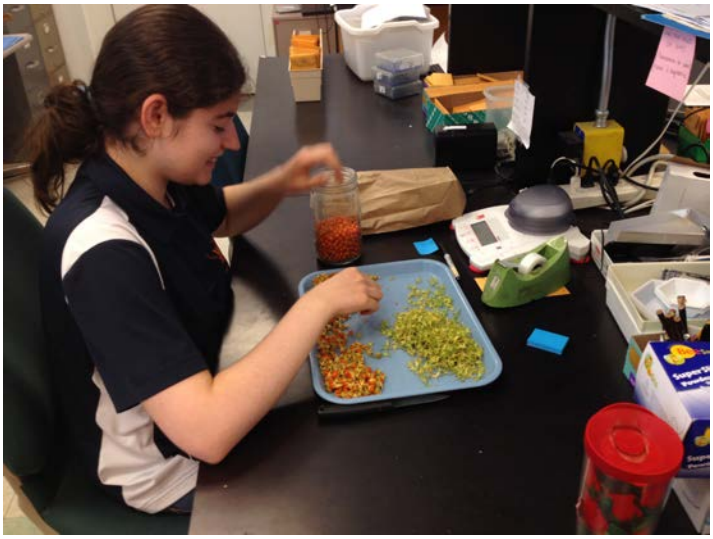
We again planted a relatively large number of cultivated tomato and selfing *S. pimpinellifolium* accessions in the field. This year our field plot was 60 rows. As usual, sequential plantings were made to spread the workload. The first transplanting was April 26, the last June 21. Early growth and establishment were satisfactory, however daytime high temperatures were

unfavorable for fruit set for extended periods of time in July and August. We again noted a relatively high incidence in the field of tomato spotted wilt virus (TSWV).

Table 2. Results of seed germination tests. Values are based on samples of 25-50 seeds per accession, and represent the % germination after 14 days at 25°C. Seed lots with a low germination rate are defined as those with less than 80% germination.

***Solanum* Species**	**Date of Tested Lots**	**# Tested**	**Avg % Germ**	**# Low Germ**
S. lycopersicum	2003-2008	467	67.9	271
S. pimpinellifolium	2003-2008	92	93.3	7
S. cheesmaniae, S. galapagense	2003-2008	32	93.8	2
S. chmielewskii, S. neorickii	2004-2008	18	95.9	1
S. chilense	1994-2008	33	71.7	15
S. peruvianum, S. arcanum, S. corneliomulleri, S. huaylasense	1997-2008	36	48.0	31
S. habrochaites	2004-2008	12	84.5	3
S. pennellii	2004-2008	14	94.0	1
Total		**707**		**331**

Most of the wild species, many mutants and certain other genetic stocks require greenhouse culture, either for isolation purposes or because they do not grow or flower well under field conditions. For the mutant stocks, we sow the weakest lines first, and finish with lines of normal vigor. Our schedule of greenhouse plantings of the wild species is based on photoperiod responses: those with the least sensitivity are planted first, in the early spring; those with intermediate reaction are planted in early summer; the most sensitive (i.e. flower best under short days) are planted in mid-summer for fall blooming. Optimal planting dates and other growing recommendations for each species are listed on our website.



Alexis McQueary processing fruit of *S. galapagense*.

Preventing the spread of seed borne pathogens is an important aspect of any seed regeneration program. We inspect all our plantings throughout the growing cycle for disease symptoms. Plants displaying signs of disease are tested with Agdia ImmunoStrips. To prevent spread of TSWV, our greenhouse managers made several changes in their spray and biological control programs, resulting in much improved control of the insect vector, Western flower thrips. All of our stocks grown for seed increase were tested for the presence of PSTVd, both at the seedling stage and the mature plant stage, and all results were negative. We continue to treat samples of all new seed lots with acid (1% HCl for 5 mins) and bleach (1% hypochlorite for 5 mins) to prevent transmission of seed borne pathogens.

Samples of all newly regenerated seed lots were catalogued then stored at 5°C – our working collection, used for filling seed requests -- and at -18°C for long term preservation of

viability. We continue to use Zeolite beads to dry seed to ultralow moisture levels prior to sealing in foil pouches, then stored at -18°C or 5°C. As in the past, large samples of newly regenerated seed lots were sent to the USDA National Laboratory for Genetic Resources Preservation in Ft. Collins, Colorado, and the Svalbard Global Seed Vault in Norway for long-term backup storage. This year 86 accessions were sent to NLGRP and 25 to Svalbard.

EVALUATION

All stocks grown for seed increase or other purposes were systematically checked to ensure that they have the correct phenotypes. New accessions were evaluated in greater detail, with the descriptors depending upon type of accession (wild species, cultivar, mutant, chromosomal stocks, etc.). Plantings were reviewed at different growth stages to observe foliage, habit, flower morphology, fruit set, and fruit morphology. Images of selected accessions were uploaded to our website.

Many genetic stocks, including various sterilities, nutritional, and weak mutants, cannot be maintained in true-breeding condition, hence have to be transmitted from heterozygotes. Progeny tests must therefore be made to verify that individual seed lots segregate for the gene in question. Other accessions may show unexpected segregation or off-types due to outcrossing, and need to be progeny tested to reestablish true breeding lines. We sowed 122 lines for progeny testing of male-steriles or other segregating mutants, as well as various other stocks with incorrect phenotypes. This year's progeny tests included stocks of the following mutant loci: *cactiflora, Lapageria, narrow cotyledons, ghost, stamenless,* and *male-sterile-2, -3, -5, -9, -10*^{35}*, -15*^{26}*, -16, -33, -38*^{40}*,* and *-48.* In addition, we grew progeny tests of other lines that showed unexpected segregation or off-types, including cultivars Rutgers, Marglobe, VFNT Cherry, Dirty Orange Cherry, Ailsa Craig, Earliana, and Globonnie, as well as a number of Latin American varieties and introgression lines.

DISTRIBUTION AND UTILIZATION

A total of 7,154 seed packets of 2,045 different accessions were sent in response to 339 seed requests from 263 scientists, breeders and educators in 32 countries. About 18% of the samples went to users in the private sector, and 82% were sent to public sector researchers. Relative to the size of the TGRC collection (4,344 accessions), the number of seed samples distributed (7,154) was equivalent to a utilization rate of 165%. Nearly half of our accessions were requested at least once in 2018, confirming that most of the collection is being actively used. Over 32 purely informational requests were also answered.

The various steps involved in filling seed requests – selecting accessions, treating and repackaging seeds, entering the information into our database, providing cultural recommendations, obtaining phytosanitary certificates and import permits, etc. – involve a large time commitment. The TGRC crew has worked diligently to fill seed requests while implementing stringent phytosanitary practices. We continue to use an online payment system to recover the costs of phytosanitary certificates and express mail shipping. Shipments are sometimes delayed or lost and need to be refilled.

Information provided by recipients regarding intended uses of our stocks is summarized in Table 3. As in previous years, there was a notable emphasis on disease insect pest resistance, both for breeding purposes and for research. The diseases generating the most requests were TSWV and the different Fusarium diseases. There was also strong demand for research and breeding resistance to insect pests. There continues to be growth in work on abiotic stress responses,

particularly drought and extreme temperatures. There were many requests for research on fruit related traits, particularly shelf life and ripening. Most requests for breeding uses did not provide specifics, but there seems to be increased interest in rootstocks and grafting. Under genetic studies, there were many requests than mentioned whole genome sequencing or sequence analysis. There were also many projects related to gene mapping or evolution. We received many requests for studies of reproductive barriers, including self- and interspecific incompatibility. There was also much interest in flowers: chemistry, color, development, etc. There were also a large number of requests for work on root biology or mycorrhizae. We again received a significant number of requests for instructional uses.

We continue to receive more and more requests for introgression lines (ILs), nearly isogenic lines (NILs), and other prebreds. A total of 44 requests and 775 seed samples were processed for the *S. pennellii* ILs, 20 requests and 419 samples for the *S. habrochaites* ILs, and 15 requests and 96 samples for the *S. lycopersicoides* ILs. We also sent out 200 samples of *S. lycopersicum* x *S. pimpinellifolium* recombinant inbred lines in response to 8 requests. These numbers show that breeders and researchers continue to find many uses for prebred germplasm.

Table 3. Intended uses of TGRC stocks as reported by requestors. Values represent the total number of requests mentioning each keyword or category. Requests addressing multiple topics may be counted more than once.

Category	
Biotic Stresses	
Viruses:	
Criniviruses	1
Potyviruses	1
TLCV	2
ToMV	2
TSWV	5
TYLCV	1
Unspecified viruses	1
Bacteria:	
Bacterial spot	2
Bacterial speck	3
Bacterial wilt	2
Other bacteria	4
Fungi:	
Alternaria	2
Botrytis	1
Early blight	4
Fusarium spp.	6
Powdery mildew	3
Septoria	1
Target spot	2
Other fungi	4
Nematodes	4
Unspecified diseases	25
Insect pests	
Aphids	1
Leafminer	1
Psyllid	3
Tuta absoluta	1
Unspecified insects	5
Parasitic plants	1
Uspecified biotic stress	1
Abiotic Stresses	
Al, Bo toxicity	2
Drought	5
Flooding	2
High temperatures	5
Low temperatures	7
Salinity	4
Unspecified abiotic	10
Fruit Traits	
Alkaloids	2
Anthocyanins	1
Blossom end rot	3
Carotenoids, color	3
Flavor, volatiles	1
Fruit devel./ripening	4
Fruit dehydration	1
Quality, nutritional	2
Shelf life	6
Postharvest chilling	1
Breeding	
Genomic selection	1
Grafting, rootstocks	7
Male sterility	2
Marker development	6
Morphological traits	1
Prebreeding	5
Seed treatments	2
Unspecified breeding	24
Genetic Studies	
Allele tests	1
DNA repair	1
Domestication	1
Epigenomics, paramut.	1
Evolution, diversity	6
Functional genomics	5
Gene discovery, clone	3
Gene expression, regul.	2
Gene sequence anal.	7
Genome sequencing	7
Genome wide assoc.	3
Mapping, QTLs	9
Other genetic studies	6
Phenomics	3
Tetraploidy	2
Transformation	5
Transposable elements	1
Physiology & Develop.	
Acylsugars, glycolipids	2
Anthocyanins	2
Brassinosteroids	2
Cell walls	1
Elevated CO2	1

Category	
Flower chemistry	1
Flower morph/develop	3
Flowering time	1
Hormone responses	3
Leaf develop./shape	4
Metabolites	8
Microbiomes	2
Nutrient uptake	2

Category	
Plastids	1
Pollen thermotolerance	2
Reactive oxygen sp.	1
Reproductive barriers	7
Roots, mycorrhizae	7
Salicylic acid	2
Seed physiology	2
Shade avoidance	2

Category	
Source : sink	1
Stomatal responses	3
Terpenes	1
Trichomes	2
Wounding, defense	4
Miscellaneous	
Instructional uses	7
Unspecified research	18

Our survey of the 2018 literature and unreviewed papers of previous years uncovered 90 journal articles, reports, abstracts, theses, and patents that mention use of TGRC stocks (see Bibliography, below). Many additional publications were undoubtedly missed, and cases of utilization by the private sector are generally not publicized. These publications, many in high impact journals, show that TGRC germplasm continues to be employed for a wide range of basic and applied research, breeding and educational purposes.

DOCUMENTATION

Additional images of mutants and wild species accessions were uploaded to our database are accessible via our website. Passport data on new accessions was added and records on existing accessions were updated as needed to correct errors or incorporate new information. We updated revised guidelines on seed germination, pollen collection, and reproducing the wild species to incorporate a number of small, but significant improvements made this year. We again provided the USDA National Plant Germplasm System with passport data on accessions sent to them for back up storage for uploading into their GRIN-Global database. Seed distribution records and summary statistics were also provided to the USDA.

RESEARCH

***S. sitiens* introgression lines show some extreme phenotypes such as reticulated fruit epidermis.**

Research by the TGRC aims to elucidate and manipulate the mechanisms of crossing barriers and to expand the genetic base of tomato by generating new types of breeding lines. With funding from the National Science Foundation, Dr. Xiaoqiong Qin isolated *FPS2,* a gene expressed in pollen that is required for compatibility on pistils of the wild tomato *S. pennellii*. She showed the *FPS2* is part of a previously unknown mechanism for rejecting foreign pollen that appears to be independent of self-incompatibility, the mechanism for preventing self-fertilization in many of the wild species. This work was published in the *Plant Journal.* Dr. Qin is currently working to isolate the corresponding pistil factor gene that establishes the requirement for FPS2

expression in *S. pennellii* (pistils of cultivated tomato do not discriminate between pollen with or without FPS2).

With funding from the USDA-NIFA, we developed a set of introgression lines representing the genome of *S. sitiens* in the genetic background of cultivated tomato. This wild tomato relative is known for its tolerance to drought, salinity, and low temperatures, as well as delayed fruit ripening, fruit desiccation, and other unique traits. The introgression lines (ILs) each contain a defined segment of DNA from the wild parent integrated into the chromosomes of cultivated tomato. Through conventional breeding and selection, aided with molecular markers, we selected a set of 55 lines capture ca. 95% of the *S. sitiens* genome in a uniform, cultivated tomato genetic background. The ILs have been genotyped to relatively high resolution using DNA markers (SNPs) to pinpoint the beginning and end of each wild species chromosome segment. Each IL has been hybridized with three different varieties in order to facilitate future testing for quantitative traits in different genetic backgrounds. Many lines display unusual or extreme phenotypes (see image above for one example) suggesting they will be a rich source of traits. This newly developed genetic resource will facilitate the study and utilization of genetic variation in *S. sitiens.*

PUBLICATIONS

Qin, X. Q., Li, W. T., Liu, Y., Tan, M. L., Ganal, M. and Chetelat, R. T. (2018) A farnesyl pyrophosphate synthase gene expressed in pollen functions in S-RNase-independent unilateral incompatibility. *Plant Journal,* **93,** 417-430.

SERVICE AND OUTREACH

Presentations and lectures on the TGRC, research projects, and related topics were given to the Plant Breeding Academy at UC-Davis, HRT 200B (a UCD graduate course in horticulture), and an Intro to Plant Breeding course from the University of Nevada-Reno. RTC was filmed in the greenhouse for a video lecture segment on plant breeding being prepared by Dr. Jeff Mitchell.

TGRC staff met with and provided tours to visitors from the Seed Central Processing Tomato Conference, the USDA-ARS, the Chinese Academy of Agricultural Sciences, the Chinese Ministry of Agriculture and Rural Affairs, the Jiangsu Academy of Agricultural Sciences, Campbell Soup Co., UCD Chancellor Gary May, ISI Sementi, East-West Seeds, Inari Agriculture, CEBAS-CSIC in Spain, and Kyungpook National University in Korea.

PERSONNEL

Scott Peacock, our Assistant Curator for many years, took a position with the Humboldt County Agricultural Office. He is replaced by Adryanna Corral, who has also worked with us for several years and is quite familiar with our operations. Tom Starbuck, our long time database manager and webmaster, has retired. Undergraduate student assistants Kyle Johnson and Emily Schoenborn both graduated. New undergraduate students are Gaby Hayes, Grace McKie, Alexis McQueary, Maxine Nixon, Kieran Bolger and Patricia Gleason. They join continuing student Anastasia Mathews. Dr. Xiaoqiong Qin continues to the research reproductive barriers and *S. sitiens* introgression, while providing the TGRC with lab services (DNA marker analysis, GMO testing, phytopathology, etc.). Two visiting scientists from China, Dr. Xiaomei Xu and Dr. Yongen Lu, joined the lab and will participate in research projects. High school student Aakash Mishra completed an internship in the lab.

TESTIMONIALS

"Thank you for your kindly help! It is a wonderful resource for the tomato research!" -- Naichong Chen, Oklahoma State University

"We deeply appreciate your kindly help." -- Zhongxiang Sun, Fujian Agriculture and Forestry University

"Thank you, and pass my regards to the TGRC for being a very valuable institution that the world needs for improving crop species and working to feed the world and push the boundaries of scientific discovery." -- Andrew James

"I wanted to say thank you for the quick response and all of the help. I really appreciate it!" – Sean Fenstemaker, Ohio State University

"We are grateful to the Tomato Genetics Resource Center at the University of California, Davis, for kind advice on the Solanum lycopersicum L. cv. Motelle and Moneymaker seeds." -- Jie Zhou, Zhejiang University, China

"Many thanks for all of your work." -- Peter DiGennaro, University of Florida

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