# STATUS OF THE NATIONAL COTTON GERMPLASM COLLECTION

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## Abstract

The US National Cotton Germplasm Collection (NCGC) distributes germplasm and associated information of cotton and crop wild relatives (other *Gossypium* species) to users in the USA and worldwide. It is part of the USDA-ARS National Plant Germplasm System, a collaborative effort to safeguard the genetic diversity of agriculturally important plants, and often including partners in the public and private sectors. Annual statistics on key activities such as cotton seed distribution, seed increase, germplasm characterization, acquisition, database development, and research are presented. Comparison with data from previous annual reports reveal trends in these activities and generate implications for the long term status of the NCGC. Recently seed increases per year of diverse germplasm is more limited. Germplasm acquisitions are severely curtailed due to Intellectual Property Rights of developed cotton seed, or International Treaties that often require permissions and subsequent benefit sharing of acquired germplasm. Rising demand for cotton genetic resources, rising costs of operations, and static financial resources have created refined priorities, policies, and operations in the NCGC. Standardized and expanded morphological descriptors and digital images have been gathered on accessions since 2010 to assist the users of the NCGC in streamlining their germplasm requests to minimize time and germplasm resources. This data was collected in several environments, a Cotton Winter Nursery in Liberia, Costa Rica, and summer field plots and year round greenhouses in College Station, TX. Two online databases are used for submitting this data, CottonGen and GRINglobal. New germplasm was acquired by collection of crop wild relatives in the US and Territories, and from cotton germplasm and cultivars whose intellectual property right protection period had expired. Collaboration within USDA-ARS and outside with Cotton Incorporated and various State and University partners (such as Texas A&M University) are essential to the maintenance, growth and utility of the NCGC. Regular reports on the activities and accomplishments of the NCGC are essential to maintain continued support in USDA-ARS and with our collaborators.

## Introduction

Activities of the NCGC are placed in sections: Seed Increase and Plantings, Data and Databases, Distribution and Acquisitions, Extension, Research, and Analysis. Methods and results are within each section. A final analysis or conclusion is drawn from the summarized activities. This format is used for annual presentation to the Cotton Crop Germplasm Committee at each Cotton Beltwide Conference, but is also being made viewable to all attendees as a poster presentation and uploading as a manuscript.

## Seed Increase and Plantings

Seed increases and characterization of the NCGC were conducted at the CWN site in Liberia, Costa Rica, and on site in College Station, Texas. In 2018 Liberia (2018-2019 growing season), 374 accessions were selected for critical seed increases from the NCGC, sent to the CWN, and planted by the manager Alfonso Palofox.October 29, 2019. The breakdown is: 10 cotton standards (8 *G. hirsutum* and 2 *G. barbadense*), 85 *G. barbadense* accessions, 270 *G. hirsutum* TX landraces/racestock accessions, 2 *G. hirsutum* cultivars, and one diploid species *G. raimondii*. The CWN staff irrigated the plots regularly with control of local insects because it is their dry season. Selfing was normally from wiring unopened flowers shut at their tips and later harvesting the cotton from open bolls that are marked with the wire. From February 19 – 28 the NCGC and collaborators visited the CWN to take descriptive

field ratings and digital images. Several scientists traveled with us to the CWN, Jodi Scheffler of Stoneville, MS and Brian Irish of Prosser, WA, to assist in the collection of descriptors and leaf, flower, boll and stems for capturing digital images. The morphological descriptors are 30 traits common recognized by cotton breeders such as leaf shape, flower color, seed fuzz color, lint color, stem hair, etc. Ratings were expanded to include range of variation seen in wild collected cotton and most species in the NCGC and viewable at www.cottongen.org/data/trait/NCGC_rating_scale. They were preloaded onto Microsoft Excel® files with rows headed by the accession, columns headings as the traits, and each cell with a data validation function listing the ratings for that trait. Digital images were collected on mature leaf, opened flower, mature but non cleft cotton bolls and stem fragments. They were placed on metered grid background (fabric rulers) and supplemental lighting used, on camera stands. Canon Mark EOS 5D digital cameras with manual focusing.

In 2019 College Station, 106 accessions were planted in the field at College Station, across the street from the USDA location on April 15, 2019. They were seeded into Jiffy pellets on April 1, 2019 in USDA greenhouses. The breakdown is: 10 cotton standards, 58 *G. hirsutum* SA accession, 10 *G. hirsutum* TX accessions, 5 *G. barbadense*, 12 *G. arboreum*, 2 *G. herbaceum*, and one each of *G. sturtianum*, *G. nandewarense*, *G. australe*, *G. costulatum*, *G. nelsonii*, *G. davidsonii*, *G. bickii*, *G. thurberi*, and *G. stocksii*. In 2019, longer plot sizes were used for priority seed increases (20 versus 5 plants) than in years past. These select nine exotic *Gossypium* species were started in the greenhouses in November of 2018 to give larger plants for transplanting and faster maturation in the summer. Much of the plantings were set up as a display for the Cotton Breeders Tour (July 22 – 25, 2019) and arranged according to ratings in specific traits in the morphological descriptors that are used in characterization of the NCGC. Descriptor ratings and digital images were also collected on these plants throughout the summer growing season. Flowers were enclosed in organza® mesh bags to ensure self-pollination and easy collection of seed.

In 2019 Liberia (2019 – 2020 growing season), 552 accessions were planted by the CWN crew on November 1, 2019. The breakdown is: 10 cotton standards, 96 *G. hirsutum* cultivars; 356 accessions of the *G. hirsutum* TX accessions, 33 *G. barbadense* and one accession of *G. barbadense* (directly into the carryover nursery), 25 of *G. herbaceum*, 25 of *G. arboreum*, and 6 *G. raimondii* (directly into the carryover nursery) Dr. Jodi Scheffler was responsible for coordinating the planting of 230 of these accessions.

Plantings in the CWN since the 2005 – 2006 season are summarized in Table one.

Table 1. Number of accessions of the NCGC planted at the Tropical Nursery according to year and species, racestocks or other germplasm (development/breeding lines). Totals within each year, over a 15 year period and averages for each germplasm category are also shown.

| Year | Asiatic Diploids | *G. barbadense* | *G. hirsutum* cultivars | *G. hirsutum* racestocks | AD (tetraploid) species | Rest of diploid species | Other | Total by year |
|---|---|---|---|---|---|---|---|---|
| 2006 | 85 | 169 | 253 | 93 | 0 | 0 | 305 | 905 |
| 2007 | 168 | 205 | 0 | 491 | 0 | 0 | 50 | 914 |
| 2008 | 225 | 23 | 328 | 46 | 25 | 9 | 0 | 656 |
| 2009 | 180 | 132 | 136 | 562 | 1 | 1 | 0 | 1012 |
| 2010 | 284 | 406 | 208 | 122 | 0 | 0 | 0 | 1020 |
| 2011 | 1074 | 173 | 379 | 371 | 1 | 1 | 0 | 1999 |
| 2012 | 647 | 161 | 178 | 828 | 0 | 0 | 0 | 1814 |
| 2013 | 0 | 0 | 1023 | 0 | 0 | 0 | 177 | 1200 |
| 2014 | 0 | 0 | 800 | 0 | 0 | 0 | 0 | 800 |
| 2015 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2016 | 11 | 90 | 86 | 0 | 0 | 0 | 5 | 192 |
| 2017 | 0 | 107 | 62 | 39 | 0 | 0 | 0 | 208 |
| 2018 | 0 | 135 | 8 | 133 | 0 | 0 | 0 | 276 |
| 2019 | 0 | 87 | 10 | 276 | 0 | 1 | 0 | 374 |
| 2020 | 0 | 91 | 10 | 267 | 0 | 1 | 230 | 522 |
| Total | 2674 | 1779 | 3481 | 3228 | 27 | 13 | 537 | 11892 |
| Average | 179 | 119 | 232 | 216 | 2 | 1 | 51 | 793 |

Much of the NCGC consists of *G. hirsutum* and *G. barbadense* cultivars, followed by Asiatic and African diploid cotton species *G. arboreum* and *G. herbaceum* and is more than one half of the NCGC. Nearly a quarter is photoperiodic accessions of *G. hirsutum* and *G. barbadense* that still flowering in short day or winter months, because of their origins from the tropics and are kept at the CWN. Still nearly one quarter of the NCGC is largely inaccessible to users and they are the most critical and diverse accessions and must be grown in USDA greenhouses, because of special germination and cultivation requirements, that make difficult their establishment in the CWN or College Station fields. Table two summarizes flowering and seed productivity of species of *Gossypium* grown in the greenhouses over the past 12 years. The columns show total years grown, total seed produced, average seed per year, range of seed produced (highest and second lowest totals, omitting zero), total years in which seed was produced, flowers in its time span and whether the flowering was photoperiodic, day neutral or variable.

Table 2. Seed production and flowering of *Gossypium* species in USDA greenhouses in College Station, TX for the past twelve years. The total years grown, seed, average seed/year, range of seed made in a year and years in which seed was produced are listed. Total flowers and if flowering was photoperiodic is listed for each species.

| Species | Years grown | Years w/seed | Total seed | Ave. sd/yr | Range of seed made in a year | Flower cycle* | flowers |
|---|---|---|---|---|---|---|---|
| *G. hirsutum* | 12 | 12 | 143823 | 11986 | 203 - 53098 | P, N | 18388 |
| *G. barbadense* | 12 | 11 | 19705 | 1643 | 13 - 6230 | P, N | 3079 |
| *G. tomentosum* | 12 | 11 | 3433 | 287 | 4 - 2596 | P | 2244 |
| *G. mustelinum* | 12 | 10 | 4897 | 409 | 18 - 1974 | P | 1695 |
| *G. darwinii* | 12 | 10 | 8481 | 707 | 35 - 4403 | P | 1886 |
| *G. herbaceum* | 12 | 11 | 27748 | 2313 | 140 - 8549 | N | 11625 |
| *G. arboreum* | 12 | 12 | 19749 | 1646 | 22 - 6155 | N | 6543 |
| *G. anomalum* | 12 | 10 | 4649 | 388 | 26 - 1428 | P | 813 |
| *G. capitis viridis* | 6 | 5 | 1027 | 172 | 9 - 387 | P | 120 |
| *G. stocksii* | 12 | 12 | 5072 | 423 | 41 - 880 | N | 1369 |
| *G. somalense* | 7 | 7 | 5224 | 743 | 4 - 1528 | N | 773 |
| *G. areysianum* | 10 | 9 | 1136 | 114 | 2 - 847 | N | 541 |
| *G. incanum* | 12 | 11 | 1036 | 87 | 3 - 210 | N | 981 |
| *G. thurberi* | 12 | 10 | 109492 | 9125 | 8 - 46695 | Variable | 4878 |
| *G. trilobum* | 12 | 12 | 16985 | 1416 | 44 – 3212 | P | 1694 |
| *G. armourianum* | 12 | 9 | 236 | 20 | 1 - 108 | Variable | 202 |
| *G. harknessii* | 12 | 1 | 35 | 3 | 6 | P | 96 |
| *G. turneri* | 12 | 6 | 323 | 27 | 2 - 279 | P | 280 |
| *G. davidsonii* | 12 | 12 | 15623 | 1302 | 259 - 3251 | P | 5064 |
| *G. aridum* | 12 | 11 | 1310 | 110 | 11- 669 | P | 941 |
| *G. schwendimanii* | 12 | 4 | 87 | 8 | 2 - 64 | P | 82 |
| *G. lobatum* | 12 | 5 | 1779 | 149 | 11 - 1058 | P | 520 |
| *G. laxum* | 12 | 5 | 615 | 52 | 11 - 420 | P | 272 |
| *G. gossypioides* | 12 | 12 | 11977 | 999 | 129 - 4305 | P | 4098 |
| *G. raimondii* | 12 | 10 | 4459 | 372 | 32 - 2131 | P | 564 |
| *G. sturtianum* | 12 | 11 | 23240 | 1937 | 187 - 8022 | N | 1386 |
| *G. nandewarense* | 12 | 10 | 7245 | 604 | 54 - 2562 | N | 251 |
| *G. bickii* | 12 | 12 | 3100 | 259 | 5 - 544 | N | 53 |
| *G. australe* | 12 | 12 | 15619 | 1302 | 5 - 7003 | N | 742 |
| *G. nelsonii* | 12 | 12 | 9048 | 754 | 30 - 2786 | N | 598 |
| *G. costulatum* | 12 | 12 | 3638 | 304 | 81 - 556 | N | 1698 |
| *G. populifolium* | 7 | 5 | 457 | 66 | 21 - 160 | N | 457 |
| *G. pulchellum* | 5 | 4 | 295 | 59 | 15 - 188 | N | 308 |
| *G. exiguum* | 12 | 7 | 442 | 37 | 9 - 140 | N | 166 |
| *G. marchantii* | 7 | 6 | 1419 | 203 | 91 - 391 | N | 800 |
| *G. nobile* | 9 | 9 | 847 | 95 | 4 - 206 | N | 492 |
| *G. rotundifolium* | 6 | 4 | 106 | 18 | 2 - 72 | N | 117 |

*P = photoperiodic flowering, or only in short day or winter months
N = Neutral or daylength insensitive flowering

Variable = flowering observed in summer or winter months

**Data and Databases**

Descriptor data collected during the 2019-2020 growing season of the CWN were sent as excel files to Jing Yu, curator of CottonGen.org, an online resource for Cotton Breeding, Genetics and Genomics. The descriptor data was also shared with Amanda Hulse at NCSU and Daniel Montoya for quantitative analyses of descriptors and morphology of *G. hirsutum* and *G. barbadense*. Pictures were filed on share drives by each season and uploaded by Jing to CottonGen.org servers as time permitted. Information on names, pedigree, descriptors, images, registration, etc. were all linked in CottonGen.org, to Plant Inventory numbers in GRINglobal, and where possible to other collection databases worldwide.

The USDA-ARS has made GRINglobal fully functional for crop curators and online for the public (ars-grin.gov). Our priority is to fully update GRINglobal with phenotypic descriptors but training is in process to utilize this new platform, define the new descriptors, and enter the backlog of data from the NCGC.

**Distribution and Acquisition**

Users contact the NCGC directly or use GRINglobal to make seed requests, which are 25 or fewer seeds depending on the quantity available. Orders are processed and mailed free of charge to users. Foreign users send import permits with at least one copy in English to accompany their seed request.

The narrow germplasm base of improved cotton requires that extensive genetic diversity be acquired, maintained and distributed by the NCGC. Genetic gaps exists in the NCGC from not having truly wild cotton because most accessions are from improved cotton where the protection period of registration has expired, or from dooryard or escaped cotton collected in tropical areas. South Florida has been reported as a habitat for wild cotton but much of it was previously eradicated efforts to control hosts of cotton pests. Isolated populations still exist in protected areas of South Florida. The previous curator, Ed Percival and another cotton scientist James McD Stewart suggested wild cotton existed as far north as Tampa Bay. Because the representation of truly wild cotton in the NCGC is scarce and the habitats are poorly described an exploration trip was planned for South Florida. ARS personnel and various Florida Park managers and scientists were contacted for recent sightings of wild cotton

The normal route of acquisitions is from cultivars or germplasm released by the National Laboratory for Genetic Resources and Preservations once the registrations with the Journal of Plant Registration (five years) or Plant Variety Protection Office (20 years) has expired. Currently only conventional cotton germplasm is accepted. Seed (100) is first tested by the NCGC for the adventitious presence of Cry1Ac, Cry2A, and CP4 EPSPS ('RR'). Positive tests for any of the transgenes means it is not accepted into the NCGC holdings.

**Extension**

In 2019, three organized tours of the NCGC facilities were provided; including the 2019 Cotton Breeders Tour, visiting researchers/administrators from the Federal Executive Board (Houston, TX), and a group of 5$^{th}$ graders from a local school (Brenham, TX). In addition, Texas A&M collaborators used some of the NCGC live plants from our greenhouses for their research saving us both save time and seed resources.

**Research**

Fifty new *G. hirsutum* breeding lines donated from the Monsanto research program in South Africa have been genotyped and analyzed with a core set of 105 SSR markers. The new germplasm will be related to current accessions from Africa in the NCGC. The observations from SSR genotyping will be compared to low pass sequencing analysis and results submitted for publication in 2020. In a separate project, approximately 70 new *G. hirsutum* germplasm sources collected from Puerto Rico in 2013 are currently growing in the greenhouse. This germplasm will be genotyped using low pass sequencing with results available in late 2020.

These accessions along with many other rare and critical accessions are micro-managed for specific fertilizer and insecticide applications as well as seasonal adjustments in watering and soil moisture to best trigger flowering and

seed increases. Surpluses of seed of species grown in College Station field plots were shared with collaborators at the NLGRP for their tests of seed longevity in the *Gossypium* genus. Various seed treatments were tested on *Gossypium* species for improved germination.

## Results

### Seed Increase and Plantings

In total, **480** (570-52) accessions were planted during 2019. At the CWN, 2 and 10 of the *G. hirsutum* and *G. barbadense* accessions, respectively, failed to germinate. 40 of TX landrace accessions failed to flower at the time of observation (February 19 – 28) these 40 accessions were moved to the 'carryover', a field section where 2-3 yr. old plants are maintained. The 2019 carryover nursery still contained plants from 2017 and 2018 seasons (24 *G. barbadense* and 10 *G. hirsutum* TX landraces) as well as a few direct seeded accessions of the 2019 planting (one *G. barbadense*, 4 *G. hirsutum* TX landraces, and one *G. raimondii*, also included above), that will need additional seasons of growth. The oldest carryover plants were large, often 8 or more feet in height and yielded ample bolls. Only 3 plants were lost from the 2018-2019 season carryover plants, and most were flowering except for 2 plants.

Six diploid species yielded seed (> 20K) for storage in College Station, the National Laboratory for Genetic Resources and Preservation (NLGRP) in Fort Collins, CO, and for experimentation with germination protocols and storage conditions. These local field plantings are primarily restricted to day neutral flowering accessions in need of critical seed increases.

Table one shows the overall reduction in volume at the CWN because of tight budgets, higher operation costs. It has declined greatly because of a relocation from Tecoman, Mexico to Liberia, Costa Rica evidenced by the gap in 2015 and that recent plantings in Costa Rica are far lower than the 15 year average. Collaborators with the NCGC increase the use of the CWN and help to add seed increases to the NCGC (years 2011, 2012, and 2020 have higher than the normal totals).

The data in Table two illustrates the variability in flowering and seed production of species in the greenhouses. The data suggests that neutral flowering species, typically originating from Australia or the Middle East (C or G genome species), should be transplanted outside in summer for much higher seed yields and to save greenhouse space. Difficult species are evidenced by where the total years in cultivation is less than 12 and indicate losses of individual plants. Also seen are the gaps in years of producing seed or where years w/seed is less than years grown. Several D genome type diploid species from Central and South America species are easily grown in greenhouse or the carryover nursery (e.g. *G. raimondii*), but others are much more difficult to flower and set seed (e.g. *G. aridum*).

Left behind is the largest proportion of the NCGC, the day neutral flowering accessions of *G. hirsutum*, *G. barbadense*, *G. herbaceum* and *G. arboreum*, which are more than half of the count in the NCGC. They must be increased in College Station, TX fields by the staff of the NCGC and in progressively longer intervals. A few mesh bags were tied on whole plants in the hopes of creating hands free means of generating the necessary self seed while saving on labor costs.

### Distribution and Acquisition

Distributions in 2,019 were 1565 accessions in 94 orders. The majority was for *G. hirsutum* accessions, split between the cultivars (1292) and racestocks (79). Requests for other species were: *G. barbadense* (126), Asiatic diploid accessions (31) and exotic species (43). Most orders originated in the USA (93), with one from abroad. The US orders were composed of Commercial (11 orders, 513 accessions), ARS (15 orders, 99 accessions), State (54 orders, 878 accessions) or Industry (18 orders, 67 accessions) orders. Orders by private individuals or educators were assigned to the State category. We often substituted more easily grown accessions for the rare ones novice users sometimes select. Some legitimate orders were unfilled due to import requirements of some foreign nations. Other unfulfilled requested were unrealistic such as those that involved thousands of selections across many crops and curators in the National Plant Germplasm System.

In 2019, accessions of wild cotton were documented and collected where permitted in South Florida during June 15 - 22. Many conservation areas of South Florida were visited, ranging from Sarasota to the southern limit in Key West. Collection zones were coastal areas with close access by car. Key contributors to finding locations and granting permissions to collect were:
Shawn McCourt, Plant Records Keeper, Marie Selby Botanical Gardens
Damon Moore, Division Manager Ecological & Marine Resources, Manatee Parks & Natural Resources
Jenny Evans, Evans, Native Landscapes and Garden Center Manager, Sanibel-Captiva Conservation Foundation

Seed was collected at the following locations:
Neal Preserve – **1 collected**
Sanibel Island – **4 collected**
Tavernier Keys – **1 collected**
Matecumbe Key – **1 collected**

Six other locations were visited but either wild cotton was not identified, or a collection permit was required. Return trips to South Florida will need official permits from the State of Florida Dept. of Env. Protection, and the National Park Service to access their locations of wild cotton. Continued dialogue with Park Rangers, Botanists and even Entomologists will quickly find additional wild and native cotton. The habitat of wild cotton appears to be a fine area between seashores and hammocks, which is just high enough to avoid waterlogged soil. The tidal action must create cleared area suitable for cotton seedlings but shade of the trees inhibits them. This habitat is susceptible to tropical storms and climate change and wild cotton is in need of *ex situ* conservation.

Fifty-eight accessions of conventional cotton germplasm were tested for the presence of Cry1Ac, Cry2A, and CP4 EPSPS ('RR'), but 20 of them showed positive results for the presence one or more of these transgenes. The accessions that tested positive will not be admitted to the NCGC unless the developers can provide a clean source of seed. We are separately considering a collection of accessions containing transgenes, because there is a backlog of those accessions in the NLGRP waiting to be sent to the working collection at College Station, TX for curation and distribution. However, we will seek advice/permission from the committee because it requires completely separate policies and procedures.

## Research

Several presentations were made of research conducted with Pima and Upland cotton of the NCGC (Hinze et al. 2019a) and new germplasm from Africa (Hinze et al. 2019b). Characterization of the NCGC crop wild relatives, particularly with collection of *G. thurberi* from Arizona was summarized in a book chapter with collaboration among curators in the National Plant Germplasm System (Jenderek and Frelichowski 2019).

## Summary

The NCGC conducts numerous activities with the ultimate goal of conserving and making available cotton and *Gossypium* seed to users that most accurately address their cotton breeding, research or educational purposes. Two challenges exist: conducting routine activities with limited resources, and distribution of information and seed of the most diverse but difficult to cultivate accessions in the NCGC. Most accessions are grown and characterized with the CWN and College Station field plantings, but at decreasing numbers each year because of financial and resource constraints. Greenhouse use is already at maximum capacity and expert care in cultivation is used to trigger flower and seed production from exotic and critical accessions. Critical assistance has been provided to the NCGC from numerous sources such as other USDA, University, organizations such as Cotton Incorporated to help with NCGC activities. NCGC activities overall were more tightly focused on identification of accessions in greatest need of characterization and seed increases. The NCGC planted reduced numbers at each location of only the most critical accessions, at the most productive environment, with additional investment in cultivation practices to maximize seed yields. It already assigned photoperiodic accessions of *G. hirsutum* and *G. barbadense* to the CWN, which alone are close to a quarter of the NCGC holdings. While successes were enjoyed with reviving critical portions of the NCGC the bulk of it remains to be regularly seed increased. The NCGC will need more support of these activities along with creative cost saving measures to continue to increase all germplasm. Prospects for acquisitions depend on proper policies and procedures approved by the CCGC so that GMO cotton can be available to users. Explorations

are limited to USA and Territories with continuation in South Florida with permission and consultation with the State of Florida and the National Park Service.

## References

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