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 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 resources of agriculture. Annual statistics on key activities such as cotton seed distribution, seed increase, germplasm characterization, acquisition, database development, and research are presented for 2021 and compared with the past 5 years. Legal challenges remain with accessing foreign cotton genetic resources and consequently acquisitions were confined to collection of crop wild relatives in the US and Territories, and with developed cotton germplasm and cultivars whose registration period had expired. Demand for cotton genetic resources remained steady but the rising costs of operations, and static financial resources have reduced to less than a third the capacity to regenerate accessions and generated new backlogs in critical activities. Descriptors and digital images have been gathered on accessions since 2010 to bolster online databases of GRIN-global and CottonGen, and help users refine their seed requests and streamline their research. Characterization and seed increases occurred in several environments, a Cotton Winter Nursery in Liberia, Costa Rica, and summer field plots and greenhouses in College Station, TX. Reductions in seed increases means that demand for cotton seed will outpace the NCGC seed inventories. The trends forecast a widening gap in seed increase and seed distribution and stressed the need for more funding and collaboration to ensure the viability of the NCGC.

Introduction

The curator of the NCGC prepares a report of activities for the full calendar year and sends it to the members of the Cotton Crop Germplasm Committee, National Program Staff members. Beltwide Cotton Conferences are an additional opportunity for these members and key stakeholders in cotton research to review and assess the activities and the status of the NCGC. The report focuses on key activities of seed increase, characterization, distribution, acquisition, and database development. A comparison of annual statistics of seed increase and distribution was performed with the past 15 years. The contents of the NCGC are nearly 10,000 accessions of cotton and *Gossypium* seed, housed in humidity controlled, cold storage vaults in College Station, TX and the NCGRP, Fort Collins, CO. It is mostly *G. hirstum* with over 3800 cultivars but also many (~1200) landraces or wild collected cotton. Smaller holdings are *G. barbadense* cultivated and wild collected (~1600), diploid cotton species of *G. herbaceum* and *G. arboreum* (~1800) and the remaining exotic tetraploid and diploid *Gossypium* species are less than 1000 accessions. Passport data, seed inventories and collection of descriptors and digital images are performed by a curator and technician and posted in online databases of GRIN-Global and Cottongen. The cotton community and public online community requests seed and information through GRIN-Global or direct contact with the NCGC staff. Distributions are made according to inventories and to best match the users' research or educational objectives. Seed increase and characterizations use a tropical Cotton Winter Nursery (CWN) in Costa Rica, Texas A&M field plots, and USDA greenhouses. Cotton Incorporated provides funding (Don Jones) and management (Kater Hake) with a local manager, Alfonso Palafox (with over 15 years' experience), for the CWN. Data collection and entry is performed by staff with visits to the nursery or daily observations in local fields and greenhouses. The DBMU, Beltsville, MD and contractor Marty Reisinger assist the staff with GRIN-Global, and CottonGen curator Jing Yu and manager Dorrie Main, assist the NCGC with data and image uploading. Seed acquisitions are from cotton germplasm released to the NCGC from the NCGRP when their protection periods ended and are typically cultivars or germplasm registered with the Journal of Plant Registration or Plant Variety Protection. Donations are accepted

as long as they are free and clear for distributions because the NCGC currently does not enter into Standard Material Transfer Agreements. Conventional cotton germplasm is admitted and distributed. A strip test for common transgenes in cotton was recently applied to incoming cotton germplasm to detect transgenes. Also genetically engineered cotton is currently not incorporated into the NCGC because of insufficient resources to separately curate such germplasm and completely eliminate risks of contamination.

Materials and Methods

Seed Increase and Characterization

The CWN in Costa Rica is normally planted to NCGC accessions that flower only in response to short days and also with conventional seed of Cotton Breeders to advance another generation in winter months. NCGC seed increases and characterization at the CWN (2020-2021 growing season) was 307 accessions (1932 hills). The breakdown according to species and sub-collection are: 170 *G. hirsutum* TX landraces/racestocks accessions, 127 G. hirstum cultivars (incl. 8 standards), 3 *G. barbadense*. Prior checks of germination are performed with the landraces to ensure more consistent stands at the CWN.

The CWN is the ideal environment for characterization with morphological descriptors and digital images for the more diverse, wild and photoperiodic cotton accessions. However, Covid 19 prevented travel to the CWN for observations because of USDA safety protocols. Plants left from germination testing of seed sent to the CWN were maintained in the USDA greenhouses or transplanted to College Station fields to fill in some gaps for descriptors and images of critical accessions. The list of descriptors is online at CottonGen https://www.cottongen.org/data/trait/NCGC_rating_scale and in GRIN-Global via descriptor tab, crop COTTON https://npgsweb.ars-grin.gov/gringlobal/descriptors. Representative leaf, flower, stem, and bolls were tagged from each accession and taken indoors for photography. Each plant part was positioned on white backed omnigrid underneath high-resolution Canon digital cameras affixed to camera stands. Alfonso helped acquired and setup supplemental lighting to remove shadows and speed up focusing. A mobile demand field tablet with shoulder straps was used for field scoring of descriptors. It was preloaded in Microsoft Excel software with the accessions and plot numbers for rows and columns for each character. Each cell had a specific drop-down menu (enabled by data validation function) of ratings for a trait.

A nearby field from Texas A&M is used each summer to plant accessions of the NCGC for observation and/or seed increase. Typically accessions in need of characterization are first observed and photographed in College Station as a germination check and if photoperiodic (failed to flower in long summer days) scheduled for replanting and seed increase at the CWN. The 2021 summer planting in College Station focused on descriptors, images and critical seed increases of select NCGC accessions that are day neutral flowering, but duplicates of some accessions at the CWN were also grown for characterization. The total planted was 174 accessions and it breaks down to: 81 *G. hirsutum* (8 were standards), 69 *G. hirsutum* landraces 4 *G. barbadense* (incl. 2 standards of 3-79 and Pima S-6), 3 *G. herbaceum*, and 3 *G. arboreum*, 1 *G. sturtianum*, 1 *G. armourianum*, 1 *G. thurberi*, 1 *G. stocksii*, 1 *G. australe*, and 1 *G. bickii*. Self-pollinations were initiated on all but the landrace accessions (for images and descriptors only). Self-pollination involved covering 'candle stage' buds with organza mesh bags. The exotic diploids *Gossypium* accessions are not cross compatible so open pollinated seed was harvested from them. Descriptor ratings were scored with same tablet, and another excel sheet of accessions as rows and each trait as columns, and cells with drop down menu to select a rating for that trait. Representative leaf, flower, stem, and bolls were tagged and brought indoors to USDA workspace with camera stands and controlled lighting.

The 2021-2022 season at the CWN began with the USDA in College Station collecting cooperators seed, arranging for the inspection to obtain a phytosanitary certificate, final packaging with lists and official documents, and FedEx shipping to Costa Rica. The procedures were the same as previous years using APHIS (PCIT online portal), the Texas Dept. of Ag., and the same Costa Rican contacts identified by Alfonso. Seed was all acid delinted, dusted with Captan, and in individual 2.5" x 4.5" envelopes according to each hill of planting at the CWN. Each user packaged their seed into is 18" x 18" x 10" boxes and contents labeled on outside and on an excel sheet. Copies of seed inventories, cover letter from the Curator, Alfonso's import permit, APHIS phytosanitary certificates (certified copies) were placed inside and outside of the boxes for final shipment. The same paperwork and original phyto were FEDex to AG AD GLOBAL SERVICIOS and a scanned pdf of everything was also emailed to them because of strict Costa Rica importing procedures. The first shipment was sent September 29 with seed of 3 cooperators. The NCGC portion involved 150 accessions. (1740 hills). Breakdown is: 2 *G. barbadense* (2 standards), 140 *G.*

hirsutum landrace sub-collection accessions and 8 G. hirsutum standards. A second shipment of 4 cooperators passed inspection and was shipped to the CWN on November 9. The NCGC portion was 23 accessions of G. hirsutum accessions (165 hills). A third shipment of seed with 3 cooperators passed inspection and was shipped December 2. The NCGC benefitted from additional support from Jodi Scheffler and Mauricio Ulloa to pay for additional increases of NCGC accessions.

Distribution and Acquisition

The NCGC distributed seed and information to users via GRIN-Global shopping carts, or after contacting James or Janna. Because of delays in processing orders (especially with foreign orders) statistics are based on orders received through GRIN-Global. Orders received in 2021 were 199 and were consolidated down to 108 for shipping to repeat users and represent 98 users. A total of 1694 items were requested and actually represents 1345 accessions. Most orders originated in the USA with 97 orders (79 users), 929 items (788 accessions). An increase in requests from overseas occurred with 22 orders (19 users from 14 countries, 765 items (691 accessions). The breakdown of items across all orders is: *G. hirsutum* cultivars (602), *G. hirsutum* landrace/racestocks (302), *G. barbadense* (558), diploid cotton (64), exotic tetraploid (13), and diploid species (155). The orders were also broadly characterized by type of users and broken down according to GRIN convention:

STA U.S. state agencies and all universities	37 orders, 27 users, 683 items
UARS Agricultural Research Service	8 orders, 5 users, 77 items
UCOM U.S. commercial company	16 orders, 12 users, 57 items
UIND U.S. individual no affiliation	27 orders, 26 users, 93 items
UPRU U.S. non-profit organizations	9 orders, 9 users, 19 items
FCOM Foreign commercial company	4 orders, 4 users, 212 items
FGEN Foreign genebank/genetic research unit	7 orders, 6 users, 348 items
FIND Foreign individual no affiliation	2 orders, 2 users, 2 items
FPRU Foreign non-commercial organizations	9 orders, 7 users, 203 items

Acquisitions were conservative to prevent duplication within the NCGC and because foreign germplasm often comes with legal compliance with international treaties on intellectual property rights and benefit sharing. Therefore, collection trips by the NCGC are currently confined to the U.S. and its territories. The list of recent acquisitions from collection trips or donations are: 1 wild cotton accessions from private land owners, conservations, or townships in South Florida, 1 *G. thurberi* from Perin McNelis at Borderlands Restoration Network (assisted by Karen Williams, PEO, Beltsville, MD), 4 more *G. thurberi* from Mike Cashman at W6 Western Regional Plant Introduction Station, Pullman, WA, (assisted by Brian Irish, PGITRU, Prosser, WA), 8 donated from Warren Conaty of CSIRO, Narrabri, Australia (assisted by Brett Rose, Cotton Seed Distributors Ltd. Wee Waa, NSW Australia), 4 high oleic lines developed by Michael Dowd and collaborators of SSRC, New Orleans, LA, CSRL, Mississippi State, MS. Availability of accessions released to the NCGC from PVP or JPR protection periods was delayed because of recent NCGC policy of initial testing for adventitious presence of transgenes and ensuring increases and distribution of pure self-pollinated seed. Known transgenic accessions were withheld from the NCGC because of resource limitations and inability to handle in a completely separate fashion to avoid cross contamination.

Database

Descriptors and images were only collected on accessions in College Station because of Covid travel restrictions to the CWN. This information was formatted and regularly given to Jing Yu, curator of Cottongen, and Dorrie Main, of Washington State University. Jing compiled accessions from the NCGC, and linked them to passport information, descriptor ratings, and digital images. Data of descriptors and digital images were also given to Daniel Restrepo-Montoya and Amada Hulse for their preparation of a manuscript with ARS collaborators on categorical analysis and bivariate associations with the data in *G. hirsutum* and *G. barbadense*. Janna Love created descriptor panels of images associated with the ratings of selected descriptors. Cottongen can be accessed at: http://www.cottongen.org/data/trait/NCGC_rating_scale.

Significant progress was made to upload more descriptors from recent plantings but required some groundwork first in GRIN-Global. Addition of new descriptor data depends on the establishment of inventories in GRIN specific to each planting. Thus, inventories are created for observation purposes and later converted to seed inventories. PI numbers are not always added automatically to new accessions until seed viability and germination is verified. Over 130 accessions of Puerto Rico *G. hirsutum* landraces (collected 2013) and Arizona *G. thurberi* (collected 2015 and

2017) were assigned PI numbers and collected locations entered into GRIN-Global. This site is: https://npgsweb.ars-grin.gov/gringlobal/search.

There is still another 20% of the NCGC that needs characterization and seed increase. They are critical seed inventory with need for special germination techniques and fresh seed increases. They are also landraces or crop wild relative *Gossypium* species with photoperiodic flowering, low yields, odd growth habits as perennial trees or vines that resprout from basal roots, and exerted stigmas that require special care with forced self-pollination.

Results and Discussion

This manuscript is the basis of a status report of the NCGC for both the Cotton Crop Germplasm Committee and for attendees of the Cotton Beltwide Meeting. Several recaps of six years of activity were included in this report to show the time course of NCGC activities and is in reference to the start of the new CWN location in Costa Rica in 2016. A review of plantings at the CWN since 2016 is in Figure 1, which shows yearly numbers, a six-year total, and averages on total accessions planted and according to each NCGC sub-collection. The average is 296 per year and the yearly amount has declined to the present because the operating expenses and per hill costs have risen to currently about 30 US$ per hill and USDA funds for NCGC increases are limited. Photoperiodic accessions of *G. hirsutum* landraces are being sent to the CWN while day neutral accessions are increased locally in College Station. The proportion of *G. hirsutum* cultivars of the NCGC has dropped and the landraces increased in the most recent year at the CWN because of the priority in recovering critical accessions of cotton landraces.

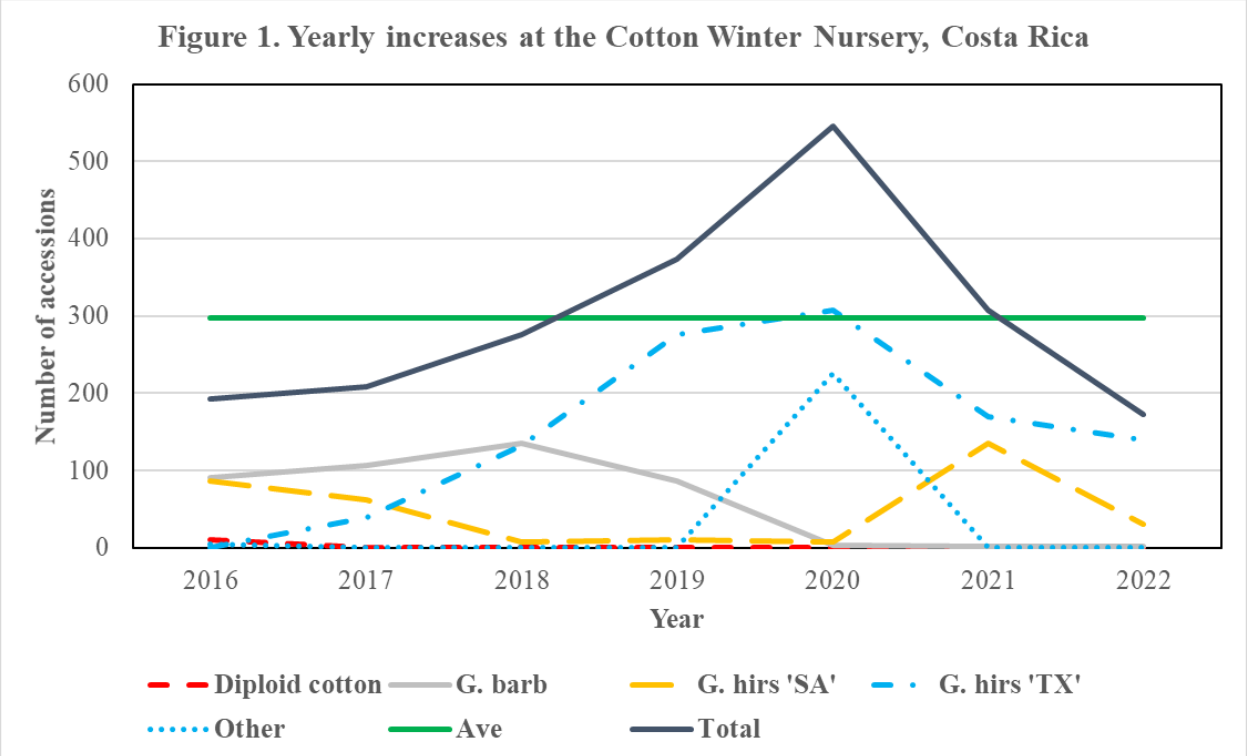

Figure 1. Cotton Winter Nursery plantings of accessions of the NCGC from years of 2016 to 2021.

No overall trend is seen in Figure 2 with yearly orders for the past six years. In 2021 a decline in domestic and an increase in foreign orders occurred relative to previous five years.

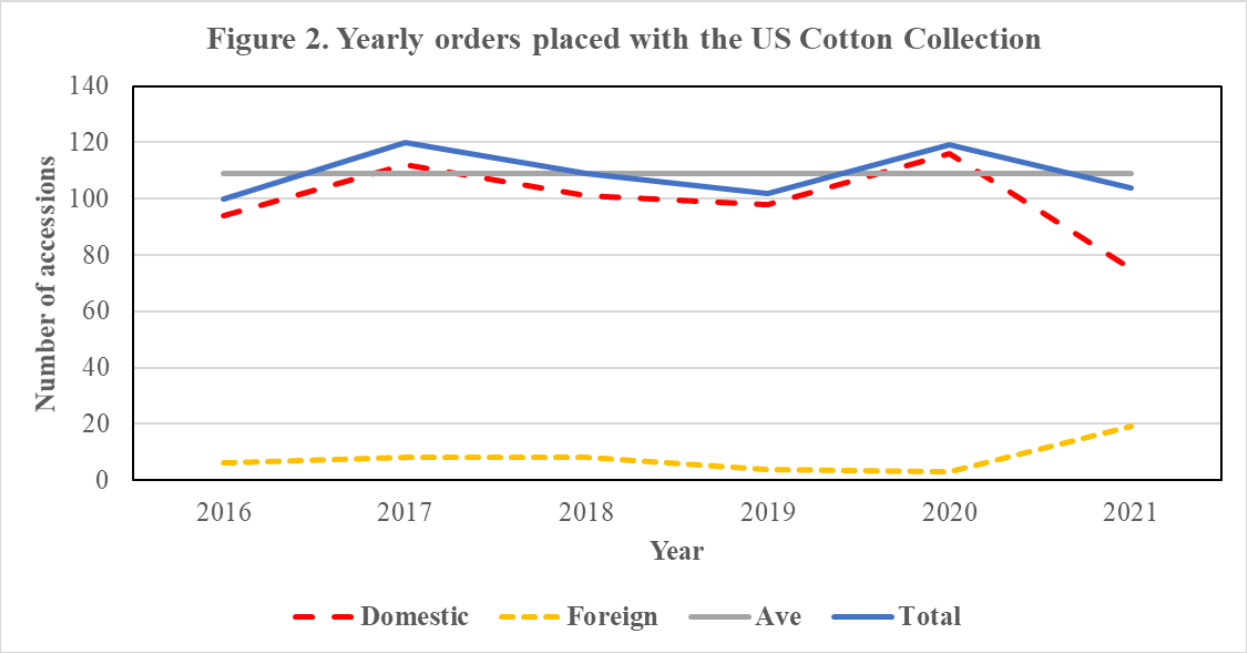

Figure 2. Yearly orders placed with the NCGC from the years 2016 to 2021.

The yearly items requested per year total and split between domestic and foreign users is in Figure 3. In 2021 a sharp increase in foreign demand was observed along with decline in order size from domestic users. A large increase in domestic items is traced to community wide priorities in addressing cotton vulnerabilities such as disease resistance. Foreign users are experiencing a delay in receiving their seed because of multiple procedures of securing import permits, and phytosanitary certificates that are needed to satisfy the requirements of the importing nation.

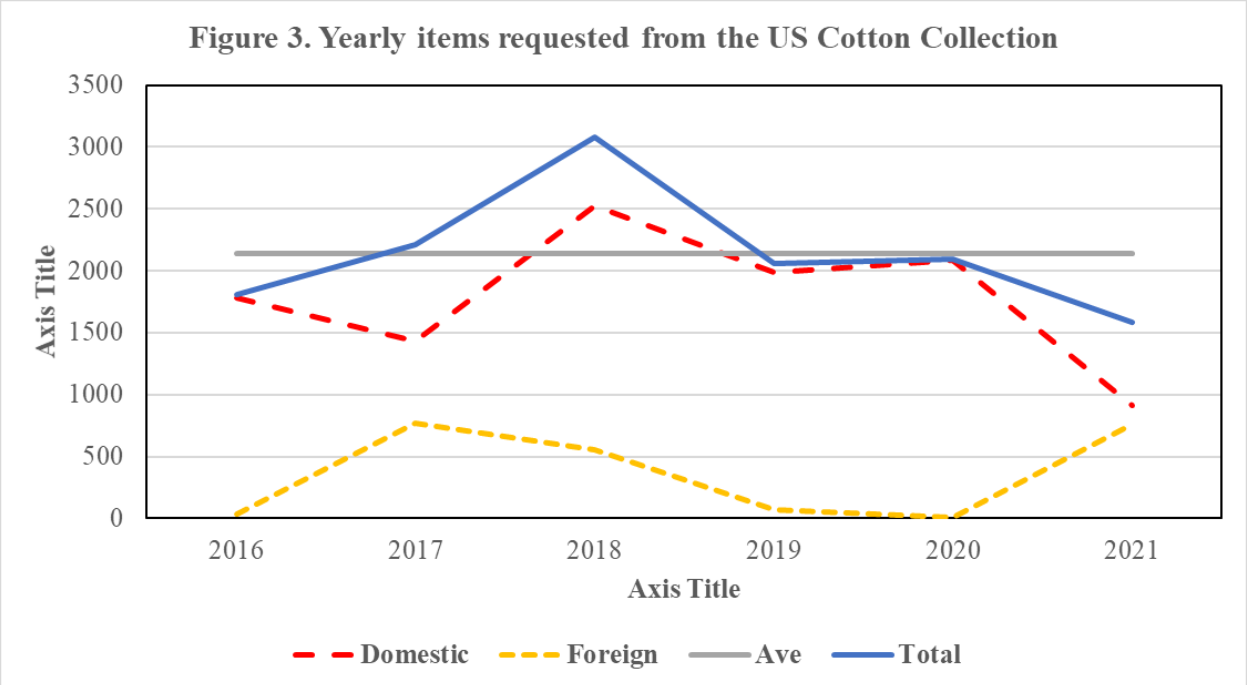

Figure 3. Yearly distributions of items from the NCGC from the years of 2016 to 2021.

Another analysis of the orders is according to sub-collection, which group them by species with useful cotton or as exotic *Gossypium* crop wild relatives. The *G. hirsutum* cultivar sub-collection typically includes an NCGC inventory prefix of 'SA' because it originated with obsolete cultivar accessions maintained in Stoneville, MS in the Delta Cotton Production Region. *G. hirsutum* landrace/racestock sub-collection has inventory prefix of 'TEX' (or TX) because USDA curators in College Station, TX accumulated wild collected and diverse *G. hirsutum* and needed to distinguish it from cultivars because of different flowering responses and other un-adapted growth characters. *G. barbadense* sub-collection originated with USDA breeders in Sacaton and Maricopa, AZ because of the development of Pima cottons for that region and their NCGC inventory begins with 'GB'. But this species has different fiber properties, longer growing season and genetic challenges in hybridization with *G. hirsutum*. Asiatic and African diploid cotton species are kept in another sub-collection because while they have fiber and are easily grown along cotton cultivars, the fiber quality is vastly inferior to commercial cotton.

The items requested per year show a general preference for *G. hirsutum* cultivars, with less for *G. hirsutum* landraces and *G. barbadense*. Recent decline in 2021 for *G. hirstum* cultivars could be simply smaller sizes of individual orders. An upswing was seen with *G. barbadense* and is traced to active programs centered around Pima breeding. In one year, the demand for diploid cotton was second highest (2020) and it was generally from a single research project but in 2021 went back to a lower level typical of most years. Demand for other species remained the lowest and is the most difficult group to utilize for cotton breeding. Keeping up with demand of the most critical and exotic accessions requires intensive maintenance in USDA greenhouses and outdoors in summer fields of College Station when possible.

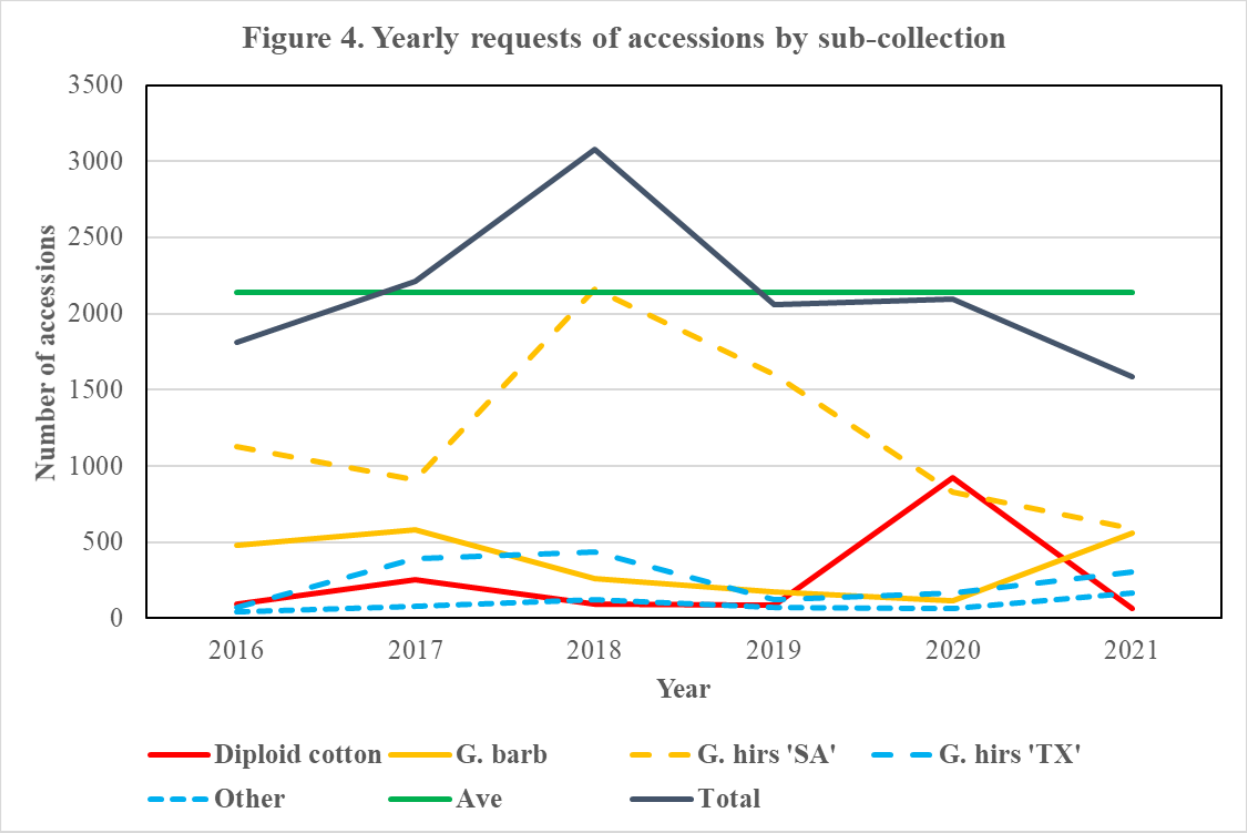

Figure 4. Yearly distributions of items from the NCGC according to sub-collection from 2006 to 2020.

Table 1 shows the most frequently requested accessions in 2021. Nine requests were made for SA 1611 or DES 119, then five requests made for Coker 310, Bleak Hall Sea Island and one other *G. barbadense* accession. Traits such as glandless or okra leaf shape or frego bracts are evident in the AUB series of accessions that were highly requested. The collective demand for exotic species is still significant and were limited to accessions with highest seed inventories. A large amount of the total requested were accessions only requested once in 2021 (1097). Not shown is over 1100 accessions requested in 2021 but not in 2020 and 130 of those were not previously requested at

all since local records were kept in 2006. This is also impacted by updating available seed inventories in GRIN-Global because users can only request those designated as available or still have the minimum seed count set in GRIN before being triggered as critical.

Table 1. Most requested accessions of the NCGC in the past six years

Inv Prefix	Inv Num	Taxon	PI	Name	Requests
SA	1611	*G. hirsutum*	606809	DES 119	9
SA	1184	*G. hirsutum*	529249	COKER 310	5
GB	679	*G. barbadense*	608115	BLEAK HALL SEA ISLAND	5
GB	991	*G. barbadense*	520734		5
SA	694	*G. barbademse*	528894	ST. VINCENT V135	4
SA	1213	*G. hirsutum*	529278	COKER 312	4
SA	1668	*G. hirsutum*	606814	PAYMASTER HS 26	4
SA	2901	*G. hirsutum*	600893	GERMAINE'S ACALA (4)	4
SA	3574	*G. hirsutum*	694489	AUB Gl-149	4
SA	3575	*G. hirsutum*	694490	AUB Gl-165	4
SA	3593	*G. hirsutum*	694516	AUB OK Fg-149	4
GB	604	*G. barbadense*	407501		4
GB	994	*G. barbadense*	520737		4
C_1	5	*G. sturtianum*	464861		4
D_1	2	*G. thurberi*	530766		4
D_1	3	*G. thurberi*	530767		4
D_1	4	*G. thurberi*	530768		4
D_1	13	*G. thurberi*	530777		4
E_1	1	*G. stocksii*	530976		4
K_1	3	*G. costulatum*	499771		4
K_8	1	*G. exiguum*	698472		4
				51 accessions	3
				176 accessions	2
				1097 accessions	1

Two databases were used to upload NCGC descriptor data and CottonGen was used for the high-resolution digital images. Progress is about 80% of NCGC accessions have descriptors and images. Descriptor panels were developed for many descriptors to illustrate how descriptors are rated and to zoom in on fine variations in certain characters. Figure 3 is the panel for descriptor seed fuzz color and eight ratings are used to score the accessions and correspond to the eight photos shown, and each represent an accession with that rating. Some accessions consistently have green fuzz, and others have no fuzz. Figure 4 is the panel for descriptor stem glanding which is the density of gossypol glands on the surface of the stem tips. All four ratings are shown, and each photo is from an accession that consistently expresses that rating of the stem glanding trait.

Figure 3. Eight ratings for descriptor of seed fuzz color.

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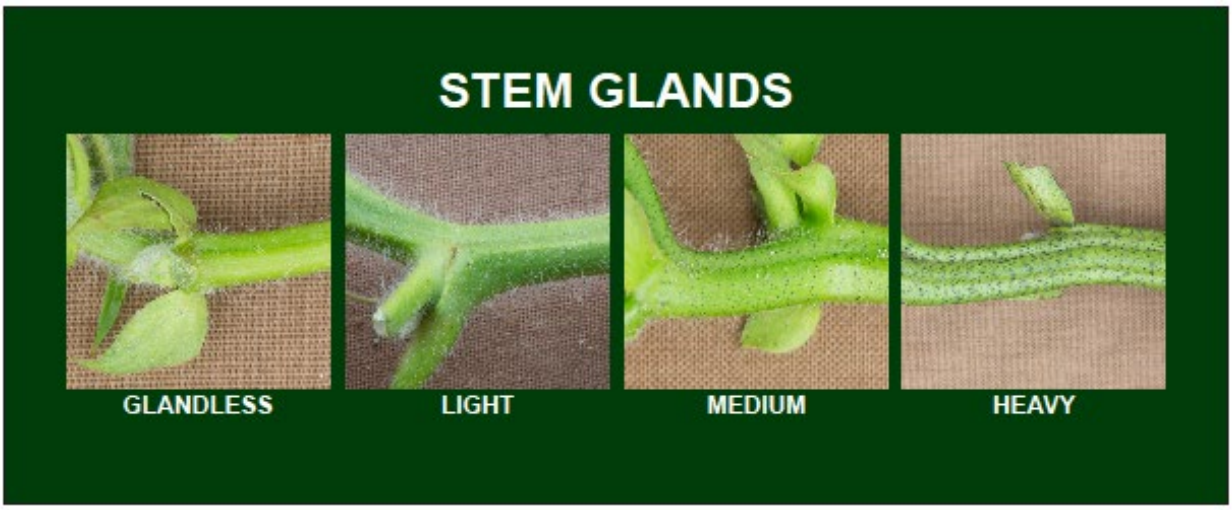

Figure 4. Four ratings for descriptor stem glanding.

Summary

The CWN was successful because of timely planting and care by the CWN crew. Seed was received from many accessions in the carryover nursery of the most critical cotton accessions in the collection. The 2021-2022 season began with challenges to meet stringent import requirements of Costa Rica and accommodate multiple shipments and use a second carrier (UPS) for faster delivery. Continued support of CWN activities by Cotton Incorporated and participation by many cotton breeders and scientist should keep this as a vital component of seed increase and characterization of NCGC and other cotton germplasm.

Distribution of diverse germplasm remains high but is delayed due to workload. We believe the demand is only curbed by the seed availability listed in GRIN-Global, because users that know us personally are sometimes insistent on seed of critical accessions even from those freshly harvested from small plots in the College Station field or greenhouses or remnant seed of older lots of stored seed. This places a strain on planning seed increases because of the existence of more critical accessions than the yearly resources will allow us to seed increase. Trying to catch up in the backlog needs a greater investment in the utility and success of the CWN.

The characterization of the NCGC has been effective because it is about 80% completed. The inertia to complete more gaps was interrupted by not being able to visit the CWN. Some data will be gathered on seedlings in the greenhouse or summer field plots. Hopefully travel to the CWN can resume because the more data that is online, the most instructive it is for the users. Interest in the NCGC is still very high with continued collaboration with researchers and fielding weekly requests to use GRIN and helping in selection of accessions.