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GERMPLASM EVALUATION OF COTTON ACCESSIONS FROM THE U.S. COTTON GERMPLASM COLLECTION, USDA-ARS (GOSSYPIUM HIRSUTUM L. LANDRACES OF MEXICO)

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Abstract

Future improvement of cotton relies on introgression of genetic variability from wild cotton resources. Characterizing, understanding and adding to cotton germplasm collections is important for future cotton improvement. A set of 434 *Gossypium hirsutum* landraces of Mexico from the U.S. Cotton Germplasm Collection was planted at Shafter, CA for phenotypic and genetic characterization of variability. Commonly used phenotypic characters were scored for each accession and tissue was bulked of each accession for DNA extraction. PCR based microsatellite markers from public sources and developed at Shafter are being used to describe genetic variation in the same accessions. A subset of accessions will be scored on individual plants for phenotypic and molecular marker variation to compare between and within accession variation. The combination of the two approaches will provide a better description of the variation in the collection, provide a template for future data collection and description of cotton germplasm, and may discover relationships between the markers and the phenotypic traits (i.e. linkage to genes). Preliminary results showed that significant variation still exists in the collection from the data collection. Diversity between and within accessions was the highest for accessions collected within the states of Guerrero, Yucatan, Oaxaca, Veracruz and Chiapas. The use of molecular markers is expected to reveal more variation not evident in the physical traits, and will become a valuable tool in the maintenance and utility of the diversity of the germplasm collection.

Introduction

The *G. hirsutum* gene pool form Mexico encompasses a wide range of habitats and is one of the primary sources for improvement of most of the Acala and Upland cotton growing in the world today. Mexico is also the center of diversity of the *Gossypium* genus with 11 of the 13 known diploid *Gossypium* species of the Western Hemisphere endemic to its geopolitical boundaries. Since the initial delineations, scientists involved in germplasm collection, maintenance and evaluation of cotton have not recently evaluated new and existing accessions of Mexican *G. hirsutum* landraces (Ulloa et al., 2005).. Despite the existence of large collections of landraces of *G. hirsutum* and *G. barbadense*, they are poorly evaluated (Percival, 1987) and difficult to characterize for their value in the collection and potential for cotton improvement. The management of large collections would be improved with knowledge of the current state of representation of the landraces in the collection and methods to compare differences in the accessions. If *in situ* diversity of the Mexican cottons is severely eroded, then current and additional accessions in the USDA Cotton Germplasm Collection assume a highly significant role in preservation of the diversity previously existing in Mexico

Gossypium hirsutum is the most widely cultivated cotton in the world, which is known by various common names including, among others, Acala or Upland cotton, short staple cotton, Mocó cotton, and Cambodia cotton (Johnson, 1926). *G. hirsutum* is the focus of this research because cultivars from the *G. hirsutum* species supplies 90% of the bulk world's cotton demand (Wendel et al., 1992). Little information is available on the *G. hirsutum* landraces in the collection, and there is still room to add more data on morphological traits as well as to incorporate the use of molecular markers for description of the genetic variation. Phenotypic markers are limited in numbers, difficult to score with environmental influences and are subjected to individual discretion in the methods of scoring. Plant

breeders may find molecular markers useful as a selection tool in monitoring cotton genome introgression in cotton breeding.

DNA markers are useful for a range of basic and applied scientific objectives in plant improvement. The USDA-ARS Cotton Enhancement Program at Shafter, CA has molecular marker development projects that will be used for the characterization of cotton genetic diversity, saturation of the cotton map and association of marker alleles with genes for cotton improvement. Microsatellite markers, alternatively called simple sequence repeat (SSR) markers are commonly identified and used for PCR based markers due to their high level of polymorphism and transferability across species. SSR markers have been developed in cotton such as JESPR (Reddy, et al., 2001), BNL and CIR (Nguyen, et al., 2004), and more are being developed by Dr. Ulloa's laboratory at the Cotton Enhancement Program, Shafter, CA in cooperation with UC-Davis for EST derived SSR's (Young et al., 2004) and with Clemson University Genomics Institute for BAC-end derived SSR's (Palmer et al., 2004). Markers polymorphic with *G. hirsutum* cultivars and accessions are the focus of this research.

The goals of this research are the assessment of the current status of *G. hirsutum* genetic resources in the USDA Cotton Germplasm Collection and comparisons of the feasibility of genetic marker (i.e. microsatellites) vs. botanical characterization for maintaining germplasm variation and preservation in cotton collections. The research falls into three main objectives: evaluation of morphological characteristics of the cotton accessions of landraces of Mexico (*Gossypium hirsutum* L.) from the USDA-ARS Cotton Germplasm Collection at College Satiation, TX, evaluation with the use of microsatellites the genetic diversity of these cotton accessions, and comparison of the feasibility of microsatellites vs. botanical characterization for maintaining germplasm variation and preservation in cotton collections.

Materials and Methods

Seed of 434 accessions of landraces were received from the National Collection of *Gossypium* Germplasm. These accessions represent collections of cotton from 18 states throughout Mexico from a period of 1946 to the present. A range of years in the collection of the landraces as well as recent collections where possible are included to observe any changes that have occurred in the natural variation in *G. hirsutum*. Fifteen seed of each accession were planted in a single row to provide at least 10 plants for data, fiber for analyses and DNA. *G. hirsutum* varieties Acala Maxxa, Phytogen 72, and TM-1 were included in the field plantings as references for performance evaluation of the traits related to cotton yield. Morphological traits were scored on the accessions and individual plants when segregation was evident. Over thirty traits were used ranging from simple qualitative traits to petal spot and color to measurements of leaf and boll dimensions to better describe their variation. Traits were evaluated according to procedures developed by Dr. Ulloa in the cotton breeding program, and according to the literature (Stanton et al., 1994, C. Jiang et al., 2000). Table 1 lists the traits selected for scoring.

Table 1. Listing of phenotypic traits and scoring methods for *G. hirsutum* Landraces

Category	Traits	Scoring range
Growth	Vigor	
	Height	Average in cm
	square load	1-10, 5=Maxxa, 6-10 less, 1-4 more squares
	peak bloom	1-10, 5=Maxxa, 6-10 less, 1-4 more squares
	Maturity	1-10, 5=Maxxa with 50% open, 6-10 <50%, 1-4 >50%
Leaf	color	green, red/light/dark/olive as descriptors
	shape	super okra, okra, intermediate, normal, normal wide leaf
	dimensions	(Jiang et al., 2000)
	other	wrinkled, cupping, folded shape
Flower	petal color	cream or yellow
	petal spot	absent (0) or 1-3 (largest spot)
	pollen color	cream, yellow, gold
Nectary	presence, size	0-2 average number on 3 main lobes

		Average width and length (mm) 4th node leaf
Glands	presence, activity	absent/inactive (0), 1-3 (most glands/activity)
Pubescence	stem, petiole, leaf	glabrous (0), 1-4 (most dense hairs)
Red coloration	stem, petiole, leaf, veins	all green (0), 1-3 (most red color)
Lint	yield, color, seed/boll quality	

BAC-end sequences of Maxxa, provided by the Clemson University Genetics Institute were used to design primer pairs flanking 800 SSR's, at the Cotton Enhancement Program, Shafter, CA. Among *G. hirsutum* varieties Maxxa and TM-1, *G. barbadense* Pima 3-79, and *G. raimondii*, 263 markers were polymorphic and 27 were polymorphic between *G. hirsutum* varieties Maxxa and TM-1. PCR markers were also designed from SSR's identified in EST's of *G. arboreum* (Young et al., 2004). Fourteen PCR markers from these EST's were polymorphic among *G. hirsutum*. Additional SSR markers derived from BNL, JESPR and CIR (Reddy et al., 2001; Nguyen et al., 2004) sources should provide a suitable quantity of markers to assay landraces of *G. hirsutum*.

Preliminary Results and Discussion

Significant morphological variation was observed among the accessions which could be distinguished by scoring of phenotypic traits. Segregation for traits was observed within 40 accessions and is an underestimate of the variation as most accessions failed to flower. Fiber traits are still being evaluated and are expected to show useful variation for potential cotton improvement. Thirteen traits that were scored on all accessions were evaluated for their diversity for accessions within each state and a summary of the observations for 14 states (that had more than one accession) are listed in Table 2. The numbers of accessions are listed for each state followed by the number of accessions that flowered in our growing season, then the number out of 13 traits that were scored differently (i.e. could be used to discriminate the accessions) for each state, and the last column indicates the number of accessions that segregated within each accession for one or more traits.

Table 2. Listing of the States of Mexico and the total number of accessions per state evaluated and that flowered in Shafter, CA. The number of morphological traits that varied among accessions within each state and the number of accessions segregating with accessions are also listed.

State	Accessions evaluated	Accessions that flowered	Traits	Segregating accessions
Guerrero	65	22	12	6
Yucatan	32	8	12	2
Oaxaca	98	35	11	12
Veracruz	29	14	11	4
Chiapas	60	52	11	4
San Luis Potosi	22	18	7	5
Colima	17	3	8	2
Michoacan	9	3	10	1
Sinaloa	4	1	5	1
Puebla	2	1	5	0
Campeche	15	4	10	0
Quintana Roo	19	4	7	0
Baja	5	4	5	0
Tamaulipas	11	8	5	0

Accession from Guerrero, Yucatan, Oaxaca, and Chiapas appeared to show the greatest among and within accession diversity. Photoperiod responses for flowering was suggested by the data as most accessions failed to flower or flowered late, suggesting a response to a different day length than in our growing season. In contrast most accessions of Chiapas flowered and set harvestable bolls suggesting that they were neutral in their response to day length. Data collection and additional analyses are ongoing and will be published elsewhere. Further evaluation of the segregating accessions will be necessary to compare between and within accession variation and also to rule out

accidental contamination. A replicate planting of a subset of these accessions at the USDA Winter Nursery at Tecoman, Colima, and Mexico will be scored for these traits to complete the morphological and fiber data for determination of the consistency of the trait scores in multiple environments.

Molecular markers are still being designed and screened for polymorphisms within *G. hirsutum* genotypes. Molecular variation as measured with SSR markers is expected to reveal more variation. A comparison of molecular variation within and between accessions will be conducted with this subset of accessions that segregate. Molecular marker variation may prove to be as informative, if not more than, phenotypic variation. Also with this amount of data that will be collected simple correlations will be attempted between the traits and markers. The long term goal of this research is that molecular markers will be associated with known and yet undiscovered traits in cotton and their diverse alleles will be measurable in cultivated and wild cotton sources to give plant breeders powerful tools for future cotton improvement.

Standardizing the means to score these traits and clear communication of the methods to score the traits will expedite future phenotypic evaluations. The authors suggest the incorporation of digital images where possible to document the plant development in each environment and to communicate the phenotypic variation and scoring methods. Difficulty still exists in scoring traits such as those involving color and fine differences between accessions. The use of live material from standard genotypes (e.g. Maxxa and TM1) would help provide consistent references for scoring material as well as determining traits of potential use in cotton improvement.

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