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PROGRESS REPORT FOR THE EVALUATION OF COTTON LANDRACES FROM THE USDA-ARS COTTON GERMPLASM COLLECTION

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Abstract

A set of 434 G. hirsutum landrace accessions were selected from the U.S. Cotton Germplasm Collection, USDA-ARS and planted in several years and environments which include Shafter, CA and Tecoman, Colima, Mexico. The objectives are the characterization of the accessions for over 30 morphological traits and 100 microsatellite (SSR) markers, including recently developed and genetically mapped SSRs. Morphological traits may have potential for cultivar improvement and are important for conservation. SSR data will be useful to compare genetic diversity of the landraces throughout the cotton genome and enable advanced analyses. Five morphological traits and 24 SSR markers were used to compute Jaccard's coefficient and construct different dendrograms of 204 select accessions, which were chosen to represent their natural origin across 18 states of Mexico. The accessions did not cluster clearly by origin in the dendrograms suggesting gene flow and introgression of traits/alleles throughout different regions of the natural habitat of the landraces. The SSR marker data did group many more accessions by their origin than the trait data. A small comparison of 7 specific markers specific to homoeologous chromosomes showed a difference in the genetic diversity revealed. This research will continue to compile more trait and chromosome specific SSR markers to describe genetic variation in the landrace collection, and to use this information to improve the maintenance and utility of the germplasm collection.

Introduction

The constant or declining genetic diversity in cultivated cotton is a restriction on future improvement of cotton varieties. Maintenance and utilization of cotton germplasm resources is essential for providing the raw genetic variability for cotton breeding programs. Updating and expanding the information on the diversity of the collection would improve the maintenance and utility of the collection. This study focuses on the genetic variability of a set of *Gossypium hirsutum* landrace accessions maintained at the U.S. Cotton Germplasm Collection, USDA-ARS. The objectives are to characterize the accessions for morphological trait diversity under several environments, to evaluate with the use of microsatellites (SSR) markers the genetic diversity of these cotton accessions, and to compare the two approaches for maintaining, preserving, and using this information for cotton improvement.

Materials and Methods

434 accessions of *G. hirsutum* landraces were selected from the USDA Cotton Germplasm Collection and planted at Shafter, CA, and Tecoman, Colima, Mexico. This collection originated from 18 states of Mexico, collected from the period of 1946-97. Morphological variation was measured on the accessions for many traits. DNA was extracted from each accession for detection of molecular variation between *G. hirsutum* accessions with approximately 100 microsatellite markers (SSR) from newly developed MUSB (BAC-end sequences, 'Maxxa' genomic DNA, Clemson Univ. Genomics Inst., Frelichowski et al. *submitted*), MUSS, MUCS (Park et al. 2005), and public databases.

Over 30 morphological traits were scored on plants of each landrace accession. Six traits were selected for the current analysis: petal spot, relative stem hair density, presence of short stem hair, red coloration of stems, relative density of (gossypol) glands in stems. Figure 1 shows examples of variation in type and density of stem hair, glands and degree of red pigmentation and Figure 2 shows examples of flower petal color, spot, and pollen color traits that were scored. These traits were coded as 0 or 1 for absence or presence of their individual scores (usually on a scale of 0-5) and resulted in forty-five individual

categories for the traits. Only 204 accessions still representing at least one accession of each state of Mexico were selected for analysis on the basis of completed morphological and SSR marker data.

Currently twenty four SSR markers were successfully scored on DNA of 204 accessions. These include 5 and 3 MUCS and MUSS SSR markers, respectively, and 16 new MUSB markers. A total of 108 individual SSR marker alleles were scored and each coded as 0 or 1. Future work will compile about 100 SSR markers with chromosome locations. A small comparison was performed with 3 SSR alleles of 3 markers from Chromosome 10 and 4 SSR alleles from chromosome 20. These marker alleles have been localized to their chromosomes by hypoanueploid deficiency analyses and genetic linkage mapping.

The morphological data and SSR marker data were treated separately for the creation of a morphological trait and an SSR marker dendrograms of the accessions. Genetic dissimilarity coefficients were calculated for each using Jaccard's Coefficient (Jaccard 1908) with the NYTSC software package (Applied Biostatistics)and PAUP*4b.10 software package (Swofford 1998). Clustering was accomplished with UPGMA and dendrograms were drawn with SAHN. Accession names also include the state of collection to enable comparisons of morphological and SSR marker relationships with their natural origin. The eighteen states represented are: Baja California (BA), Campeche (CMP), Chiapas (CHIA), Colima (COL), Guerrero (GUER), Jalisco (JAL), Michoacan (MCH), Morelos (MOR), Oaxaca (OAX), Puebla (PBL), Quintana Roo (QRO), San Luis Potosi (SLP), Sinaloa (SIN), Sonora (SON), Tabasco (TB), Tamaulipas (TAM), Veracruz (VRCZ) and Yucatan (YUC). Average genetic distances for the selected markers on chromosome 10 and 20 were also calculated with the above programs.

Results and Discussion

A dendrogram from the selected morphological traits (Figure 3) showed pairwise dissimilarity coefficients ranging from 0 to 0.37. Overall the accessions do not cluster into defined clades on the basis of their origin. For example, at the top of Fig. 3 a clade contains four accessions from YUC, BA, CHIA and GUER which are widely separated geographically. With just six traits nearly all accessions could be distinguished from another but 59 accessions were listed with a dissimilarity coefficient of 0. Only four clades were found that clustered at least four accessions from the same state with the tightest cluster consisting of five CHIA, 1 VRCA and 1 SLP accessions. The most distant accessions are U5353OAX, U5430SIN and U5508YUC. The states with the highest and lowest average dissimilarity coefficients respectively were SIN (0.18) and CMP (0.15).

The dendrogram from the SSR marker alleles (Figure 4) showed pairwise dissimilarity coefficients ranging from 0 to 0.77.. Also accessions from the distant states of YUC and BA are not clustered together in this dendrogram. More accessions were clustered according to their state of Nine clades were identified that possessed at least 4 or more accessions from the same state with the tightest cluster at the top of Figure 4 containing 17 CHIA accessions origin than in Fig 3. Only 25 accessions had a dissimilarity value of 0. The most distant accessions are U5355OAX, U5373OAX and U5505YUC. The states with the highest and lowest average dissimilarity coefficients respectively were YUC (0.20) and SIN (0.16). Accession U5505YUC is separated by a genetic dissimilarity coefficient of 0.3 from the nearest clade. From visual observation (also shown in Figure 5) this accession is clearly different from others and this is in agreement with the SSR marker alleles. This research has found the value of using photographic records and planting standard cultivars to help resolve accessions that are apparently different but difficult to describe morphologically. In general accessions from Guerrero, Yucatan and Oaxaca were highly variable and within accession variability was highest for over 30 accessions, most from these three states.
The markers of chromosome 10 resulted in an average dissimilarity coefficient of 0.44 whereas the markers of chromosome 20 averaged 0.17. We propose to perform more analyses of this kind to see if additional evidence will suggest differences in genetic diversity between the A and D subgenome of the tetraploid cottons when factoring in cotton landraces. Extending this research to A and D genome diploid species will be more practical by using this approach of genome and chromosome specific SSR markers.

Work is continuing with more morphological traits (e.g. fiber quality, leaf shape, etc.) and SSR marker alleles. At this point both dendrograms from our current data suggest that there is some gene flow among

the accessions in different regions or possibly introgressions from accessions transported across Mexico by man. Perhaps factoring in habitat types of the accessions would improve the interpretation of the morphological trait dendrograms, but so far the SSR markers are more consistent in the expected clustering accessions by origins. Recent trends in studying cotton genetic diversity also prefers low copy, chromosome specific markers like these SSRs which are also amenable to further analysis of populations and gene flow. Continued analyses may reveal marker associations with morphological traits and provide tools with potential for cotton improvement.

References

Frelichowski, JE., Palmer M, Main D, Tomkins JP, Cantrell RG, Stelly D, Yu J, Kohel RJ, Ulloa M. (Submitted) Genetic mapping of microsatellites derived from BAC-end derived sequences of *Gossypium hirsutum* Acala 'Maxxa'.

Jaccard, P. 1908. Nouvelles rescherches sur la distribution florale. Bull. Soc. Vaud. Sci.

Park Y-H, Alabady MS, Ulloa M, Sickler B, Wilkins, TA, Yu J, Stelly DM, Kohel RJ, El-Shihy OM, Cantrell RG. 2005. Genetic mapping of new cotton fiber loci using EST-derived microsatellites in an interspecific recombinant inbred (RIL) cotton population. Mol Gen Genomics 274:428-441.

Swofford, DL. 1998. PAUP8. Phylogenetic analysis using Parsimony. Version 4b.10. Sinauer Associates, Sunderland, MA.