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RESEARCH ARTICLE

Genome-wide assessment of genetic diversity and fiber quality traits characterization in *Gossypium hirsutum* races

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Abstract

Gossypium hirsutum races are believed to be potential reservoirs of desirable traits, which can play crucial roles to overcome the existing narrow genetic base of modern Upland cotton cultivars. However, prior to utilizing the races in cotton improvement programs, understanding their genetic constitutions is needed. Thus, this study used molecular and morphological techniques to characterize 110 *G. hirsutum* germplasm including 109 semi-wild accessions and one Upland cotton cultivar, CRI12. In the study, 104 SSR markers detected 795 alleles, with an average of 7.64 alleles per marker, ranging from 3 to 14, and average polymorphism information content (PIC) value of 0.71. And 96 of the markers were found to be highly informative, with PIC value≥0.50. Pairwise genetic similarity coefficient across the accessions ranged from 0.19 to 1.00, with an average value of 0.46. Morphological characterization was done using fiber length, fiber strength, micronaire, fiber uniformity index, and fiber elongation. Pairwise taxonomic distance within the accessions ranged from 0.17 to 3.41, with a mean of 1.33. The SSR and fiber quality traits data set based unweighted pair group method of arithmetic mean (UPGMA) analysis grouped the accessions into 7 and 12 distinct clusters, respectively, that corresponds well with the results of principal component analysis (PCA). Our study revealed the existence of vast molecular and morphological diversities within the accessions and provided valuable information on each semi-wild accession for quick and better informed germplasm utilization in cotton breeding programs.

Keywords: semi-wild accessions, *Gossypium hirsutum*, SSR markers, genetic similarity, taxonomic distance

1. Introduction

The *Gossypium* genus, the most important natural fiber crop (Campbell *et al*. 2010) and the second leading oilseed crop (Yu *et al*. 2012) is grown throughout the tropics and subtropics regions of the world (Percival *et al*. 1999). The *Gossypium* genus includes about 46 diploid (2n=26) and 5 allotetraploid species (2n=52) (Wallace *et al*. 2009). *Gossypium hirsutum*, an allotetraploid species, is the most

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extensively cultivated and industrial crop among all the *Gossypium* species, accounts over 90% of the world cotton production (Campbell *et al*. 2010). Following early domestication, *G. hirsutum* evolved into seven morphological and geographical races designated as Latifolium, Marie-galante, Morrilli, Palmeri, Punctatum, Richmondi, and Yucatanense (Hutchinson 1951; Brubaker *et al*. 1999).

SSR, also known as microsatellite, is co-dominantly inherited, uniformly distributed throughout genome and highly transferable (Park *et al*. 2009); these merits make them still to be markers of choice for genotyping plant genetic resources. Like other morphological markers, fiber quality traits confound with environmental conditions (Song *et al*. 2015), as a result luck enhanced power of discrimination in cotton genetic resources characterization (Agarwal *et al*. 2008). Nevertheless, if fiber quality traits are accompanied with SSRs; better genetic information and comprehensive genotyping of germplasm collections are inevitable.

Characterization studies of cotton germplasm collections have been done to provide vital information on genetic diversity of accessions. Broad genetic diversity avoids massive economic losses by serving as a buffer against biotic and abiotic uncertainties and ensures the development of segregating populations from which crop genotypes with preferable gene combinations can be selected (Esbroeck *et al*. 1999). However, over the past years, a wide array of DNA based molecular studies, such as random amplified polymorphic DNA (Tabar *et al*. 2004), restriction fragment length polymorphism (Wendel and Brubaker 1993), amplified fragment length polymorphism (Iqbal *et al*. 2001), and SSR (Rungis *et al*. 2005; Zhang *et al*. 2005; Bertini *et al*. 2006; Abdurakhmonov *et al*. 2008; Kalivas *et al*. 2011; Dahab *et al*. 2013) have revealed the presence of high genetic similarities within *G. hirsutum* cultivars, which arisen as a consequence of the recurrent utilization of limited number of elite cotton cultivars in breeding programs (Zhang *et al*. 2005). Such practice has been threatening the quality and quantity of global cotton production (Abdurakhmonov *et al*. 2007; Zhang *et al*. 2011). Thus, for substantial recovery of the genetic diversity that lost during the past breeding history and sustainable cotton production, paying attention to exotically conserved *G. hirsutum* races may play an important role.

The GenBank of the Institute of Cotton Research, Chinese Academy of Agricultural Sciences (ICR, CAAS) where the seeds of the studies accessions were obtained has maintained a considerable number of exotic germplasm collections and is believed that the accessions are important source of resistance to biotic stress (Zheng *et al*. 1995; Knutson *et al*. 2014) and abiotic stress (Bibi *et al*. 2010; Wu *et al*. 2014), besides to agronomically important traits such as fiber quality and yield potential (Iqbal *et al*. 2001; Abdurakhmonov *et al*. 2007), which could be exploited in the evolution of broad based new cultivars. However, before starting the mining and introgressing processes of these potentially valuable alleles into Upland cotton cultivars, a systematic molecular and morphological characterization, which is a perquisite for efficient and successful GenBank management and for better informed germplasm utilization in breeding programs has to be done (Montilla-Bascón *et al*. 2013). Therefore, the intents of this study were to characterize exotic germplasm collections of *G. hirsutum* races using SSR markers and fiber quality traits.

2. Materials and methods

2.1. Plant materials and DNA extraction

In the study, 110 *G. hirsutum* accessions, including 109 semi-wild accessions, which were introduced from Texas, USA and one Upland cotton cultivar, CRI12 were characterized. The semi-wild collection were represented by 42 Latifolium, 18 Marie-galante, 18 Morrilli, 17 Punctatum, 8 Richmondi, 4 Palmeri and 2 Yucatanense accessions. The complete list of the accessions is reported in Appendix A. From each accession, a bulk of young fully expanded leaves were collected and stored at –80°C. Total genomic DNA was extracted from the frozen leaf tissues using pestle and mortar based on cetyltrimethylammonium bromide (CTAB) extraction protocol as described by Zhang and Stewart (2000) with slight modifications. DNA quality was evaluated by spectrophotometer using 260/280 nm absorbance ratio and 1% (w/v) agarose gel electrophoresis. The stock DNA samples were stored at –20°C and working DNA samples at 4°C.

2.2. SSR markers selection

A total of 1 040 randomly selected SSR markers, including 140 DPL, 60 GH, 208 HAU, 280 MON_CGR, 60 MUCS, 150 NAU, 42 STV, 60 TMB, and 40 ICRC were assayed for their polymorphism using 12 representative accessions, namely CRI12, Latifolium 79, Latifolium 127, Marie-galante 11, Marie-galante 66, Morrilli 2, Morrilli 34, Palmeri 37, Punctatum 16, Punctatum 23, Richmondi 8 and Yucatanense 2. Of which, 104 polymorphic markers that produced clear bands were used for genotyping the accessions.

2.3. PCR amplification

PCR mixture was prepared in a volume of 10 μL containing 2 μL template DNA, 0.25 μL forward primer (10 μmol L^{-1}), 0.25 μL reverse primer (10 μmol L^{-1}), 1 μL 10× Easy *Taq* buffer, 0.5 μL dNTP (10 m mol L^{-1}), 0.1 μL Easy *Taq* DNA polymerase (5 U μL^{-1}), and 5.9 μL ddH_2O. PCR amplifica-