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Research Article

A Novel Segregation Distortion in Intraspecific Population of Asian Cotton (*Gossypium arboretum* L.) Detected by Molecular Markers

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Abstracts: The segregation ratio of markers in an F_2 population derived from Rudongjijiaoyaguo (Rdjjyg) and Zhongmian971 (Zm971) was studied using 3 morphological markers, 20 SSR markers, and 11 SRAP markers. Totally, 24 markers (77.42%) showed a distorted segregation and all of them skewed toward the female genotype, which was peculiar in recent cotton research. All the three types of SSR markers and SRAP marker showed distorted segregation, but the morphological markers (Purple stem, Okra leaf, and Red spot color) were normally segregated. This indicated that such a novel segregation distortion phenomenon resulted from interior genetic factors. The allele frequency and the distribution of different genotype frequencies in the F_2 population were analyzed in codominant markers, to find out factors attributed to distorted segregation. Most of them implied distorted allele frequency, but it was normal genotype frequency, which showed that these markers were influenced at the gamete level.

Keywords: *Gossypium arboretum* L.; segregation distortion; SSR; EST-SSR; SRAP

Segregation distortion, which is defined as a deviation of the observed genotypic frequencies from their expected values, violates the Mendel's segregation ratio and cannot be analyzed by traditional genetic methods. Segregation distortion was first reported in maize by Mangelsdorf using morphological markers [1], and subsequently studied in other crop species including rice, barley, sorghum, and tomato, using morphological markers, enzyme markers, and molecular markers [2−6]. Compared to other markers, molecular markers are immune to the influence of phenotype and more convenient for analysis of segregation distortion. Most of the SSR markers were codominant, so allele frequency and the distribution of different genotype frequencies could be analyzed, to see whether the existing gametic or zygotic selections could be identified. It is helpful for studying the genetic mechanism of segregation distortion, crop improvement, and breeding. During the construction of an intraspecific genetic linkage map of Asian cotton (*Gossypium arboretum* L.), a novel segregation distortion was found. Among the 31 molecular markers, 24 markers (77.4%) showed segregation distortion, but morphological markers were normally segregated in F_2 population. This article is aimed at reporting this novel segregation distortion phenomenon.

1 Materials and Methods

1.1 Plant materials

Rdjjyg and Zm971, provided by the Cotton Research Institute, Chinese Academy of Agricultural Sciences (CRI, CAAS) were crossed as female and male parent respectively, in Wuhan, Hubei in 2004. The F_1 plant was self-pollinated in 2005, and 137 F_2 plants were obtained. Total DNA was extracted from

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the young leaves of each F_2 plant according to Paterson *et al* [7].

1.2 Morphological markers

Rdjjyg shows Green stem (Gs), Okra leaf (Ol), White spot color (Wsc), and White petal color (Wpc), whereas Zm971 is Purple stem (Ps), Normal leaf (Nl), Red spot color (Rsc), and Red petal color (Rpc). In the F_2 population, three morphological markers were scored as either Ps (dominant) or Gs (recessive), Ol (dominant) or Nl (recessive), Rsc (dominant) or Wsc (recessive), following three separate observations made at three-week intervals, but the flower color was too complex to be scored.

1.3 SSR and SRAP statistical analysis

Sequences of SSR primers were downloaded from the CMD database (http://www.mainlab. Clemson.edu/cmd/Primer.shtml), and synthesized by Shanghai Shengong Inc. PCR amplifications were carried out according to Wu *et al* [8].

Sequences of SRAP primers were adopted according to the previous publication, and synthesized by Shanghai Shengong Inc. PCR amplifications were carried out according to Lin *et al* [9].

1.4 Analysis of segregation distortion of markers

For each locus in this research, the expected Mendelian ratio was 1:2:1 for codominant markers and 3:1 for dominant markers in F_2 populations. A chi-square test was used to compute segregation distortion by biparents' genotypes to ascertain whether they skewed toward the female genotype or the male genotype. For the codominant markers, allele frequency ($p = q$) and the distribution of different genotype frequencies in the F_2 population (p^2:2pq:q^2) were analyzed, to characterize factors resulting in distorted segregation.

2 Results

2.1 Polymorphism screen for SSR and SRAP primers

For molecular marker analysis, a total of 1,250 pairs of SSR (BNL, CIR, and NAU) were screened for polymorphism between parents and generated 11, 1, and 8 polymorphic markers respectively; additionally, 153 SRAP primer combinations were screened for polymorphism between parents and generated 11 polymorphic loci. The result showed that polymorphism among Asian cotton (*Gossypium arboretum* L.) was relatively low.

2.2 Analysis of morphological markers

For the three morphological markers, Ps:Gs was 100:37, Ol:Nl was 105:32, and Rsc:Wsc was 102:35; χ^2 ($p < 0.5$) values were 0.197, 0.003, and 0.119 respectively, indicting that the three morphological markers showed normal (3:1) segregation ratio.

2.3 Analysis of segregation distortion of genomic SSRs

The 12 genomic SSRs (BNL and CIR) were codominant (Table 1); BNL2260、BNL2660, and BNL2812 showed normal segregation, the other nine markers showed distorted segregation and they all skewed toward the female genotype.

2.4 Analysis of segregation distortion of EST-SSR

For EST-SSR analysis, NAU series primers were used, which were derived from *G. arboretum* and upland cotton (*Gossypium hirsutum* L.). Among the EST-SSR from *G. arboretum*, only NAU220 was polymorphic, but segregated distortedly and skewed toward the female genotype. Among the EST-SSR from *G. hirsutum*, five codominant markers and two dominant markers were all distorted, and skewed toward the female genotype (Tables 2 and 3).

2.5 Analysis of segregation distortion of nonspecies specific marker

The nonspecies specific marker, SRAP, was used to detect this population. Among the eleven poly morphic SRAP markers, Me1Em12 and Me4Em12 showed codominant segregation, andMe1Em12 was distorted (Table 4); six of the nine dominant markers were distorted. All of the dis torted markers skewed toward the female genotype (Table 5).