Genetic Structure and Changes of Indica/Japonica Components of Major Inbred Rice Varieties in China

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Abstract: A total of 39 SSR markers were used to analyze the genetic structures and compare with the changes in the indica or japonica components of 304 Chinese major inbred rice varieties planted widely from 1950s to 1990s. The results showed that the genetic structure of major inbred rice varieties in China was distinctly divided into two populations, indica and japonica. The sub-structure of indica varieties was more complex than that of japonica ones. Of which, late-season indica and early-season japonica varieties had rather simplex genetic background. The seasonal ecotypes were not quite in conformity with the subtypes of genetic structure. Moreover, 12 SSR loci with specific differentiation between indica and japonica were used to calculate the indica/japonica components. The differences of indica/japonica components among the five decades were not significant except late-season indica varieties. The japonica components of late-season indica varieties planted in 1990s were increased obviously. These results will help to understand the genetic structure of Chinese major inbred rice varieties and indica-japonica hybridization breeding in China.

Key words: rice; major variety; genetic structure; indica/japonica component

Rice is one of the main food crops in China. Knowing the genetic structure of Chinese major inbred rice varieties would helpful to further understand the dynamic mechanism of rice varietal development, help breeders to construct parent selection for variety improvement, and broaden the genetic basis of rice (Zhang et al, 2009).

Chinese cultivated rice is composed of two subspecies, indica and japonica (Ding, 1957). Indica rice has comparable good tolerance to wet, heat and hard light conditions, and poorer cooked quality with higher amylose content. Japonica rice has comparable rice tolerance to cold, weak light, and perfect cooked quality with lower amylose content. Thus, indica-japonica hybridization breeding may not only synthesize both excellent traits, but also make use of heterosis to breed excellent varieties with high yield potential (Yang, 1962, 1973; Zhu, 1964; Zeng et al, 1980). Indica-japonica hybridization breeding began in the 1950s, already for over 50 years. A series of varieties or combinations with high yield potential (Hua et al, 2007). However, the genetic structure based on model and indica or japonica components of Chinese major inbred rice varieties were not reported. Till now, it is difficult to evaluate the effect of indica-japonica hybridization breeding on the genetic background of Chinese major inbred rice varieties.

The present study selected 304 Chinese major inbred rice varieties planted from 1950s to 1990s for analyzing genetic structure and comparing with the indica and japonica components based on 39 SSR markers to generate a lot of inspiration on recognizing indica-japonica hybridization breeding in China.

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MATERIALS AND METHODS

Rice materials

A total of 304 Chinese major inbred rice varieties with an annual cultivated area more than 66,700 hm² each during 1950s to 1990s were selected. Moreover, these varieties included 190 indica and 114 japonica (Table S1, see online at http://www.ricescience.org; http://www.sciencedirect.com/science/journal/16726308). All materials tested came from the rice germplasm bank of China National Rice Research Institute (CNRRI).

DNA extraction and SSR analysis

The uppermost fresh young leaves (1−2 cm) at the tillering stage were ground in liquid nitrogen. Genomic DNA was extracted and purified following the extraction procedure of Zheng et al (1995). Totally 39 pairs of SSR markers from each chromosome (Shanghai Shenggong Biological Engineering Technology Co., LTD, China) were used for SSR analysis (Table S2, see online at http://www.ricescience.org; http://www.sciencedirect.com/science/journal/16726308).

PCR reaction was carried out in 10 μL reaction system containing 2.0 μL of 10 × buffer, 1.0 μL of 2 mmol/L dNTPs, 1.0 μL of 25 mmol/L MgCl₂, 0.6 μL each of 10 μmol/L forward and reverse primers, 0.1 μL Taq polymerase (5 U/μL) and 20 ng of template DNA. DNA amplification was performed in a PTC-100 96v Thermocycler (MJ Research Inc.). The PCR reaction was conducted with following steps: pre-denature at 94 °C for 2 min, followed by 30 cycles of 94°C for 45 s, 55°C for 45 s (61°C for RM161, RM162 and RM135; 67°C for RM142, RM178 and RM169), 72°C for 1 min, and final extension at 72 °C for 8 min. The amplified products were electrophoresed on 6% non-denaturing polyacrylamide gels, and detected using silver staining (Panaud et al, 1996).

Data analysis

The number of alleles (Na) was evaluated using PowerMarker 3.25 (Liu and Muse, 2005). F-statistics (Fa) were used to analyze the special loci of the indica and japonica based on the allelic discrepancy at each locus by the procedure of AMOVA (Analysis of Molecular Variance) in ARLEQUIN ver 3.0 (Excoffier et al, 2005). The model-based program STRUCTURE were used to infer population structure (Falush et al, 2003), and indica or japonica component of each variety was calculated by the special loci of the indica and japonica. To determine the K value (the number of clusters), we used both the LnP(D) value and α. LnP(D) is the log likelihood of the observed genotype distribution in K clusters, and α is the value of the model parameter for degree of admixture. Small α implies that most individuals are essentially from one population or another, while α > 1 implies that most individuals are admixed. The significance test was conducted for evaluating the difference of indica or japonica component among the five decades by applying ‘Duncan Test’ of SAS software.

RESULTS

Genetic structure

Thirty-nine SSR markers were used for PCR amplification in 304 tested varieties. There were 223 detected alleles with 5.7 alleles per locus (Na) in average, ranged from 2 (RM142, RM427 and RM463) to 11 (RM21).

The genetic structure based on the model was constructed using Structure 2.2 software. LnP(D) value maintained at a high level and α value was stable at K = 2 (Fig. 1). This indicated that the major inbred rice varieties can be divided into two populations, corresponding to subspecies: indica and japonica (Fig. 2-A, green corresponds to indica, and red to japonica). This result was consistent with original indica/japonica description, and suggested that the classification of indica and japonica is obvious and no middle type materials exist. The sub-structures within indica and japonica were in conformity with the each early-season and late-season ecotypes at K = 4, showing that the difference between early-season and late-season rice was more significant, whereas the genetic basis of mid-season could be considered as the mid-type between early-season and
late-season. However, except late-season indica varieties clustered alone at $K = 5$ and $K = 6$, the different memberships in the early-season indica and mid-season indica were mixed, there were much more sub-structures within season ecotypes. The genetic structure of japonica varieties were sampler than indica ones and consisted of one group and two groups respectively. In addition, instead of the six
ecotypes: early-season, mid-season, late-season indica/japonica were divided into their corresponding populations independently at $K = 6$ (Fig. 2).

Fig. 3 and Fig. 4 showed the photograph corresponding to the genetic structure of indica and japonica, respectively. Similar to Fig. 2, two subspecies, both indica and japonica, could be differentiated into two populations: early-season and late-season rice. The six season ecotypes did not cluster independently. The genetic structure of indica varieties showed more complex than japonica ones. The average values of early-season indica and late-season indica membership in mid-season indica type were 0.445 and 0.555 at $K = 2$, respectively, i.e. the number of late-season indica membership was a little more than early-season indica ones. The late-season japonica membership in mid-season japonica type was significantly fewer than early-season japonica ones. The average values of early-season japonica and late-season japonica membership were 0.607 and 0.393, respectively.

**Difference of indica/japonica components of major inbred rice varieties among five decades**

AMOVA analysis showed that the indica/japonica genetic differentiation degree among the different SSR loci differed from each other. The indica/japonica genetic differentiation values on 12 loci, RM125, RM147, RM211, RM277, RM288, RM293, RM427, RM161, RM178, RM135, RM221 and RM142 were large ($Fst > 75\%$), occupying 30.8% of the total loci.

A total of 12 SSR loci with specific differentiation between indica and japonica rice were used to calculate the indica or japonica components. The mean indica component in indica rice was 0.988 with a range from 0.728 to 0.999, whereas the mean japonica component in japonica rice was 0.989 with a range from 0.742 to 0.999, i.e. the indica/japonica components of indica/japonica rice were extremely high. There were certain differences in indica/japonica components during the five decades. However, the significant test showed that the differences of both the indica component of japonica and japonica component of indica among the five decades were not significant (Table 1). Meanwhile, Table 1 showed that variance in indica/japonica components in early-season, mid-season and late-season ecotypes among the five decades had no obvious regulation. The japonica component of late-season indica rice planted in 1990s was increased obviously, but the differences of indica/japonica components of the other ecotypes were not significant.

**DISCUSSION**

As shown in the results from analysis of genetic structure based on the model, Chinese major inbred rice varieties could be divided into two populations, indica and japonica, marching consistently with original indica/japonica description. This suggests that ‘Structure analysis’ based on SSR data is available to study on genetic structure such as indica/japonica classification. Our result showed that the genetic structure feature of early-season and late-season rice was more evident. The late-season indica membership in mid-season indica rice was much more, but the late-season japonica membership was fewer than those of early-season in mid-season japonica ones. It was noticed that our result was different from the study on Chinese cultivated rice landraces by Zhang et al (2009). Major inbred rice varieties since 1950s were bred basically about hybridization process, cross-using of late-season, mid-season and late-season parents lead to the complexity of sub-structure of each ecotype, especially in mid-season rice. Taking indica rice for example, as early key parents e.g. Aizizhan, Nantehao, Dijiaowujian and Shenglixian were used for new late-season, mid-season and late-season variety breeding widely, whose derived varieties also became main parents of indica varieties in China. The varieties released by International Rice Research Institute (IRRI), such as IR8, IR24, IR26 were introduced to China in 1970s, they were widely used as parents for hybridization of late-season, mid-season and late-season rice breeding programs due to strong temperature sensitivity and weak or very weak photoperiod sensitivity.

<table>
<thead>
<tr>
<th>Period</th>
<th>Indica component</th>
<th>Japonica component</th>
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<tbody>
<tr>
<td></td>
<td>Early-season</td>
<td>Mid-season</td>
</tr>
<tr>
<td>1950s</td>
<td>0.012 a</td>
<td>0.024 a</td>
</tr>
<tr>
<td>1960s</td>
<td>0.015 a</td>
<td>0.001 a</td>
</tr>
<tr>
<td>1970s</td>
<td>0.011 a</td>
<td>0.016 a</td>
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<tr>
<td>1980s</td>
<td>0.007 a</td>
<td>0.001 a</td>
</tr>
<tr>
<td>1990s</td>
<td>0.013 a</td>
<td>0.024 a</td>
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</table>

Within a column, data followed by the same letters indicate no significant differences at the 0.05 level.
Our result also indicated that the japonica component of late-season indica varieties planted in 1990s was increased obviously by comparing the indica/japonica components of Chinese major inbred rice varieties among the five decades. This phenomenon was related to increasing quality requirement of late-season indica varieties in the late 1980s. Since the late 1980s, the requirement of indica cooked quality was raised, improving mid-season and late-season varieties through selecting superior long grain japonica parents e.g. Basmati and Jasmine 85 had great progress. For example, Xiangwaxian 11, which was bred systematically from Cuba rice with japonica background, released in 1999 and extended more than 100 000 hm² in 2000. Its japonica component reached up to 0.272. Nevertheless, the fact that the number of indica-japonica hybridization combinations with larger planted area in rice production is extremely limited, and the difference of indica or japonica components in the most bred varieties among the five decades were not significant, though indica-japonica hybridization breeding was carried out over 50 years since 1950s. Therefore, how to synthesize the advantages of indica and japonica more efficiently, and to breed super-high yielding rice variety still need more thorough research.

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