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Temporal changes in SSR allelic diversity of major rice cultivars in China

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Abstract

Forty simple sequence repeats (SSRs) were used to assess the changes of diversity in 310 major Chinese rice cultivars grown during the 1950s−1990s. Of the 40 SSR loci, 39 were polymorphic. A total of 221 alleles were detected with an average of 5.7 alleles per locus (*Na*). The Nei's genetic diversity index (*He*) varied drastically among the loci (0.207 to 0.874, mean 0.625). Comparing the temporal changes in *Na* and *He*, the cultivars from the 1950s had more alleles and higher *He* scores than the cultivars from the other four decades. Analysis of molecular variance (AMOVA) indicated that the genetic differentiation among the five decades was not significant in the whole set, but significant within *indica* and *japonica*. More changes among the decades were revealed in *indica* cultivars than in *japonica* cultivars. Some alleles had been lost in current rice cultivars in the 1990s, occurring more frequently in *indica*. These results suggest that more elite alien genetic resources should be explored to widen the genetic backgrounds of rice cultivars currently grown in China.

Keywords: rice (*Oryza sativa* L.); major varieties; SSR; genetic diversity; AMOVA

Introduction

Genetic diversity is an important component in the development of improved rice (*Oryza sativa* L.) cultivars that are resistant to diseases and pests, and tolerant to abiotic stresses. Nevertheless, scientific and public concern over practices of modern intensive plant breeding that leads to a reduction of genetic diversity has been emphasized since the 1970s (Frankel, 1970; Harlan, 1972; Hawkes, 1983; Ramanatha and Hodgkin, 2002). Recently, molecular techniques have been employed to detect genetic diversity and its impact on crop breeding. Some reports demonstrated that a significant decline of genetic diversity has occurred in current crop cultivars. Roussel et al. (2005) analyzed 480 European bread wheat (*Triticum aestivum* L.) cultivars released from 1840 to 2000 using a set of 39 SSRs, and found a clear reduction in genetic diversity after 1970. Similar results were reported for oat (*Avena sativa* L.) (Fu et al., 2003), and maize (*Zea mays* L.) (Clerc et al., 2005). In contrast, Donini et al. (2000) characterized 55 UK cultivars of winter wheat in the period 1934–1994 using combined data from morphology, storage protein content, AFLP, and SSR analysis, and indicated no significant decline in genetic diversity over time. This view was also supported by later studies on Argentinean wheat (Manifesto et al., 2001), UK barley (*Hordeum vulgare* L.) (Koebner et al., 2003), and European barley (Backes et al., 2003; Malysheva-Otto et al., 2007). Indeed, in some cases, the levels of genetic diversity had increased in the current cultivars (Smale et al., 2002; Maccaferi et al., 2003).

Rice is the major food crop in China (Cai and Zhu, 1992). In 2006, it occupied 28.85 million hectares with a total production of 180.6 million tons (National Bureau of

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Statistics of China, 2006). Two subspecies of rice are cultivated in China. *Indica* rice is principally cultivated in lower latitude and altitude regions of southern, central, and southwestern China, while *japonica* rice is mostly grown in higher latitude and altitude regions of northern, central (roughly above 29°N), and southwestern China. Although the cultivation history of rice in China can be dated back at least 10,000 years (Tang, 2004), systematic rice breeding only started in 1919 (Ying, 1993). During the period from the 1920s to the 1940s, pure line selection was the commonly used method in rice breeding. After 1949, extensive national cooperation in rice breeding occurred using various effective breeding methods (Lin and Ming, 1992). During the past five decades, at least 5,132 improved rice cultivars have been developed. However, only a few studies have dealt with distribution of rice genetic diversity over time. Zhuang et al. (1996) analyzed 25 Chinese *indica* varieties with 50 RFLP markers, and showed very low genetic variability in the early 1990s. Yang et al. (1998) analyzed 238 Chinese rice cultivars using microsatellite markers and found no significant genetic differentiation between modern cultivars and landraces. More recently, Qi et al. (2006) studied 257 Chinese modern rice cultivars selected from the primary core collection (containing 520 accessions representing over 96% of phenotypic traits of total rice cultivars in the China seed bank by 1998) with 36 SSRs and 42 phenotypic traits, and demonstrated that the genetic diversity declined from the 1950s to the 1980s and increased greatly in the 1990s. These different results might be caused by the differences in sample sizes of accessions and molecular markers used.

In the present study, the genetic diversity of 310 major Chinese rice cultivars, planted over large area annually from 1951 to 2000, were analyzed using 40 SSR markers. The main objectives were: 1) to characterize the allelic diversity, both in the whole set and in subsets of *indica* and *japonica* subspecies; and 2) to assess the genetic variation within and among decadal periods.

Materials and methods

Plant materials

A representative set of 310 cultivars, which included 199 *indica* and 111 *japonica* cultivars, were chosen from major rice-growing areas in China (Supplemental Table 1). Two criteria were considered when selecting material samples: 1) the planted area of the cultivars must be over 100,000 hectares annually in certain years during the period of the five decades (1951–2000); and 2) the sample number (size) both for each decade and for *indica* and *japonica* subspecies should be approximately equal. Based on their year of release year to farmers, these cultivars are divided into five decadal groups (Table 1). Seeds were obtained from the National Medium Rice Genebank at the China National Rice Research Institute (CNRRI), Hangzhou, China.

DNA extraction and SSR analysis

Total genomic DNA was isolated from five plants for each cultivar following a modification of the SDS "mini-extraction" protocol developed by Zheng et al. (1995). Forty SSR primer pairs, which are randomly distributed throughout the 12 rice chromosomes, were used in this study. Details of the primer characterization are available at http://www.gramene.org/. DNA amplification was carried out with a PTC-100 programmable thermal cycler (MJ Research, Inc., USA) in a 10 μL reaction mixture. Each reaction contained 10 × buffer 1.0 μL, 2 mmol/L dNTPs 1.0 μL, 25 mmol/L $MgCl_2$ 1.0 μL, 0.6 μL each of the forward and reverse primer (10 μmol/L), 5 U/μL *Taq* polymerase 0.1 μL, and 20 ng template DNA. The PCR cycle profile was as follows: 94°C, 5 min; 35 cycles of 94°C, 1 min, 55°C (61°C for RM161 and RM162, and 67°C for RM169, RM174 and RM178, respectively), 1 min, and 72°C, 2 min; and 72°C, 5 min. PCR products were separated on a 6% non-denaturing polyacrylamide gel at 8 V/cm for 2–3 h and silver stained as described by Panaud et al. (1996). Alleles were scored according to molecular weight, and between-gel differences were controlled by inclusion of molecular weight markers on each gel.

Table 1
The number of rice cultivars for each of the five decades used in this study

Decade	*Indica*	*Japonica*	Total
1950s	41	22	63
1960s	38	24	62
1970s	42	20	62
1980s	40	21	61
1990s	38	24	62
Total	199	111	310