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Construction and Testing of a Primary Microsatellite Database of Major Rice Varieties in China

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Abstract: Sixty-three major inbred varieties and parental lines of major F_1 hybrids used in the commercial rice production in China were assayed with rice microsatellites screened in a previous study and additional microsatellites on four chromosomes. A set of 24 markers was selected and proposed for its application in the variety identification of rice, which are distributed on all the 12 rice chromosomes with 2 markers on each chromosome. The 63 major varieties and parental lines, as well as 41 major F_1 hybrids, were genotyped with the markers. Alleles detected in each line at each marker locus were verified. By matching marker genotypes of corresponding F_1, maternal and paternal lines of hybrid rice, high reliability of the maternal lines was verified, data on the paternal lines were modified, and a false hybrid was removed. A database containing genotype data of 103 major rice varieties and parental lines at the 24 marker loci was constructed and analyzed.
Key words: simple sequence repeat; rice (*Oryza sativa*); variety identification; frequency of polymorphism; database

The determination of distinctness, uniformity and stability (DUS) is prerequisite to the registration of a new plant variety. Morphological and physiological characters have long been used as the descriptors in granting the variety right and settling variety disputes. The identification is usually a long procedure, as some of the descriptors cannot be characterized until harvest or post-harvest. It is also noted that the description maybe confounded as many of the characters are inherited quantitatively, showing continuous variations and high sensibility to environmental factors. Furthermore, a great number of varieties have been released and the number is increasing over time. It is impossible for any testing authority to distinguish a new variety from all existing varieties by traditional means. It has become recognized that DNA profiling might provide a feasible solution to such problems, among which microsatellites also called simple sequence repeats (SSRs) are especially advantageous [1-5].

Databases of DNA profiles of major varieties are essential to the application of DNA marker-based variety identification. While such databases have become available in tomato [6] and wheat [7], the attention in rice has been drawn to the variety authentication and purity assessment of few given hybrid rice [4, 8-12]. Only recently a DNA profiling of parental lines of major hybrid rice in Sichuan Province, China, was reported [13]. In this study, a set of 24 rice SSRs were selected and proposed for the variety identification in rice, and these markers were used to construct a database for 103 major rice varieties and parental lines in China.

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MATERIALS AND METHODS

Rice materials

Inbred varieties and F_1 hybrids of rice that have a planting area of 66.7 kha or more (1 million mu) in China in 2002 according to the national statistics, as well as parental lines of these hybrids, were sought. Altogether, 63 inbreds (a male sterile line and its maintainer line was counted as a single genotype) and 41 hybrids were obtained from 11 research groups of

the China National Rice Research Institute (CNRRI), Hangzhou, China and 16 other organizations. These included 7 indica inbred varieties, 14 japonica inbred varieties, 14 maternal and 28 paternal lines of indica hybrid rice, and 36 three-line and 5 two-line indica hybrids (see Online Supplementary Materials: Supplementary Table 1). Five of the inbreds, Fengyuan A, You I A, 926, Xiushui 04 and Minghui 70, did not satisfy the planting area in the given year, but they have become widely used and were included in a previous screening of SSRs for the variety identification of rice [14]. Of the target rice lines, 2 indica inbred varieties, 1 japonica inbred variety, 2 maternal lines and 5 paternal lines of indica hybrid rice, and 10 indica hybrids, were not available.

To reduce the risk of using false material, rice lines in the same name were collected from different sources when practicable. Of the 63 inbreds, 23 were obtained from two sources and 19 from three or more sources, bringing the total number of the inbred accessions to 150.

DNA extraction and SSR analysis

Two seedlings of each of the 150 inbred accessions were subjected to mini-preparation of genomic DNA as per the protocol of Zheng et al [15]. They were tested with five rice SSRs (RM17, RM72, RM171, RM297 and RM1195) selected from the previous survey [14]. In the presence of within-variety heterogeneity, the plants having the major type, or the sample of the breeder's seed if no major types were observed, were considered the true type samples. In addition, one male sterile line shown to be a hybrid was replaced by the true line. Larger amount of DNAs were then extracted from the 'true-type' plants of each inbred and pools of twenty plants of each hybrid following the sodium dodecyl sulfate (SDS) method of Lu and Zheng [16]. PCR products were detected on non-denaturing polyacrylamide gels by using silver staining as suggested in our previous report [14].

The 63 'true-type' inbreds and 7 of the hybrids were assayed to screen 24 highly discriminative SSRs with two markers on each chromosome. Markers on the same chromosome should not be tightly linked, preferably, on either arms. In addition, SSRs with annealing temperature of 55°C were preferential when multiple choice were available. This selection was begun with the 58 SSRs used previously [14] and followed with additional SSRs on chromosomes for which the 58 SSRs were not sufficient.

Having all the inbreds and hybrids detected with the 24 SSRs, the authenticity of the hybrids and the parental lines were analyzed by comparing their allelic compositions. Questionable samples and their duplicates from other sources were re-assayed, and the DNA profile was modified (see the RESULTS Section 'Authenticity examination and data modification' for details). After this work was completed at the CNRRI, Hangzhou, China. DNA samples of 10 inbreds, including 2 indica varieties (Xianxiaozhan and Xiangzaoxian 31), 2 japonica varieties (Kendao 8 and Kendao 10), 3 male sterile lines or maintainer lines (II-32B, V20B and Guangzhan 63S), 2 restorer lines (Enhui 58 and Milyang 46), and 1 paternal line of two-line hybrid (9311) were selected and assayed with the 24 SSRs at the South China Agricultural University (SCAU), Guangzhou, China and the International Rice Research Institute (IRRI), Manila, the Philippines.

Data analysis

SSR bands were scored as present (1) or absent (0). Stutter patterns with multiple bands of synchronous migration were observed for most samples, but only the major band was recorded. Frequently, one or more bands of higher molecular weights that were not present in either parents were visible in the hybrid. These bands were not scored, but they were regarded as an additional evidence of the heterozygosity.

The frequency of polymorphism (FP), calculated as the proportion of the number of polymorphic varieties in the total number of variety pairs [14, 17], was used to measure the discrimination power of the SSRs. Ratio of shared DNA fragments [18] of the 24 SSRs was used to measure the genetic similarity between rice lines in the groups of maternal parents, paternal parents and F_1 hybrid rice, respectively. This analysis was carried out using the software NTSYSpc [19].

RESULTS

Selection and detection of the 24 SSRs

A set of 24 SSRs with annealing temperature of