遗 传 学 报 *Acta Genetica Sinica*, December 2006, 33 (12): 1127–1131 ISSN 0379-4172

Analysis of the Phylogenetic Relationships Among Several Species of *Gramineae* Using ACGM Markers

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Abstract: To study the transferability of rice (*Oryza sativa* L.) genome data, we used amplified consensus genetic markers to analyze the phylogenetic relationships among several species and genera in Gramineae. Ten accessions representing five grass genera (*Oryza*, *Zea*, *Setaria*, *Triticum*, and *Phyllostachys*) were used. According to the genetic distances, a cluster tree was constructed. The relationships among the five genera could be simply described as ((*Oryza* + (*Zea* + *Setaria*)) + *Triticum*) + *Phyllostachys*. The results suggest that the genetic distance between rice and maize (*Z. mays* L.) or rice and millet (*Setaria italica* L.) is closer than that between rice and wheat (*Triticum aestivum* L) or rice and bamboo.

Key words: ACGM markers; phylogenetic relationship; *Gramineae*; genome; transferability

Botstein *et al.*[1] were the first to use restriction fragment length polymorphisms as genetic markers to construct a human genetic map. Since then, many new molecular marker techniques have been developed, such as random amplified polymorphic DNA[2], amplified fragment length polymorphism[3], microsatellite or simple sequence repeat[4,5], sequence-related amplified polymorphism[6], and single-nucleotide polymorphism[7]. Good molecular marker systems are very useful tools for genetic research (e.g., constructing genetic maps, mapping genes or quantitative trait loci) and breeding (e.g., marker-assisted selection).

Draft genome sequences of two rice (*Oryza sativa* L.) cultivars, 93-11[8] and Nipponbare[9], representing *indica* and *japonica* subspecies, respectively, have been completed. A set of over 28 000 full-length cDNA sequences from Nipponbare has been released[10]. Complete sequences of chromosomes 1, 4, and 10 of Nipponbare have been published[11-13]. In addition, a tentative assembly of all chromosomes of rice has been released (The Institute of Genomic Research, TIGR; http://www.tigr.org). These data provide an opportunity to systematically search for DNA polymorphisms and to exploit DNA markers on a large scale in rice.

Gramineae is a major family among the angiosperms, consisting of five to six subfamilies, 60–80 tribes, 720–765 genera, and more than 10 000 species[14]. Most food crops, e.g., wheat (*Triticum aestivum* L.), rice, and maize (*Zea mays* L.), and forage plants belong to this family. It is impossible to sequence the genome of each species. Comparative genomics studies have shown that linear organization exists among different genomes in Gramineae[15]. Therefore, the information of rice genome could be used to study the genetic basis of other plants in Gramineae. The closer the genetic relationship of a species to rice, the better the utilization of the information.

Amplified consensus genetic marker (ACGM) is a polymerase chain reaction (PCR)-based marker

Received: 2005-11-14; Accepted: 2006-04-10

This work was supported by the China National Programs for High Technology Research and Development (863 Program) (No. 2003AA207160).

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with primers designed in conservative regions of coding sequences[16]. Therefore, ACGM would be quite useful for phylogenetic studies. In this study, we applied the ACGM markers to analyzing the phylogenetic relationships among several species and genera in Gramineae. Our purposes were to examine the feasibility of using ACGM markers exploited from rice for the phylogenetic study in Gramineae and to provide reference for the use of rice genome data to the genetic study of other species in Gramineae.

1 Materials and Methods

1.1 Plant materials

Ten accessions representing five grass genera were used (Table 1), including *japonica* rice variety Nipponbare and *indica* rice variety 93-11; two maize inbred lines F683 and F743 (bred by Zhejiang University); two millet (*Setaria italica* L.) lines 4a and 21; two wheat species *T. macha* and *T. spelta* (collected from the Plant Garden of Zhejiang University); and two bamboo species *Phyllostachys atrovaginata* C. and *P. propinqua* C. (collected from the Plant Garden of Hangzhou).

Table 1 Plant materials used in this research

No.	Plant materials	Genome
1	Oryza sativa ssp. *japonica* var. *Nipponbare*	2n=2x=24
2	*O. sativa* ssp. *indica* var. 93−11	2n=2x=24
3	*Zea mays* var. F683	2n=2x=20
4	*Z. mays* var. F743	2n=2x=20
5	*Setaria italica* var. 4a	2n=2x=18
6	*S. italica* var. No.21	2n=2x=18
7	*Triticum macha*	2n=6x=42
8	*T. spelta*	2n=6x=42
9	*Phyllostachys atrovaginata*	2n=2x=48
10	*P. propinqua*	2n=2x=48

1.2 Detection of ACGM

Thirty-eight pairs of ACGM primers[17] were used in the experiment. Modified CTAB (cetyltrimethylammonium bromide) method[18] was used to extract DNA from young leaves of each plant material. PCR was performed in a 15 μL reaction mixture containing 50 ng of template DNA, 0.5 μmol/L of each primer, 200 μmol/L of each dNTP, 1.5 mmol/L of $MgCl_2$, 0.1% of Triton X-100, 1 unit of *Taq* polymerase, and 1.5 μL of 10× PCR reaction buffer. A touchdown-PCR[19] program was used: 5 min at 94℃; 10 cycles of 30 s at 94℃, 30 s at 59℃ minus 0.3℃/cycle, 1 min at 72℃; 20 cycles of 30 s at 94℃, 30 s at 56℃, 1 min at 72℃; and 5 min at 72℃ for a final extension. For primer pairs that did not generate good amplification results, we adjusted the initial annealing temperature from 55℃ to 60℃. Each of the primer pairs was tested twice to confirm the repeatability of the observed bands in each genotype. PCR products were separated on 6% nondenaturing polyacrylamide gel electrophoresis (80 volts, 2.5 h). Gels were silver stained for visualizing DNA bands, following the procedure of Xu *et al*[20].

1.3 Statistical analysis of phylogenetic relationship

Bands were scored as 1 (presence) or 0 (absence). Genetic distances between accessions were calculated according to Nei and Li[21]. The distance matrix was used to construct the Unweighted Pair-Group Method Using Arithmetic averages (UPGMA) dendogram using the NEIGHBOR model of the PHILIP Version 3.6c[22].

2 Results

All the primers used could yield stable PCR products in each accession (Fig. 1). In general, the

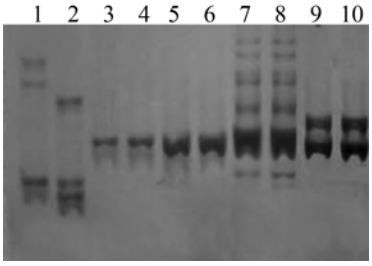

Fig. 1 PCR products obtained from primer pair GA24 in different accessions

The lane codes 1−10 are consistent with the accession codes shown in Table 1.