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SSR Marker Analysis on indica-japonica Differentiation of Natural Population of *Oryza rufipogon* in Yuanjiang, Yunnan Province

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Abstract: By using 19 pairs of primers that could identify two subspecies (indica and japonica) of cultivated rice (*Oryza sativa* L.), the indica-japonica differentiation of 56 individuals from the natural population of common wild rice (*Oryza rufipogon* Griff.) in Yuanjiang was analyzed by SSR (microsatellite DNAs, or simple sequence repeat). Of the 19 pairs of primers, 17 pairs (89.47%) could amplify only one kind of band type among all of the individuals, but primers RM251 and RM18 could amplify polymorphic band types. The bands amplified by 16 pairs of primers (84.21%) were identical to the indica-japonica diagnostic bands of relevant locus in cultivated rice, including 11 japonica-like loci and 4 indica-like loci. The bands amplified by the other three pairs of primers (RM18, RM202, RM205) were different from indica or japonica diagnostic bands of cultivated rice. The results showed that according to 19 loci analyzed, 84.21% of SSR loci in genomic DNA of common wild rice in Yuanjiang displayed indica-japonica differentiation and 13.79% of the loci still kept primitive, and most of the detected loci were homogenetic in the natural population.

Key words: *Oryza rufipogon*; indica-japonica differentiation; simple sequence repeat; population

It was generally recognized that the common wild rice (*Oryza rufipogon* Griff.) was the ancestor of cultivated rice (*O. sativa* L.) and there was indica-japonica differentiation in common wild rice before it evolved into cultivated rice [1-10]. The analysis of cpDNA, mtDNA, nuclear DNA and isozyme indicated that there was indica-japonica differentiation in most of Chinese wild rice; however, the indica-japonica differentiation of common wild rice lines was not synchronistic in cpDNA, mtDNA and nuclear DNA. Most of Chinese wild rice was approximately half japonica-like and half indica-like in cpDNA; RFLP analysis showed that common wild rice was indica-like in mtDNA; but Chinese wild rice could be divided into primitive common wild rice, japonica-like and indica-like in nuclear DNA [8, 11-12].

Yuanjiang common wild rice, which was a perennial type and homogenetic in the self-offsprings, was distributed on the hill areas in Yuanjiang County, Yunnan Province (whose altitude is 780 meters and it was well isolated from cultivated rice) and was recognized as one of the pure and primitive common wild rice populations in the world [12]. Another result showed that Yuanjiang common wild rice was a more primitive population, though it had shown potential indica-japonica differentiation [13]. The compatibilities of Yuanjiang common wild rice crossed with indica cultivar or japonica cultivar were similar, indicating that there were no indica-japonica differentiation in morphological characters but in esterase [14]. The result of analysis on DNA also showed that there was potential indica-japonica differentiation in Yuanjiang common wild rice population. Some of Yuanjiang common wild rice was japonica-like type; the others could not be clustered into indica group or japonica group but was a independent group whose morphological characters were relatively primitive, belonging to ancestral wild rice type [15]. On the other hand, mtDNA RFLP of Yuanjiang common wild rice was closer to that of common wild rice from India and Burma than that from other places of China such as Guangdong Province, Guangxi Municipality, Dongxiang of Jiangxi Province and Chaling of Hunan Province [12].

In this study, the indica-japonica differentiation of common wild rice in Yuanjiang, Yunnan was analyzed using indica-japonica diagnostic SSR primers. We hope the study would help to clarify the position of the Yuanjiang common wild rice in the origin and evolution of Chinese cultivated rice, and provide the useful information for protection and utilization of the natural resources of common wild rice and improvement of the resources of cultivated rice.

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MATERIALS AND METHODS

Materials

Common wild rice

Yuanjiang common wild rice was mainly distributed in three pools of Hongguang Farm of Yuanjiang County, Yunnan Province. In this study, 56 individuals of common wild rice were randomly collected from the three pools.

indica and japonica diagnostic SSR primers

Based on the information provided by Zhu et al [16] and Fan et al [17], a total of 19 pairs of the indica and japonica diagnostic SSR primers were synthesized by Beijing Dingguo Biotechnology Limited Company, which could distinguish indica and japonica cultivars. The primers included RM4, RM13, RM16, RM18, RM20, RM23, RM25, RM50, RM202, RM205, RM217, RM228, RM234, RM240, RM242, RM250, RM251, RM258 and RM259.

Test of indica and japonica diagnostic SSR primers

The primers above mentioned were tested with 14 typical indica cultivars (such as IR36 from IRRI) and 13 typical japonica cultivars (such as Akihikari from Japan) to determine the correct fragment size and discrimination ability. Each amplification locus was tested for the coincidence of indica or japonica type. The locus was considered as indica locus when more than 85% of indica cultivars could be amplified the correct fragment size; on the contrary, as japonica locus when more than 85% of japonica cultivars could be amplified the correct fragment size.

DNA Extraction and PCR reaction

Rice genomic DNA was extracted using the CTAB method (Jeffrey).

PCR reaction was carried out under the following conditions: the 20 μL reaction mixture consisted of 2 μL 10×PCR buffer, 2.2 μL $MgCl_2$ (25 mmol/L), 1 μL dNTP (10 mmol/L), 1 μL primer, 0.2 μL *Taq* polymerase (1 U), 1 μL DNA and 12.4 μL ddH_2O. The PCR reaction was carried out based on the information provided by Fan et al [17]. The amplified DNA was separated by electrophoresis on 3% agarose gel in TBE buffer system.

RESULTS

Test of indica-japonica diagnostic SSR primers

Nineteen pairs of SSR primers were tested with 14 typical indica cultivars and 13 typical japonica cultivars. On 3% agarose gel, a pair of primers could amplify one clear band (indica diagnostic band or japonica diagnostic band) (Fig. 1, samples 9 to 12). Indica diagnostic band was possessed in more than 85% of the indica cultivars and japonica diagnostic band was done in more than 85% of the japonica cultivars. The sizes of the fragment were consistent with those provided by Zhu et al [16] and Fan et al [17]. The result indicated that 19 pairs of SSR primers could be used as diagnostic primers to differentiate between the indica and japonica cultivars and to analyze the indica-japonica differentiation of common wild rice.

indica-japonica differentiation of the common wild rice

The genomic DNA of 56 individuals of Yuanjiang common wild rice populations was analyzed with 19 pairs of SSR primers (Table 1). Each pair of primer could amplify one band for all of the wild rice individuals, whose size was similar or same to indica or japonica band. The bands amplified by 16 pairs of primers (84.21%) were similar with the indica or japonica bands of relevant locus in cultivated rice; but the bands amplified by the other 3 pairs of primers (RM18, RM202,

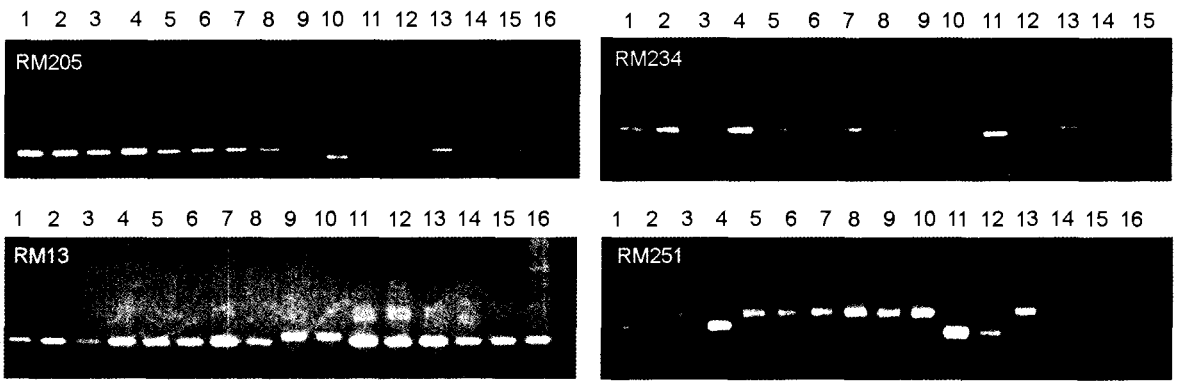

Fig. 1. Amplified products of genomic DNA of some common wild rice individuals with SSR primer RM205, RM13, RM234, and RM251.

Lane 9, IR36 (indica control cultivar); Lane 10, Nanjing 11 (indica control cultivar); Lane 11, Ballila (japonica control cultivar); Lane 12, Hexi 30 (japonica control cultivar); The other lanes are wild rice individuals.

Table 1. indica-japonica differentiation of Yuanjiang common wild rice population in 19 SSR loci.

Primer	No. of band types	Number of individuals with indica-like band	No. of individuals with japonica-like band	No. of individuals with indica-japonica mixed bands	No. of individuals with special bands
RM4	1	0	56	0	0
RM13	1	0	56	0	0
RM16	1	0	56	0	0
RM18	2	0	0	0	56
RM20	1	0	56	0	0
RM23	1	56	0	0	0
RM25	1	0	56	0	0
RM50	1	0	56	0	0
RM202	1	0	0	0	56
RM205	1	0	0	0	56
RM217	1	0	56	0	0
RM228	1	0	56	0	0
RM234	1	56	0	0	0
RM240	1	0	56	0	0
RM242	1	0	56	0	0
RM250	1	0	56	0	0
RM251	3	40	6	10	0
RM258	1	56	0	0	0
RM259	1	56	0	0	0

RM205) could not be found in cultivated rice, whose molecular weights were similar to the indica-japonica diagnostic bands of relevant locus (Fig. 1). The results showed that 84.21%of SSR loci in genomic DNA were in indica-japonica differentiation and 13.79% loci still kept primitive in Yuanjiang common wild rice population.

For 16 loci that could amplify indica-like or japonica-like bands, 15 loci only amplified an indica-like or japonica-like bands, including 11 japonica-like loci and 4 indica-like loci (Fig. 1). RM251 could amplify three type bands, i.e. indica-like, japonica-like, and indica-japonica mixed bands.

In the detected 19 loci, only RM251 and RM18 could amplify polymorphic band types among all of the individuals. It indicated that the common wild rice population was homogenetic in most of the detected loci (89.47%). RM18 could display two band types, which were different to the cultivated rice. The RM251 locus was special because the amplified products could be displayed three polymorhpic band types, i.e. indica band type, japonica band type and indica-japonica mixed band type. Of 56 individuals analyzed, 34 individuals from two pools showed indica-like band type, 22 individuals from another pool displayed three polymorhpic band types, i.e. 6 individuals showed japonica-like band type (Fig. 1, samples 1, 4), 6 individuals showed indica-like band type (Fig. 4, samples 2, 3 and 5-8) and 10 individuals showed indica-japonica mixed band type.

DISCUSSION

It is generally recognized that there was indica-japonica differentiation in common wild rice before it evolved into cultivated rice. According to the results, there was indica-japonica differentiation in 84.21% SSR loci of genomic DNA in Yuanjiang common wild rice population and 13.79% loci was different to cultivated rice and still kept primitive. As most of Chinese wild rice, Yunjiang common wild rice was japonica-like in nuclear DNA.

Most of modern common wild rice is the crossing product between the common wild rice and cultivated rice. Morishima [13] recognized that the common wild rice in Yuanjiang, Yunnan was one of the pure and primitive common wild rice populations by an on-the-spot investigation. His conclusion was supported by our results and the samples of Yunjiang common rice population was homogenetic in most of the detected loci (89.47%).

In the two polymorphic loci, RM18 was a primitive locus because the primer could display two band types different to cultivated rice. RM251 could display three polymorphic band types (indica-like type, japonica-like type and indica-japonica mixed type), whose two types (indica-like and japonica-like) could be found in cultivated rice and the third type (indica-japonica mixed type) could be a crossing product of indica-like type with japonica-like type or a primitive type but not a crossing product of common wild rice with cultivated rice, because there was no crossing evidence in other loci detected and the wild rice was far away from cultivated rice. If it was a crossing product of indica-like type with japonica-like type, then its self-offsprings would be non-homogenetic. If it was a primitive type, indica-like and japonica-like types could be

derived from the indica-japonica mixed type. It was more interesting that there was two differentiation ways in a locus. Further analysis should be done to understand the doubtful point by increasing number of samples and analyzing self-offspings of plants with indica-japonica mixed type.

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