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Evaluation of Genetic Diversity by Using Link Maker for Amylose Content of Some Iranian Local Rice Cultivars

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Abstract: Molecular markers are the best method for evaluation of genetic diversity in life. In this study 72 cultivars those indicated types *Indica* and *Japonica*, performed at Iranian Rice Research Institute, Rasht Iran. For assessment of genetic diversity, the *Waxy* gene locus link to controller trait amylose content, used from two oligo nucleotide (484 and 485). Consequently, perform PCR reactions and scoring. Rice cultivars have been screened representing current important Iran germplasm using primers flanking the *Waxy* microsatellite. According to earned information in this study, seven classes of *Waxy* microsatellite, containing $(CT)_n$ repeats were detected, ranging from n = 7 to 20. The amplified PCR products ranged from 102 to 128 bp in length and represented the $(CT)_n$ repeats of $(CT)_7$, $(CT)_8$, $(CT)_{14}$, $(CT)_{17}$, $(CT)_{18}$, $(CT)_{19}$ and $(CT)_{20}$, that according to Iranian germplasm cultivars earn seven classes for in gene locus, that regularity explained per classes of 70, 72, 78.95, 80 and 70%.

Key words: Rice, *Waxy* microsatellite, oligo nucleotide, amylose content

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the staple foods of over half the world's population and is the most important for third world people. Amylose content is the most important determining factor in eating and cooking quality of rice. Breeders pay more attention to enhancing rice quality as well as improving yield. In rice accession represent low, intermediate and high amylose lines. Many studies showed that trait control by *Waxy* locus in short arm chromosome 6 (Tan *et al*., 1999).

A polymorphic $(CT)_n$ microsatellite was identified in the 5'-untranslated region of the *Waxy* gene that has been located closely linked to the *Waxy* gene of rice (Bligh *et al*., 1995). Ayres *et al*. (1997) and Shu *et al*. (1999) identified eight *Waxy* microsatellite alleles which together explained more than 82% of the variation in AAC of non-*Waxy* rice. This same microsatellite explained 88% of the variation in AAC of 198 U.S. cultivars and breeding lines grown in different locations (Bergman *et al*., 2001); so, microsatellite can thus be used as a molecular marker by rice breeders to more rapidly develop cultivars with desirable amylose content.

Pratherpha (2003) reported sixty-eight strains belonging to two species of *Oryza* were characterized by using *Waxy* microsatellite, $(CT)_n$ repeats that are closely linked to the rice *Waxy* gene. The Polymerase Chain Reaction (PCR) was used to amplify a DNA fragment including the beginning of exon 1 and the beginning of the intron 1 of the *Waxy* gene. Polymorphism was observed between rice strains with low amylose and intermediate and high amylose content.

Bao *et al*. (2002) studied for 56 accessions of *Waxy* rice (*Oryza sativa* L.). Four $(CT)_n$ microsatellite alleles, $(CT)_{16}$, $(CT)_{17}$, $(CT)_{18}$ and $(CT)_{19}$, at the *Waxy* locus were detected in this set of *Waxy* rice, of which $(CT)_{17}$ was the most frequent. Temnykh *et al*. (2000) reported that in OSR19 gene locus showed seven alleles. Tian *et al*. (2005) using QTL analysis revealed for AC the largest proportion of variance was located on the short arm of chromosome 6, centered at RM190 (found in the *Waxy* gene). Experiments showed that amylose content governed by a single gene having a major effect in low or intermediate amylose content. The purpose of this study was the evaluation of diversity in locus of gene *Waxy* by using from molecular marker in rice of Iranian cultivar.

MATERIALS AND METHODS

A total of 72 rice cultivars used in this study whose population are listed in Table 1. All accessions were planted in investigation center rice of Iran, in late April 2006 and harvested in late August 2006.

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DNA was extracted from fresh leaves using the CTAB method (Doyle, 1991) and afterwards detected extracted DNA quality and quantity by using both two method spectrophotometer and agarose gel. Amylose content (%) was measured base on Juliano (1971) protocol by using spectrophotometer method. The primer used for amplifying microsatellite in short arm number chromosome 6 that indicated two oligo-nucleotide 484 (CTTTGTCTATCTCAAGACAC) and 485 (TTGCAGATGTTCTTCCTGATG) (Bligh *et al*., 1995). For the microsatellite assay, PCR reactions consisted of 10 μL containing 2 microgram of total rice DNA, 0.5 μL each of primer pairs, 0.48 μL $MgCl_2$, 1.2 μL dNTPs, 1 μL PCR buffer and 0.13 units Taq polymerase. The PCR reactions followed by 26 cycles of 94°C for 45 sec, 55°C for 45 sec and 72°C for 60 sec. The final extension was at 72°C for 5 min.

After dye formamide was added to PCR product being denatured at 95°C for 5 min and immediately chilled on ice, 6 μL of sample was run through a 6% poly acryl amide gel for 1 h at power (100 w) using sequencing gel Biorad. PCR products were detected by silver staining and scoring.

RESULTS AND DISCUSSION

We screened 72 rice cultivars representing current important Iran germplasm using primers flanking the *Waxy* microsatellite (Table 1).

All PCR reactions performed well and the bands scoring carefully. Value of amylose content cultivar earn in this study (Table 1). According to the International Rice Research Institute (IRRI, 1989), the non-glutinous rice represents *Waxy* (1-2%), low (10-20%), intermediate (20-25%) and high (25-30%) amylase strains. In this study, calking relationship between these values by bands earned of reaction chain polymerase.

According to earned information in this study, seven classes of *Waxy* microsatellite, containing $(CT)_n$ repeats were detected, ranging from n = 7 to 20. The varieties could be divided into seven classes on this basis, with the majority falling into four of the groups (Table 1). The amplified PCR products ranged from 102 to 128 bp in length and represented the $(CT)_n$ repeats of $(CT)_7$, $(CT)_8$, $(CT)_{14}$, $(CT)_{17}$, $(CT)_{18}$, $(CT)_{19}$ and $(CT)_{20}$, that according to cultivars germplasm Iranian earn seven classes in gene

Table 1: The rice accessions and the microsatellite in the *Waxy* gene

Code	Accession	*Waxy* $(CT)_n$	Amylose (%)
1	Ghasroldasht	8	21.30
2	Tarom amiri	19	23.69
3	Gharib	14	23.89
4	Shah pasand	18	24.14
5	Hasan saraei	14	23.39
6	Zirbandi	17	23.26
7	Salari	8	21.00
8	Rashti	8	21.96
9	Sadri	7	20.40
10	Abji boji	8	20.02
11	Qashangeh	8	12.47
12	Mohammadie chaparsar	8	27.76
13	Mosa tarom	17	19.83
14	Rashti sard	8	21.96
15	Ahlami tarom	8	22.00
16	Dasht	8	22.40
17	Domsiah	8	22.30
18	Local tarom	8	22.00
19	Anbarbo	14	20.30
20	Sdang tarom	14	24.90
21	Sang jo	14	24.01
22	Hasani	14	22.54
23	Tarom mantagheh	18	22.26
24	Hasan saraei atashgah	8	22.33
25	Hasan saraei pichideh ghalaf	8	21.05
26	Zireh	8	25.33
27	Tarom amiri	8	22.60
28	Ali kazemi	8	20.84
29	Dadras	14	20.62
30	Mohammadi chaparsar	14	23.00
31	Sorkheh	18	24.91
32	Hashemi	18	24.79
33	Champa bodar	8	24.56
34	Farsi 1	20	25.79
35	Dom sorkh	8	17.31
36	Tarom dashtor	18	23.29
37	Mianeh	8	20.86
38	Dom siah	18	26.30
39	Zaiandeh rod	14	18.52
40	Pach gharib domdar	14	23.78
41	Binam	14	21.26
42	Ieki	8	17.33
43	Marjou	14	15.25
44	Shahi	14	15.19
45	Shastak maleki	8	20.86
46	Alam sabz	18	23.87
47	Hasani rezvanshahr	14	24.61
48	Goli	20	21.07
49	Sari chaltick	14	20.10
50	Deilamani	14	24.50
51	Drodzan farsi	8	20.87
52	Bzhi	17	15.49
53	Sefid	18	12.53
54	Gharib domdar	18	23.21
55	Garm sadri	18	24.92
56	Champa bodar	8	22.25
57	Sadri domsiah	8	18.36
58	Sadri dour	17	18.55
59	Shaltok champa sar khon	17	16.30
60	Rezajou	7	14.58
61	Sadri dom sorkh	7	12.30
62	Nugoran	7	13.72
63	Gardeh lorestan	14	11.96
64	Tarom domsiah	8	20.00
65	Farsi 2	7	10.51
66	Rice champadoplon	7	13.97
67	Farsi 4	7	22.95
68	Sadri dom sefid	14	20.37
69	Mir tarom	7	13.23
70	Sangari	7	12.02
71	Anbarbo ilam	14	22.94
72	Sadri moulai	7	20.47

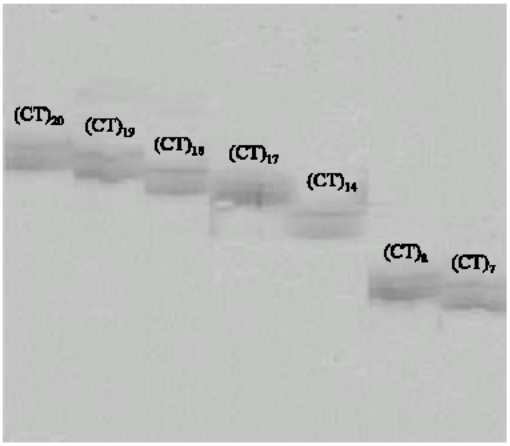

Fig. 1: Silver-stained polyacrylamide gel showing eight *wx* microsatellite alleles

locus (Fig. 1). The allele frequencies in the nonwaxy rice samples were as follows: 10 accessions had the $(CT)_7$ allele, that 70% of this cultivars included classes low amylose toward low, 25 accessions had the $(CT)_8$ allele, that 72% of this cultivars included intermediate amylose toward low, 19 accessions had the $(CT)_{14}$ allele, that 78.95% of this cultivars were included intermediate amylose, 5 accessions had the $(CT)_{17}$ allele, that 80% of this cultivars were included low amylose upgrade, 10 accessions had the $(CT)_{18}$ allele, that 70% of this cultivars were included intermediate amylose upgrade and the remaining accessions had the $(CT)_{19}$ and $(CT)_{20}$ alleles.

The $(CT)_4$, $(CT)_7$, $(CT)_9$, $(CT)_{10}$, $(CT)_{11}$, $(CT)_{12}$ and $(CT)_{13}$ repeats were not found in the Iranian rice germplasm, while these classes have been reported in US rice (Bligh *et al*., 1995; Ayres *et al*., 1997), also seven alleles said above have been reported in the Thai germplasm (Prathepha, 2003). The $(CT)_8$ class is prominent in the Iranian gene pool, 25 of the strains tested contained this class.

The $(CT)_{18}$ class was found only in traditional glutinous rice strains: both cultivated rice (*O. sativa Indica*) and wild rice (*O. nivara*). In contrast, all strains of non-glutinous rice with high amylose content (>25%) contained the $(CT)_{11}$ class. Similar results have also been reported in US rice samples (Aryes *et al*., 1997).

These strains could be differentiated by using the $(CT)_n$ classes. In the Thai strains, therefore, the *Wx* microsatellite classes are polymorphic enough to distinguish most rice strains in different amylose classes.

A microsatellite such as this which is tightly linked to the *Waxy* gene has the potential to be used by rice breeders to follow the lineage of the *Waxy* gene from a selected line without having to assess the grain quality of every single product from a cross.

However, a possible reason for the observed differences in the *Waxy* microsatellite among glutinous rice strains is that sampling may have been inadequate. In addition, the pedigrees of the rice strains analyzed in this study are not known. However, the differences may reflect a real distinction between the rice genome domesticated by people and farmers in Iran. The strains used in this study also showed a difference in frequency distribution of the *Waxy* microsatellite between different regions. Rice domestication and evolution studies can provide valuable information about genetic diversity. The domestication pattern of rice in Iran should be further investigated using molecular evidence. Therefore an extensive survey of domesticated rice germplasm for the diversity of the *Waxy* microsatellite in Iran will be useful.

Previous studies have indicated that there are seven $(CT)_n$ microsatellite alleles located 55 bp upstream of the putative 5'-leader intron splice site in the *Waxy* gene (Bligh *et al*., 1995; Ayres *et al*., 1997; Shu *et al*., 1999; Bao *et al*., 2002). These alleles could explain a high percentage of variation in AC of rice (Ayres *et al*., 1997; Shu *et al*., 1999; Bergman *et al*., 2001; Bao *et al*., 2002). According to what have been reported in germplasm US and Thai, no research that showed $(CT)_7$ classes. Same in results showed intermediate amylose surmount to low amylose and high amylose did not show except one cultivar dom siah.

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