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Lowering Grain Amylose Content in Backcross Offsprings of indica Rice Variety 057 by Molecular Marker-Assisted Selection

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Abstract: To lower the amylose content (AC) of the indica rice restorer line 057 with high AC, backcrosses were made respectively by using four indica varieties (R367, 91499, Yanhui 559, Hui 527) as low AC donor parents and 057 as the recurrent parent. A molecular marker (PCR-*Acc* I) was used to identify the genotypes (GG, TT and GT) of the waxy (*Wx*) gene. Plants with GT genotype were selected and used as female parent and crossed with 057 to advance generation. The ACs of rice grains harvested from plants with different *Wx* genotypes were measured and compared to analyze the efficiency of marker-assisted selection. The ACs of the rice grain, harvested from the plants of *Wx* genotypes GG, GT and TT, were higher than 20%, in the range of 17.7–28.5%, and less than 18%, respectively. The PCR-*Acc* I marker could be used for efficiently lowering the AC of 057 through backcrossing, and there were some influence of parental genetic background on the AC of rice grains with the same *Wx* genotype.

Key words: molecular marker-assisted selection; indica rice; amylose content; grain quality

In recent years, breeders pay more attention to enhancing rice quality as well as improving yield. Many studies showed that the eating and cooking quality of rice was largely determined by amylose content (AC), and appropriate AC was the key index of good quality [1]. Breeding experience indicated that one parent with low AC and the other parents with intermediate or high AC, or either of two parents with intermediate AC, were necessary to get indica hybrids with intermediate AC [1, 2]. Traditional rice breeding can not select the major gene conferring for AC of rice for which is governed by the major gene as well as several minor genes and environment. Whereas molecular marker-assisted selection can overcome the shortage of traditional breeding and select the major gene directly in early generation in rice breeding, which enhance the efficiency in quality breeding.

The AC of rice is controlled directly by granule-bound starch synthase (GBSS) encoded by waxy (*Wx*) gene. After the *Wx* gene was identified by Wang [3], the research on the regulation and expression of *Wx* was conducted, which showed that AC was highly associated with the splicing efficiency of *Wx* intron 1[4]. Further research [5–9] discovered that the splicing efficiency of *Wx* intron 1 was related to the first base of intron 1. If the first base of intron 1 was G in wild-type *Wx*, the *Wx* could be spliced effectively in the intron 1 and more mature mRNA was transcripted. So, much more GBSS would be translated accordingly, and AC in rice endosperm will increase. On the contrary, if the first base of intron 1 was mutated into T, *Wx* couldn't be spliced effectively in the intron 1 and less mature mRNA could be transcripted, and less GBSS would be translated accordingly, and AC in rice endosperm would decrease. Nucleotide sequence analysis of *Wx* showed that the enzyme splicing site was just the region of the G base in the intron 1 of wild-type *Wx* and its adjacent sequence, while the base was changed into T, the region couldn't be digested by *Acc* I. Therefore, the polymorphism of electrophoretic band of products from polymerase chain reaction (PCR) with appropriate primer in the upstream DNA segment of *Wx*, digested with *Acc* I, could be used to determine whether the first base of

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Wx intron 1 was G or T. Cai et al [10] used the PCR-*Acc* Ⅰ marker to screen the genotype in 63 rice cultivars collected from different areas and proved that the PCR-*Acc* Ⅰ marker could be used for the assisted selection of AC. Much fundamental research have been reported on improving the rice quality by molecular marker-assisted selection, but few of them have succeeded in developing a new variety with high grain quality.

The indica hybrid rice Xieyou 57, one of the farmers' favorite hybrid combinations, displayed strong heterosis, high yielding potential and wide adaptability, but its AC is so high that its eating and cooking quality is not good enough, owing to the fact that the AC of its male parent 057 is too high. In this study, backcrosses were made respectively by using four indica varieties as AC donor parents with good eating quality and 057 as a recurrent parent. As a consequence, the AC of 057 was reduced successfully through PCR-*Acc* I molecular marker-assisted selection.

MATERIALS AND METHODS

Genetic materials

Rice line 057, the restorer line of a three-line hybrid combination, i.e. Xieyou 57, was used as a receptor parent, and four varieties (91499, R367, Yanhui 559, Hui 527) were used as donor parents respectively in this study. 057 was bred by Rice Research Institute, Anhui Academy of Agricultural Sciences (RRI, AAAS). 91499 is a conventional indica variety with good quality bred by RRI, AAAS also. R367 is a conventional indica variety introduced from America with good quality. Yanhui 559 is an elite restorer line bred by Jiangsu Province. Hui 527 is an elite restorer line developed by Sichuan Province. The four donor parents, from different areas, had diversity of genetic background.

Methods

Total DNA extraction from leaves

DNA was extracted according to the method from Lu and Zheng [15].

PCR and Acc I digestion

PCR and *Acc* I digestion was carried out as described by Cai et al [10]. Upstream and downstream primers of PCR are 5'-GCTTCACTTCTCTGCTT GTG-3' and 5'-ATGATTTAACGAGAGTTGAA-3', which specifically amplify the 460 bp DNA segments in the upstream of *Wx* gene covering the first base of intron 1. The PCR products of GG genotype whose first base of intron 1 is G, produce 403 bp and 57 bp segments when they are digested by *Acc* I, those with TT genotype whose first base of intron 1 is T couldn't be digested by *Acc* I and shows 460 bp band in gel electrophoresis, while those with GT heterozygous genotype whose first base of intron 1 partly is G and partly is T, can be digested partly by *Acc* I and shows 460 bp and 403 bp bands in gel electrophoresis clearly. So the three genotypes can be identified in agar gel after PCR products are digested by restriction endonuclease *Acc* I.

Procedure for backcross of 057 and donor parents

Backcrosses were proceeded respectively using four donor parents and the recurrent parent 057 according to Fig. 1.

During the backcross, PCR-*Acc* I molecular marker was used to select the single plant with GT genotype to backcross with 057 partly and go on self-crossing partly. The experiment started from the summer of 2000, and we have obtained BC_6F_1 in the summer of 2003 through advancing the generation in Hainan. All the generations in this study refer to the generation of plants, for example, BC_1F_1 means the plants that grow up from the seeds of the plant backcrossed one time, and the AC of BC_1F_1 represented that of seeds harvested from the plants of BC_1F_1, and so on.

Analysis of AC

AC was analyzed as described in Standardization NY 147-88 issued by Ministry of Agriculture, the People's Republic of China.

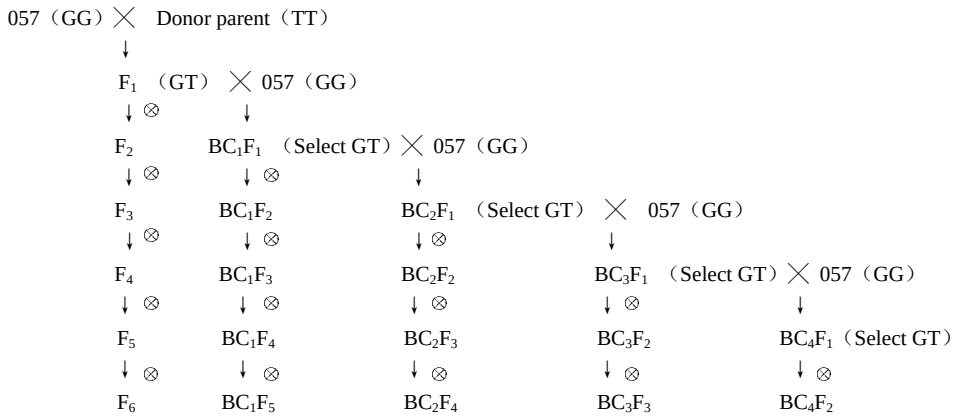

Fig. 1. Procedure for lowering the AC of 057 by PCR-*Acc* I molecular marker-assisted selection.

RESULTS

Polymorphism of PCR-*Acc* I marker and its correlation with AC

Polymorphism analyzed with PCR-*Acc* I was highlighted between the donor parents and the receptor parent 057 (Fig. 2). Xieqingzao A was the female parent of Xieyou 57, which showed 403 bp band as 057, so its genotype was GG. The four donor parents only showed 460 bp band, so their genotypes were TT. The AC of 057, Xieqingzao A, R367, 91499, Yanhui 559 and Hui 527 were 28.4%, 25.8%, 17.7%, 16.1%, 14.4% and 14.2% respectively, and the AC of 057 was the highest among them. The results showed that the AC of GG genotype was higher than that of TT genotype and the levels of AC varied from varieties (lines) even though they had the same genotype GG or TT.

PCR-*Acc* I molecular marker-assisted selection

057 was crossed and backcrossed according to the procedure in Fig. 1. Before the backcross, the plants (GT genotype) were selected with PCR-*Acc* I marker and backcrossed with 057 and the agronomic traits of the generation become more similar to 057 after each backcross. In the summer of 2003, the lines or single plants, which came from different backcross generations of different donor parents and 057, were screened with PCR-*Acc* I marker to identify their genotypes (Fig. 3 showed the genotypes of part of plants in BC_4F_2 identified by PCR-*Acc* I marker), and the following measurements of AC were carried out. The results showed as follows: When the *Wx* of 057 was replaced by that of the four donor parents respectively, the AC of 057 can be reduced significantly. The AC in TT genotype plants were all less than 18% in this test and the difference in AC between GG and TT genotypes were significant, which proved that it is credible and valid to reduce the AC by PCR-*Acc* I marker-assisted selection.

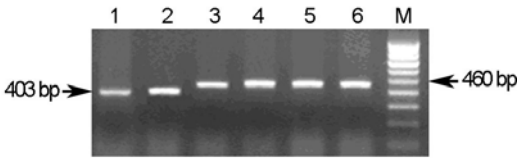

Fig. 2. PCR-*Acc* I pattern of receptor and donor parents.

Lane 1, 057; Lane 2, Xieqingzao A; Lane 3, R367; Lane 4, 91499; Lane 5, Hui 527; Lane 6, Yanhui 559; Lane M, Marker.

Influence of genetic background on amylose content

Influence of genetic background on amylose content in GG genotype plants

As listed in Table 1, there were wide variations in amylose contents even in the same genotypes, GG or TT. The *Wx* gene with GG genotype identified by PCR-*Acc* I marker should be that of 057 to a great degree, because the segment identified by the PCR-*Acc* I marker was part of the *Wx* gene and the frequency of exchange around the segment among the *Wx* genes coming from different genetic background was very low (the same to the *Wx* gene of the TT genotype). So we could deduce that the environment as well as the other genetic background except for

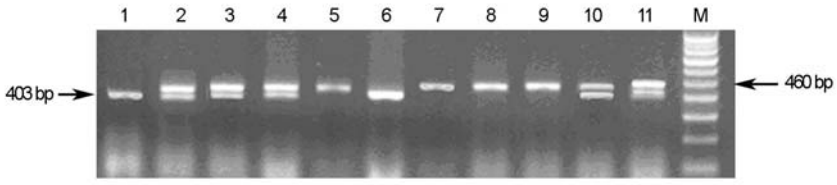

Fig. 3. PCR-*Acc* Ⅰ pattern of the improved generations(BC_4F_2) of 057.

Lanes 5, 7, 8 and 9, TT type; Lanes 1, 6, GG type; Lanes 2, 3, 4, 10 and 11, GT type; M, Marker.

Table 1. Comparison of AC of single plant or line of the three genotypes of *Wx*.

Donor parent	Geno-type	No. of plants or lines	Mean±SD (%)	Range of AC (%)
R367	GG	16	23.9 ± 2.0 a A	20.4–27.7
	GT	32	22.3 ± 2.9 b A	19.6–28.2
	TT	17	14.3 ± 1.7 c B	12.9–16.1
91499	GG	18	24.0 ± 1.7 a A	19.7–26.5
	GT	18	23.7 ± 1.7 a A	17.7–28.5
	TT	12	14.1 ± 0.9 b B	12.1–17.5
Yanhui 559	GG	17	25.4 ± 2.2 A	22.6–29.4
	GT	33	21.9 ± 2.4 B	20.0–25.1
	TT	37	13.9 ± 1.4 C	10.8–15.7
Hui 527	GG	14	25.4 ± 1.8 A	21.8–29.1
	GT	13	22.1 ± 1.5 B	17.8–25.9
	TT	40	13.3 ± 1.3 C	11.2–17.0

Data within a column followed by different uppercase and lowercase letters were significantly different at 1% and 5% levels, respectively.

Wx might influence the AC. The average AC of GG genotype in all the backcross offsprings of the four donor parents were lower than that of 057, indicating that some factors leading to the reduction of AC might exist in the four donor parents. However, a little backcross offsprings with GG genotype had higher AC than 057, with the highest percentage of 29.4%, showing heterobeltiosis.

Influence of receptor genetic background on amylose content in TT genotype plants

The data in Table 1 also showed the average AC of TT genotype in backcross offsprings of the four donor parents was lower than that of the donor parents respectively, which might be influenced by the genetic background of the receptor parent 057, whose genetic background replaced that of donor parent to a different degree. The results seemed to indicate that some factors leading to the reduction of AC might also exist in the receptor parent 057. The influence of genetic background from the recurrent parent 057 on the AC of the offsprings, which came from different donor parents and various generations, existed difference to a certain degree, according to the analysis of the AC of TT genotype in different generations (Table 2), but the influence became less different by degrees as the backcross going on. For example, the AC of offspring backcrossed 3 times was almost the same as that of offspring backcrossed 4 times with each donor parent. Variation coefficient of AC appeared to be great in the backcross generations of the most donor parents and 057 with less backcrossing times (BC_1F_5 and BC_2F_4) and small in the backcross generations with more backcrossing times relatively (Table 2). In BC_1F_5, the AC of some generations which could be regarded as 'donor parents' (e.g. R367 and 91499) was lowered sharply, which seemed to be caused by the introgression of the genetic background of 057. It suggested that there are some factors in the genetic background of 057 that can interact with some factors in the genetic background of donor parents or TT genotype of *Wx* and lead to reduced AC.

Influence of the Wx from different donor parents on the amylose content of the offsprings

The AC of the four donors with TT genotype of *Wx* was different. Table 1 showed that the higher AC of a donor was, the higher AC of its backcross offspring was, which might be attributed to the difference among *Wx* genes of the same TT genotype from different genetic background. However, this presumption can not be examined until they are under the same genetic background. The data of BC_4F_2 generation whose genetic background is similar to 057, also showed this trend in Table 2. Whether it was affected by sampling needs to be examined and the backcross with 057 is still to be going on.

Table 2. Amylose content for various backcross generations with TT genotype of the different varieties. %

Donor parent	Backcross generation					
	BC_1F_5	BC_2F_2	BC_2F_4	BC_3F_2	BC_3F_3	BC_4F_2
R367	13.1±0.8	-	13.4±1.7	14.7±0.2	15.3±1.4	15.6±1.5
91499	14.1±1.7	-	-	14.3±0.9	13.7±0.4	14.6±0.9
Yanhui 559	13.0±1.3	15.9±0.5	15.1±0.7	15.7±1.9	13.8±0.9	14.5±0.5
Hui 527	12.2±1.8	13.4±1.4	13.7±1.4	-	13.9±1.3	13.9±1.0

DISCUSSION

To lower the AC of the hybrid rice combination in breeding, the traditional measurements through determining AC in rice seeds were inefficient, and the results might be easily influenced by environment. A new method was put forward to select the major effect genes by using PCR-*Acc* I molecular marker, which correlated directly with the genotypes of *Wx* gene that controlled the synthesis of AC. So we can use the PCR-*Acc* I molecular marker to select the target genotype. In this study, different donor parents were used to backcross with 057, and the genotype of the backcross generations were identified with PCR-*Acc* I molecular marker and confirmed by the measurement of AC. At last, we have succeeded in selecting a set of stable lines with similar agronomic traits to 057 and markedly reduced AC, indicating the feasibility and advantage of PCR-*Acc* I marker.

The AC of TT genotype plant is lower than that of backcross offsprings of the donor parent with low AC and 057 (Table 1). Additionally, there are some TT genotype plants showing negative heterobeltiosis and some GG genotype plants showing heterobeltiosis. This result is consistent with Qi et al [16]. On the other hand, the genetic background of donor parent and the receptor parent had effects on AC during the backcross. There are some other factors affecting the AC besides *Wx* gene. Taken together, the factors affecting AC are complicated. The *Wx* first intron, especially the first base of the intron, affected the expression of *Wx* gene except for the nucleotide sequence of *Wx* gene, which had been proved in prior study [14,17] and in this study. It is reported that the variation of the polymorphic $(CT)_n$ microsatellite sequence in the leader region of the *Wx* gene also affected the expression of *Wx* gene [14, 17]. As a consequence, the molecular mechanism controlling the amylose synthesis and accumulation is complicated, and we should take account comprehensively of these factors in rice breeding.

REFERENCES

1 Zhang L F, Liang J, Liang C G. Prime study on the law of breeding the intermediate amylose content indica hybrid rice. *Guangdong Agric Sci*, 1991, (5): 10–12. (in Chinese)

2 Huang C W, Li R. The genetic analysis of amylose content of rice. *J South China Agric Univ*, 1990, **11**(1): 23–29. (in Chinese with English abstract)

3 Wang Z Y, Wu Z L, Xing Y Y, Zheng F Q, Guo X L, Zhang W G, Hong M M. Nucleotide sequence of rice waxy gene. *Nucl Acids Res*, 1990, **18**: 5898.

4 Wang Z Y, Zheng F Q, Shen G Z, Gao J P, Snustad D P, Li M G, Zhang J L, Hong M M. The amylose content in rice endosperm is related to the post-transcriptional regulation of the waxy gene. *Plant J*, 1995, **7**(4): 613–622.

5 Cai X L, Wang Z Y, Xing Y Y, Zhang J L, Hong M M. Aberrant splicing of intron 1 leads to the heterogeneous 5′ UTR and decreased expression of waxy gene in rice cultivars of intermediate amylose content. *Plant J*, 1998, **14**(4): 459–465.

6 Bligh H F J, Larkin P D, Roach P S, Jones C A, Fu H, Park W D. Use of alternate splice sites in granule-bound starch synthase mRNA from low-amylose rice varieties. *Plant Mol Biol*, 1998, **38**: 407–415.

7 Hiranto H Y, Eiguchi M, Sano Y. A single base change altered the regulation of the waxy gene at the posttranscriptional level during the domestication of rice. *Mol Biol Evol*, 1998, **15**(8): 978–987.

8 Isshiki M, Morino K, Nakajima M, Okagaki R J, Wessler S R, Izawa T, Shimamoto K. A naturally occurring

functional allele of the rice waxy locus has a GT to TT mutation at the 5' splice site of the first intron. *Plant J*, 1998, **15**(1): 133–138.

9 Cheng S J, Ge H F, Wang Z Y, Gu M H, Wang Z Y. Analysis of influnce of *Wx* intron 1 on gene expression in transgenic rice plant. *Acta Phytophysiol Sin*, 2001, **27**(5): 381–386. (in Chinese with English abstract)

10 Cai X L, Liu Q Q, Tang S Z, Gu M H, Wang Z Y. Development of a molecular marker for screening the rice cultivars with intermediate amylose content in *Oryza sativa* subsp. *indica*. *J Plant Physiol & Mol Biol*, 2002, **28**(2): 137–144. (in Chinese with English abstract)

11 Tan Y F, Zhang Q F. Correlation of simple sequence repeat (SSR) variants in the leader sequence of the waxy gene with amylose content of the grain in rice. *Acta Bot Sin*, 2001, **43**(2): 146–150. (in Chinese with English abstract)

12 Bao J S, Corke H, Sun M. Microsatellites in starch-synthesizing genes in relation to starch physicochemical properties in waxy rice (*Oryza sativa* L.). *Theor Appl Genet*, 2002, **105**: 898–905.

13 Zhou P H, Tan Y F, He Y Q, He Y Q, Xu C G, Zhang Q. Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. *Theor Appl Genet*, 2003, **106**: 326–331.

14 Shu Q Y, Wu D X, Xia Y W, Gao M W, McClung A, Ayres N M, Park W D. Microsatellites polymorphism on the waxy gene locus and their relationship to amylose content in indica and japonica rice, *Oryza sativa* L. *Acta Genet Sin*, 1999, **26**(4): 350–358. (in Chinese with English abstract)

15 Lu Y J, Zheng K L. A simple method for isolation of rice DNA. *Chinese J Rice Sci*, 1992, **6**(1): 47–48. (in Chinese)

16 Qi H X, Li J B, Zhang J H, Xia M Y, Wang B L. Molecular marker assisted selection on the intermediate amylose content rice breeding. The corpus of dissertation in National Plant Genetics Breeding Academic Conference, 2003. 351–354. (in Chinese)

17 Ayres N M, McClung A, Larkin P D, Blight H, Jones C, Park W. Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germplasm. *Theor Appl Genet*, 1997, **94**: 773–781.