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Molecular marker assisted selection for improvement of the eating, cooking and sensory quality of rice (*Oryza sativa* L.)

Liang Jin [a], Yan Lu [a], Yafang Shao [a], Gan Zhang [a], Peng Xiao [a], Shengquan Shen [a], Harold Corke [b], Jinsong Bao [a,*]

[a] *Institute of Nuclear Agricultural Sciences, Key Laboratory of Zhejiang Province and Chinese Ministry of Agriculture for Nuclear-Agricultural Sciences, Zhejiang University, Hua Jiachi Campus, Hangzhou 310029, PR China*
[b] *School of Biological Sciences, The University of Hong Kong, Hong Kong, PR China*

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ABSTRACT

II-32B is a key maintainer line used for hybrid rice breeding in China. However, it is of poor quality for most Chinese consumers because of its high apparent amylose content (AAC), high gelatinization temperature (GT) and non-fragrance. It is well known that the AAC, GT and fragrance traits are largely controlled by the *Wx*, starch synthase *IIa* (*SSIIa*), and fragrance (*fgr*) genes, respectively. With the aid of functional markers, we improved the quality of II-32B by introgressing the *Wx*, *SSIIa*, and *fgr* genes from Yixiang B, a fragrant maintainer line that has low AAC and low GT. The microsatellite allele $(CT)_{17}$ of *Wx*, the contiguous single nucleotide polymorphism TT allele of *SSIIa* and the 8-bp deletion allele of *fgr* were transferred to II-32B by two backcrosses and three selfings. Molecular marker assisted selection was applied in the series to select for individuals carrying *Wx*-$(CT)_{17}$, *SSIIa*-TT, and *fgr*-deletion alleles. According to the marker genotypes, seventeen homozygous lines for *Wx*-$(CT)_{17}$, *SSIIa*-TT, and *fgr* gene markers were finally selected. The improved II-32B lines were fragrant with reduced AAC and GT.

1. Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop for half the world's population. Eating, cooking, and sensory qualities that affect cooked rice palatability and consumer acceptability have received increasing research attention in rice-producing countries in recent years. Currently, there is a strong emphasis in China on improving the quality of hybrid rice varieties, especially the quality of *indica* hybrids (Zhou et al., 2003).

Apparent amylose content (AAC) is one of the most important parameters determining the eating and cooking quality. Waxy rice has near zero amylose, and is used for special foods such as desserts and snacks. High amylose cultivars (>25%) are common in *indica* rice, and are dry and fluffy on cooking, often becoming hard after cooling. Low amylose cultivars (15–20%) are soft and sticky, and include nearly all-temperate *japonica* cultivars. Intermediate amylose (20–25%) rice is soft but not sticky, and is widely preferred by most consumers. AAC is largely genetically controlled by the *Wx* locus on chromosome 6, or specifically by the amount of Wx protein present (Wang et al., 1995). The level of Wx protein is related to the level of mature *Wx* mRNA, which is related to the stage of intron 1 excision from pre-mRNA (Isshiki et al., 1998). A $(CT)_n$ microsatellite in the 5′-untranslated region of *Wx* gene is significantly associated with amylose content (Ayres et al., 1997; Bao et al., 2006a).

Gelatinization temperature (GT) is another important quality predictor in determining the cooking quality of rice. Low GT rice needs less energy input during cooking than high GT rice. GT in rice is mainly controlled by the *starch synthase IIa* (*SSIIa*) gene which is located on chromosome 6 (Bao et al., 2006b; Gao et al., 2003; Umemoto et al., 2002, 2004; Umemoto and Aoki, 2005; Waters et al., 2006). Functional single nucleotide polymorphisms (SNPs) found in the *SSIIa* closely associate with GT and amylopectin structure (Bao et al., 2006b; Umemoto et al., 2002, 2004; Umemoto and Aoki, 2005; Waters et al., 2006). Especially, two contiguous SNPs (GC/TT) in *SSIIa* have a very strong association with GT (Bao et al., 2006b; Umemoto and Aoki, 2005; Waters et al., 2006).

Rice grains with a fragrance are appealing to consumers, and the retail price of fragrant rice is higher than that of conventional rice (Shi et al., 2008). Thus, fragrance is an important trait for many breeding programs. Genetic analysis has revealed that a recessive gene (*fgr*) on chromosome 8 is responsible for rice fragrance (Jin et al., 2003; Lorieux et al., 1996). Bradbury et al. (2005a, 2005b) reported that the *badh2* gene could most likely be the *fgr* gene since it has an 8-bp deletion and three SNPs in its exon 7 compared to the

* Corresponding author. Tel.: +86 571 86971932; fax: +86 571 86971421.
E-mail address: jsbao@zju.edu.cn (J. Bao).

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functional *Badh2* gene which encodes putative betaine aldehyde dehydrogenase 2 (BADH2), and developed molecular markers for fragrance genotyping. Shi et al. (2008) found a novel null *badh2* allele (*badh2-E2*), which has a sequence identical to that of the *Badh2* allele in exon 7, but with a 7-bp deletion in exon 2. They developed the functional markers for genotyping the *badh2-E2* locus. Chen et al. (2008c) confirmed that the full-length BADH2 protein encoded by *Badh2* renders rice non-fragrant by inhibiting biosynthesis of 2-acetyl-1-pyrroline (2AP), a potent flavor component in rice fragrance.

In breeding programs for improvement of rice quality, the eating, cooking and sensory quality have to be measured on a single plant at early generations (Lestari et al., 2009); the total grains on a single plant, however, are not enough for measurements of all necessary quality traits. Moreover, the measurements are usually time and cost consuming. Selection of DNA markers, so-called marker assisted selection (MAS) has a comparative advantage over conventional breeding, and has been widely used in plant breeding programs. MAS for improvement of eating quality using microsatellites of the *Wx* gene has been conducted before. Bao (2002) and Bao et al. (2008) studied the relationship between *Wx* microsatellite alleles and starch quality parameters with offsprings derived from the crosses Longtefu A [*Wx* allele $(CT)_{11}$]/371 [*Wx* allele $(CT)_{18}$] and Jiayu 293 [*Wx* allele $(CT)_{11}$]/Lemont [*Wx* allele $(CT)_{20}$], respectively. Significant differences in AAC, gelatinization temperature (GT), gel consistency (GC) and starch paste viscosity parameters were found among different *Wx* alleles. Zhou et al. (2003) demonstrated that the *Wx* marker can be used to improve eating and cooking qualities of hybrid rice, such as Shanyou 63, a hybrid rice derived from Zhenshan 97A (a male-sterile line) and Minghui 63 (a restorer line). Liu et al. (2006) successfully transferred a single nucleotide polymorphism (*Wx*-G/T) located at the first nucleotide of the splice donor site of *Wx* intron 1 to the maintainer line Longtefu B and Zhenshan 97B with MAS, leading to good quality rice with intermediate AAC. However, all these reports only employed the *Wx* gene markers to improve the eating quality of rice by MAS. Simultaneous improvement of the eating, cooking, and sensory qualities of rice involving the *Wx*, *SSIIa* and *fgr* genes have not been reported before.

The objective of this study was to improve the eating, cooking, and sensory qualities of II-32B (a key *indica* maintainer line used in rice breeding in China) using functional marker assisted selection, i.e. transferring the *Wx*-$(CT)_{17}$/$(CT)_{17}$, *SSIIa*-TT/TT, and *fgr* genotypes from Yixiang B to II-32B by two backcrosses and three selfings.

2. Experimental

2.1. Plant materials

A total of 416 rice accessions were collected, mostly from the germplasm centers and various rice breeding programs in China and several from USDA-ARS, Rice Research Unit, USA. These rice materials were used for validation of the *fgr* gene markers.

A cross between II-32B and Yixiang B was conducted to generate the F_1 hybrids (Fig. 1) in the winter/spring season of 2005/2006 at Lingshui, Hainan province. A total of 300 F_2 lines were genotyped for *Wx*, *SSIIa*, and *fgr* genes, respectively in the winter/spring season of 2006/2007. The F_2 lines homozygous for *Wx*-$(CT)_{17}$, *SSIIa*-TT and *fgr*-deletion alleles were selected for backcrossing with II-32B as a recurrent parent. The offspring of each generation was screened to determine their *Wx*, *SSIIa*, and *fgr* genotypes. Finally, the BC_2F_2 lines homozygous for *Wx*-$(CT)_{17}$, *SSIIa*-TT, and *fgr*-deletion alleles were selected, and advanced to BC_2F_4 generation.

The field experiments were performed at Zhejiang University campus farm during late May to Late Sep. (Hangzhou season) and at

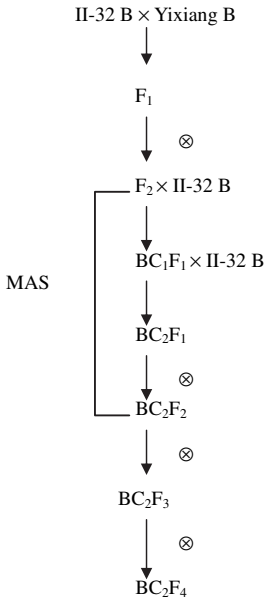

Fig. 1. The flow chart of marker assisted selection (MAS).

Lingshui, Hainan province (Hainan season) during the late Nov. to Early April. For evaluation of the agronomic traits, five to ten plants from each advanced line (BC_2F_4) were randomly selected and the major agronomic traits of the selected plants were investigated.

2.2. Markers for genotyping

Fresh leaf tissue was harvested at the seedling stage from plants grown in the field. DNA was extracted following a CTAB procedure (Doyle, 1991). The functional markers tagged for the *Wx* and *SSIIa*, and *fgr* genes were based on published results with some modifications (Table 1). The primers for *SSIIa* were modified so that the PCR products were less than those reported before (Bao et al., 2006b). The primers for amplification of the 8 bp deletion in the *fgr* gene were designed according to the *Badh2* gene sequence using the GeneTool software. Each 20 μL PCR reaction consisted of 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 0.1% Triton X 100, 2 mM $MgCl_2$, 0.1 mM dNTPs, 200 nM primers, 1 unit of *Taq* polymerase, and 50 ng of genomic DNA. All amplifications were performed on a MG96G thermal cycler (Hangzhou LongGene Scientific Instruments Co. Ltd., Hangzhou, China) under the following conditions: (1) pre-denature at 95 °C for 5 min; (2) 35 cycles of run, each followed by denature at 95 °C for 1 min, anneal at 55 °C for 45 s and extension at 72 °C for 1 min; (3) final extension at 72 °C for 10 min. The PCR products for *SSIIa* marker were separated by electrophoresis in 1.5% agarose in 0.5× tris–borate EDTA (TBE) buffer, and the others in 8% denaturing polyacrylamide gel (PAGE) with 3.4% cross-linker (ratio of bis-acrylamide to acrylamide) in 1.0× TBE buffer.

Table 1
Details of PCR markers for marker assisted selection (MAS).

Gene marker	Forward primer (5′→3′)	Reverse primer (5′→3′)	Reference
Wx	484: ctttgtctatctcaagacac	485: ttgcagatgttcttcctgatg	Ayres et al. (1997)
SSIIa	NF1: cgaggcgcagcacaacag F22: caaggagagctggaggggggc	NR1: ggccgtgcagatcttaaccat R21: acatgccgcgcacctggaaa	Bao et al., (2006b) and this study
fgr	1F: ggagcttgctgatgtgtgtaaa 2F: cctctgcttctgcctctgat	1R: ggaaacaaaccttaaccatag 2R: gattgcgcggaggtacttg	this study Shi et al., (2008)