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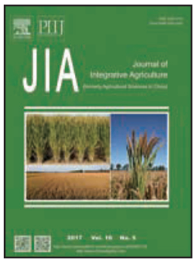

RESEARCH ARTICLE

Discussion on strategy of grain quality improvement for super high yielding *japonica* rice in Northeast China

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Abstract

japonica rice is mainly distributed in Northeast China and accounts for 44.6% of the total cultivated area of *japonica* rice in China. The comprehensive using of inter-subspecies heterosis is the main breeding mode of super *japonica* rice varieties in this region. Improving rice quality at relative high yielding level is the current research focus. Performing crosses between *indica* and *japonica* lines allows for the recombination of regulatory genes and genetic backgrounds, leading to complicated genetic rice quality characteristics, which can be used to explore patterns of quality improvement. In the present study, we utilize recombinant inbred lines (RILs) derived from *indica-japonica* hybridization to analyze the effect factors of rice quality derived from genetic factors, which contain both regulatory genes concerning rice quality and genetic backgrounds' random introduction frequency coming from *indica* (Di value), and the improvement strategy was further discussed. The regulatory genes involved in amylase content (*Wx*) and nitrogen utilization efficiency (*NRT1.1B*) were the major factors affecting the amylose content (AC) and protein content (PC) in RILs, respectively. Both the Di value and the major grain width gene (*GS5*) had regulatory effects on milled rice width (MRW) in RILs, and their interaction explained the major variance of MRW in the RILs. With the mediation of MRW and chalkiness degree (C), Di value had a further impact on head rice rate (HR), which was relatively poor when the Di value was over 40%. In Northeast China, the Di value should be lowered by backcrossing or multiple crosses during the breeding of *indica-japonica* hybridization to maintain the whole better HR and further to emphasize the use of favorable genes in individual selection.

Keywords: super rice, *indica-japonica* hybridization, genetic dissection, quality improvement

1. Introduction

indica and *japonica* are two subspecies in Asia (Li *et al*. 2004; Sun *et al*. 2012), and has formed respectively unique inherent characteristics owning to the long-term evolutionary process (Cheng *et al*. 2007; Xiong *et al*. 2010; Sun *et al*. 2012). Therefore, *indica-japonica* hybridization has become an important rice-breeding method in China to integrate desired subspecies traits and use inter-subspecies heterosis (Chen and Xu

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2007). The crossing of *indica* and *japonica* was performed earlier in northeast rice growing areas, and the unit yield was significantly improved. Currently, optimizing rice quality while maintaining a high yield is a key research focus.

Substantial differences exist in the quality traits between *indica* and *japonica*. The grain is short and round in *japonica*, but slender in *indica*. In addition, the rice milled quality, chalkiness degree (C) and gel consistency (GC) of *japonica* are more desired than those of *indica*, its amylose content (AC), gelatinization temperature (GT) and protein content (PC) are lower than those of *indica* rice (Yang *et al*. 2000; Cheng *et al*. 2002; Olsen *et al*. 2006). With the wide application of *indica* and *japonica* hybrids, the variation pattern in offspring has become a hot research topic. Wu *et al*. (2003) showed that the differentiation degree of *indica* and *japonica* rice was significantly correlated and closely linked to the GC, GT, PC, and crude fat content. Mao *et al*. (2010) determined that the interactions of subspecies characteristics and ecosystem environments had certain effects on the appearance and milled quality of rice (Mao *et al*. 2010; Gao *et al*. 2013; Zhang *et al*. 2015). The above studies had put emphasis on phenotypic analysis, the analysis on concerning gene was missing. In the northeast rice growing area of China, the variation patterns of rice quality in the offspring of *indica* and *japonica* crosses need to be further determined.

Many genes involved in rice quality have been successfully cloned, such as *GS5*, *GW5*, *GS3*, *GW8*, *Wx*, *SSII-3*, and *NRT1.1B* (Cameron and Wang 2005; Fan *et al*. 2006; Song *et al*. 2007; Shomura *et al*. 2008; Wang *et al*. 2012; Hu *et al*. 2015), which provided a new approach for the above study. *GS5*, as the major quantitative trait locus (QTLs) controlling grain width, was successfully cloned in 2011, with a 6-bp insertion/deletion in the coding region resulting in two alleles, Zhenshan 97 (*GS5*) and H94 (*gs5*). The *GS5* allele from wide grained rice is a dominant QTL, which can significantly improve the grain width and grain plumpness to further increase the yield (Song *et al*. 2007). The major regulatory gene for amylose content is the waxy gene (*Wx*). The G-T single nucleotide polymorphism at the first base of the splice donor in the first intron produces two mainstream alleles, Wx^a (prevalent in *indica* rice) and Wx^b (*japonica* rice), which determine AC variation between *indica* and *japonica* (Cameron *et al*. 2005; Crofts *et al*. 2012). The successful cloning of the major QTL responsible for the regulation of the nitrogen utilization rate, *NRT1.1B*, was considered as "a great discovery". Two alleles of *NRT1.1B* are present in *indica* and *japonica*, respectively. The *indica* version of the gene significantly improves the nitrogen utilization rate by increasing the absorption of nitrate nitrogen (Hu *et al*. 2015), but its effect on PC has not been reported.

The majority of loci affecting rice quality are QTLs, and the differences in their explained phenotypic variation were relatively great under different genetic backgrounds (Cui *et al*. 2003; Huang *et al*. 2009; Xu *et al*. 2014). Additionally, the number of confirmed regulatory genes is extremely limited. Therefore, in addition to regulatory genes, the effects of genetic backgrounds on rice quality should be considered. There is a wide range of evaluation indicators for rice quality, and there exist a subordinate relationship. Therefore, evaluating rice quality with simple and effective indicators is helpful for the determination and implementation of the breeding objectives. The rice milling quality includes the brown rice rate (BR), milled rice rate (MR) and head rice rate (HR), among which the HR is the ultimate goal of processing quality owning to consideration of the commodity circulation characteristics of the rice and its applications. The chalk traits include the chalky grain percentage, chalky area and C, with the latter being the comprehensive evaluation indicator of grain chalk trait. In the present study, the effects of genetic factors on rice milled quality were analyzed using MRW, C and HR, along with the genetic investigation of the core nutritional indicators, AC and PC, to provide a theoretical basis for the quality improvement of super rice breeding in Northeast China.

2. Materials and methods

2.1. Plant materials

The 125 recombinant inbred lines (RILs, F_{15} and F_{16}) from Akihikari (AKI)×Qishanzan (QSZ) were planted for two growing seasons during 2012–2013 in a field trial at the Liaoning Institute of Saline-Alkali Land Utilization, located in Panjin, China (41.07°N, 122.03°E). Akihikari is a typical *japonica* originated in Japan, and Qishanzan is a typical *indica* originated in Guangdong, China. The seedlings were cultivated using factory technology. The RILs were arranged in a randomized block design with three replications, each repetition was planted in three rows, 10 plants per row, with a plant spacing of 30 cm×13.3 cm. The RILs were planted on 15 April, 2012 and 20 April, 2013 in consecutive growing seasons, and transplanted on 20 May, 2012 and 25 May, 2013, respectively. N (225 kg ha^{-1}), P_2O_5 (105 kg ha^{-1}), and K_2O (45 kg ha^{-1}) were applied, and the other cultivation and management practices were the same as in the local production field.

2.2. Detection of rice quality

The tested rice grains were prepared after stored 3 mon