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RESEARCH ARTICLE

Identification of QTLs for seed storability in rice under natural aging conditions using two RILs with the same parent Shennong 265

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Abstract

Seed storability (SS) is an important trait for agronomic production and germplasm preservation in rice (*Oryza sativa* L.). Quantitative trait locus (QTL) for seed storability in three storage periods was identified using two sets of recombinant inbred lines (RILs) derived from the crosses with a common female parent Shennong 265 (SN265). Ten QTLs for seed storability were detected on chromosomes 1, 2, 3, 4, 6, 8, and 12 in SL-RILs (SN265/Lijiangxingtuanheigui (LTH)), and a total of 12 QTLs were identified on chromosomes 2, 3, 4, 6, 9, and 10 in SH-RILs (SN265/Luhui 99 (LH99)) in different storage periods. Among these QTLs, five major QTLs were identified in more than one storage period. The *qSS3-1*, *qSS3-2*, *qSS12-1*, and *qSS12-2* were detected in SL-RILs. Similarly, *qSS2-2*, *qSS2-3*, *qSS6-2*, *qSS6-3*, *qSS6-4*, *qSS9-1*, and *qSS9-2* were detected in SH-RILs. In addition, the maximum phenotypic variation was derived from the *qSS6-1* and *qSS9-2*, explaining 53.58 and 29.09%, respectively, while *qSS6-1* was a new stable QTL for seed storability. These results provide an opportunity for pyramiding and map-based cloning major QTLs for seed storability in rice.

Keywords: rice, recombinant inbred lines, natural aging, seed storability, quantitative trait locus

1. Introduction

Rice (*Oryza sativa* L.) is one of the most widely distributed dietary crops in the world, accounting for more than 50% of the staple calories intake (Khush 2005; Ashikari and Matsuoka 2006; Xing and Zhang 2010). Seed storability is a major constraint in rice storability and germplasm conservation, as it is a key factor in determining storability and reproductive frequency (Agacka-Mołdoch *et al.* 2015). Strong storability reduces the risk of rapid seed deterioration, which is a serious problem for rice production in tropical Asia. During warehousing period, seeds begin to deteriorate, lose vigor, and as a result, become more sensitive to stresses during germination, and ultimately die (Lin *et al*. 2015a). Many factors, such as inherited character, seed size, harvesting time, dormancy, and storage conditions, are reported to be related to storability (Yamauchi and Winn 1996; Cheng *et al*. 2012; Lin *et al*. 2015b).

The seed germination capability is affected not only by environments (e.g., storage condition) but also by genetic factors (Bewley and Black 1994; Miura *et al*. 2002; Clerkx

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et al. 2004). Previous studies have shown that the variation in seed dormancy is controlled by multiple genetic factors, known as quantitative trait loci (QTLs) (Gan *et al*. 2005; Jiang *et al*. 2005, 2011; Zeng *et al*. 2006; Xue *et al*. 2008; Wang *et al*. 2010; Li *et al*. 2012; Ngo *et al*. 2014; Zhang *et al*. 2014). Over the last decades, numerous QTLs associated with the storability have been identified using many mapping populations and methods. Miura *et al*. (2002) identified three QTLs related to seed longevity on chromosomes 2, 4 and 9, using a backcross population of Nipponbare/Kasalath under artificial aging method. Three QTLs for seed storability were mapped on chromosomes 9, 11 and 12 (Zeng *et al*. 2006). Xue *et al*. (2008) detected three QTLs associated with seed storability on chromosomes 1, 3 and 9 using recombinant inbred lines (RILs) derived from the cross IR24/Asominori. Sasaki *et al*. (2005) detected four QTLs for seed longevity under natural aging condition. Jiang *et al*. (2011) found seven QTLs associated with seed storability using two sets of RILs by employing similar aging treatments. These QTLs associated with seed storability were useful for better understanding the molecular mechanism. However, QTLs detected under natural and artificial aging treatments were significantly different, and hence, the available information applied by breeders was not entirely consistent. Artificial aging with elevated ambient temperature and relative humidity (RH) was utilized to rapidly assess seed storability of rice, which abound with false positive and distortion phenomenon. Natural aging was intrinsically time-consuming, thus not suitable for rapid screening of a large amount of samples and germplasm, but it was similar to normal process of rice storage. In addition, the success of QTL mapping was affected by genetic background, population size, map density, and environment, among which, population genetic background (e.g., *indica-japonica* and *japonica-japonica*), was the most important (Wan *et al*. 2006; Song *et al*. 2007; Huang *et al*. 2013). Despite it is a powerful tool to use comparative analysis to process quantities of QTL data, QTLs were obtained from different experimental studies, with varying mapping populations, environments and statistical methods, which hindered the alignment of multiple QTL in different data sets (Xing and Zhang 2010; Deng *et al*. 2015).

In this study, two RIL populations with diverse seed storability and genetic backgrounds were used to detect QTLs for seed storability traits across three environments. The two sets of RIL populations were derived from crosses between a common female parent, super rice variety Shennong 265 (SN265) which was a super high yield *japonica* cultivar of strong storability in the northern China and two male parents, *japonica* cultivar Lijiangxingtuanheigui (LTH) and *indica* cultivar Luhui 99 (LH99). Among the two male parents, the former (LTH) was a high altitude area *japonica* cultivar, having a significant difference with the temperate *japonica* rice (SN265) in seed storability, and the latter (LH99) was an important restorer line for *indica* hybrid rice cross. The SL-RILs (*japonica*/*japonica*) and SH-RILs (*indica*/*japonica*) populations had significant differences in both seed storability and genetic background. Therefore, we used them as research materials in order to discuss their influence on the QTLs of seed storability and detect novel QTLs. The objectives of this study were (1) to detect QTLs for seed storability; (2) to compare the number and distribution of QTLs in different natural aging conditions; and (3) to perform a systematic analysis and confirm whether genetic background affects rice seed storability.

2. Materials and methods

2.1. Plant materials

Two sets of RIL populations, developed from the crosses SN265×LTH (RILs-JJ (RILs from the cross *japonica*/*japonica*), SL-RILs) and SN265×LH99 (RILs-IJ (RILs from the cross *indica*/*japonica*), SH-RILs), consisting of 96 and 158 lines, respectively, were used in this study (Jiang *et al*. 2012; Zhang *et al*. 2014). Parental accessions (SN265, LTH and LH99) and the RIL populations (SL-RILs and SH-RILs) were both planted in 2012, 2013 and 2014, respectively. The field management followed normal agricultural practice. Harvested seeds were stored at room temperature for 1-, 2- and 3-year, respectively.

2.2. Evaluation of seed storability

Germination rate was used to determine the degrees of seed storability (Li *et al*. 2012). Firstly, 200 full grains were randomly selected from each line under each storage year, then the wet cleaning process was used to eliminate immature grains. The remaining grains were disinfected with 70% alcohol for 1 h and rinsed for 2–3 times. Finally, three replications of 50 treated seeds of each line were placed on doubled sheets of moistened filter paper in 9 cm Petri dishes. Germination rates were recorded after 7 d in the biochemical incubator, which was set for 14 h, at 26°C on the day while for 10 h, at 24°C at night.

2.3. QTL and statistical analysis

Linkage maps were constructed using MAPMAKER/EXP 3.0 (Lincoln *et al*. 1993). Polymorphic simple sequence repeat (SSR) and insertion/deletion (InDel) markers distributed on all 12 chromosomes were used for genotyping the two RILs: 121 markers for the SL-RIL populations (Jiang *et al*. 2012)