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RESEARCH ARTICLE

Genetic mapping of quantitative trait loci for the stigma exsertion rate in rice (*Oryza sativa* L.)

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Abstract

The efficiency of hybrid rice seed production can be improved by increasing the percentage of exserted stigmas. To identify quantitative trait loci (QTLs) for this trait, we conducted QTL mapping using 75 chromosome segment substitution lines (CSSLs) developed from a cross between the donor parent, Xieqingzao B (XQZB), a maintainer line which has high stigma exsertion and the recurrent parent, Zhonghui 9308 (ZH9308), a restorer line which has low stigma exsertion. A total of nine QTLs (*qSSE5*, *qSSE10*, *qSSE11*, *qDSE10*, *qDSE11*, *qTSE5*, *qTSE6*, *qTSE10*, and *qTSE11*) for single stigma exsertion (SSE), dual stigma exsertion (DSE) and total stigma exsertion (TSE) were assessed in two environments (Hainan and Zhejiang). Six of these QTLs (*qSSE10*, *qSSE11*, *qDSE10*, *qDSE11*, *qTSE10*, and *qTSE11*) were found in both environments, while one QTL (*qTSE6)* was found in only Hainan, and two QTLs (*qSSE5* and *qTSE5*) were found in only Zhejiang. The *qSSE10*, *qSSE11*, *qDSE10*, *qDSE11*, *qTSE6*, *qTSE10*, and *qTSE11* alleles, which are derived from the parent XQZB, exhibited a positive additive effect. In contrast, the *qSSE5* and *qTSE5* alleles, which are derived from the parent ZH9308, exhibited a negative additive effect. The SSE, DSE and TSE traits were significantly correlated with each other in an environmentally dependent manner. These results indicated that the lines showing higher values for SSE were more likely to exhibit increased values for DSE, which would ultimately increase TSE. To evaluate the advantage of exserted stigmas for cross-pollination, single, dual and total stigma exsertion should be considered separately in future attempts at genetic improvement to achieve increased production of rice hybrid seeds. This study also provides information for fine mapping, gene cloning and particularly marker-assisted selection (MAS), on the latter and with an emphasis the phenotypic effects and implications of the QTLs for practical use in hybrid rice breeding.

Keywords: hybrid rice, stigma exsertion rate, QTL mapping

1. Introduction

Rice (*Oryza sativa* L.) is the most important staple food for billions of people worldwide. Hybrid rice breeding, which was initiated in China in the 1970s, has led to great improvements in rice productivity (Virmani 1994). Breeding

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high-yielding hybrid rice is one promising potential solution for addressing food shortages caused by a marked increase in the global population. Virmani and Athwal (1973) hypothesized that stigma exsertion is controlled by polygenes in rice species, based on the continuous phenotypic variation in this trait and the fact that continuous phenotypic variation is broadly observed. Traditional selection for stigma exsertion based on direct observation in the field is inefficient because this trait is complex and is easily affected by environmental factors. Recent progress in DNA marker technology and the construction of linkage maps have enabled the detection of quantitative trait loci (QTLs) controlling complex genetic traits (e.g., stigma exsertion) that are applicable to actual breeding. QTL analysis for the percentage of exserted stigmas has been conducted using segregating populations. During the past three decades, hybrid rice has made great contributions to increase in rice yields (Virmani 1996; Yuan 2004). However, a major unresolved issue is how to improve the efficiency of hybrid seed production. The stigma exsertion rate is a key contributing factor in hybrid seed production.

A high stigma exsertion rate is expected to cause more pollen to be trapped and improve cross-pollination and the efficiency of hybrid seed production in rice. Exserted stigmas remain viable for approximately 4 days and continue to accept pollination (Long and Shu 2000; Tian *et al*. 2004). Recent progress in DNA marker techniques has been providing genetic information about stigma exsertion that is applicable to actual breeding. For example, nine QTLs for the frequency of stigma exsertion were detected in the recombinant inbred lines (RILs) derived from a cross between a *japonica* variety, Asominori, and an *indica* variety, IR24 (Yamamoto *et al*. 2003), and two QTLs for the rate of exserted stigmas were identified in RILs derived from a cross between an *indica* variety, Pei-Kuh, and a wild rice, W1944 (*Oryza rufipogon* Griff.) (Uga *et al*. 2003). However, whether these QTLs function effectively in the genetic background of current candidates for serving as the maternal parent in hybrid rice is still uncertain.

Lou *et al*. (2014) used an F_2 population derived from two *indica* cytoplasmic male sterility (CMS) maintainers, Huhan1B and K17B, to map QTLs that influence stigma exsertion in rice. QTLs that influence single stigma exsertion (SSE), dual stigma exsertion (DSE) and total stigma exsertion (TSE) rates were detected using a linkage map of 92 SSR markers. A total of 1, 3 and 1 QTLs were detected for SSE, DSE and TSE on chromosomes 5, 6 and 7, respectively. Yan *et al*. (2009) conducted an association mapping experiment on the stigma exsertion rate in a minicore of 90 accessions with 109 DNA markers and found that the simple sequence repeat (SSR) marker RM5 played a major role and was highly correlated with both the dual and single stigma exsertion rates. Using two segregating populations derived from the *indica* cultivar Guangluai 4 and the wild cultivar W1943 (*O. rufipogon*), Li *et al*. (2014) found that the stigma exsertion rate is a complex quantitative trait that is governed by polygenes. Furthermore, they performed genetic mapping and validated QTLs for the stigma exsertion rate in rice. To clarify the genetic basis of the stigma exsertion rate, a recombinant inbred line population was developed from two superior rice cultivars, Zhongguoxiangdao (ZX) and Chuanxiang 29B (CX29B), and a total of 11 QTLs for the stigma exsertion rate were detected over 2 years. Two QTLs on chromosome 1 and four QTLs on chromosome 6 formed two QTL cluster regions, *qSe1* and *qSe6*, respectively.

In this study, three flowering-related traits, including SSE, DSE and TSE, were quantified in two different environments (Hainan and Zhejiang) in a chromosome segment substitution lines (CSSL) population. Here, we identified QTLs influencing the stigma exsertion rate. The results will be useful for facilitating the development of male sterile lines with a high stigma exsertion rate, which would be of great value in hybrid rice seed production. The phenotypic effects and the implication of QTLs for practical use in rice breeding are discussed.

2. Materials and methods

2.1. Plant materials

The CSSL populations consisted 75 lines that were developed from a cross between Xieqingzao B (XQZB) and Zhonghui 9308 (ZH9308), followed by a series of self-crosses and backcrosses. XQZB is a high percentage stigma exsertion maintainer line, and ZH9308 is a low percentage stigma exsertion restorer line. The scheme for population development is illustrated in Fig. 1.

2.2. Field experiments

The CSSL populations and their parents were cultivated in Lingshui, Hainan Province, China on December 20, 2014 and in Fuyang field of the China National Rice Research Institute, Hangzhou, Zhejiang Province, China on June 15, 2015. Progenies were established in six rows with eight plants per row with spacing of 30 cm×20 cm within rows and inter-rows, respectively. Standard cultivation practices were followed.

2.3. Trait evaluation

The stigma exsertion rate was subdivided into three traits: SSE, DSE, and TSE. At 5–7 days after heading, five normal panicles were sampled from different plants of each line and