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SHORT COMMUNICATION

Fine mapping of a novel *wax crystal-sparse leaf3* gene in rice

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Abstract

Cuticular wax plays an important role in protecting plants against water loss and pathogen infection and in the adaptations to environmental stresses. The genetic mechanism of the biosynthesis and accumulation of epicuticular wax in rice remains largely unknown. Here, we show a spontaneous mutant displaying wax crystal-sparse leaves and decreased content of epicuticular wax that was derived from the cytoplasmic male sterility (CMS) restorer line Zhenhui 714. Compared with the wild type Zhenhui 714, the mutant exhibited hydrophilic features on leaf surface and more sensitivity to drought stress. The mutation also caused lower grain number per panicle and thousand grain weight, leading to the decline of yield. Genetic analysis indicates that the mutation is controlled by a single recessive gene, named *wax crystal-sparse leaf3* (*wsl3*). Using segregation populations derived from crosses of mutant/Zhendao 88 and mutant/Wuyujing 3, respectively, the *wsl3* gene was fine-mapped to a 110-kb region between markers c3-16 and c3-22 on chromosome 3. According to the rice reference genome and gene analysis, we conclude that a novel gene/mechanism involved in regulation of rice cuticular wax formation.

Keywords: rice, cuticular wax, wax crystal-sparse, fine mapping

1. Introduction

The cuticular wax, which forms a protective barrier against water loss, pathogen infection, the external environment, is a complex mixture and consists of very-long-chain fatty acid (VLCFA; >18 carbons long) and the secondary alkanes, aldehydes, ketones, alcohols, and esters derived from VLCFA (Kunst *et al*. 2009; Javelle *et al*. 2011; Yeats and Rose 2013). To the present time, only a few genes associated with the biosynthesis of cuticular waxes have been characterized in rice (Jung *et al*. 2006; Yu *et al*. 2008; Islam *et al*. 2009; Qin *et al*. 2011; Mao *et al*. 2012; Wang *et al*. 2012; Zhou *et al*. 2013, 2015; Zhu and Xiong 2013). For example, *Wax Crystal-sparse Leaf1* (*WSL1*), which is located on chromosome 6, encodes a β-ketoacyl-CoA synthase (KCS) family protein. *WSL1* catalyzes the elongation of VLCFAs and participates in a wide range of rice growth and developmental processes beyond biosynthesis of cuticular waxes (Yu *et al*. 2008). *WSL2* (also designated as *OsGL1-1*) was isolated by using map-based cloning and found to be located on chromosome 9 (Mao *et al*. 2012). Analysis of the overall composition of the wax revealed that

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the *wsl2* mutant produces a substantially reduced quantity of C22–C32 fatty acids, suggesting that *WSL2* functions in the elongation of VLCFA (Mao *et al*. 2012). Totally, the genetic mechanism of the biosynthesis and accumulation of cuticular waxes in rice remains largely unknown.

In this study, we identified a spontaneous mutant from the cytoplasmic male sterility (CMS) restorer line Zhenhui 714 (R714) that showed hydrophilic leaf blade phenotype, caused by epidermal wax crystal sparse. This mutant carries much decreased cuticular wax on leaves and reduced grain yield compared to wild type. We further report the fine-mapping of the *wax crystal-sparse leaf3* (*wsl3*) gene and found that *WSL3* is a novel gene that regulates rice epidermal wax formation.

2. Materials and methods

2.1. Plant materials

A plant which shows light green leaves and easily water hydrophilic was found in the propagation population of R714, an *indica* restorer line developed by Zhenjiang Agricultural Research Institute, China. After several generations of self-pollination, we obtained its homozygous line and named it rice cuticle wax-deficient mutant. The mutant was crossed with its wild type R714 parent for analyzing its heredity mode. For mapping the *wsl3* gene, the mutant was crossed with two elite *japonica* varieties, ZD88 and WYJ3, respectively, to construct two independent F_2 segregating populations. A total of 1 353 F_2 individuals with mutant phenotype were selected and used for fine genetic mapping of *WSL3* at the 4-leaf stage.

2.2. Phenotype data collection

For drought stress test, 2-wk-old seedlings of mutant and wild type were subject to 12 d of drought then re-watered and allowed to recover for 7 d. The survival rates were recorded respectively. At the heading stage, the chlorophyll content of the flag leaf was measured using a chlorophyll meter (SPAD-502 plus, Konika Minolta, Japan) in addition to measuring the water loss rate. To analyze the water loss rate, we incubated the detached fresh leaves from wild type (WT) and the mutant in an air oven (28°C) for 7 h then measured the water loss (described as relative water content, RWC). The method of the cuticle wax extract was conducted according to that described by Zhou *et al*. (2007).

Agronomical traits, including plant height (PH), heading date (HD), panicle length (PL), panicle number (PN), grain number per panicle (GNP), seed setting rate (SESR), thousand grain weight (TGW), and length/width of flag leaves (L/WFL), were measured at the mature stage. For each trait, 10 plants were measured. Statistical analysis of the agronomic trait measurements was performed using the Student's *t* test program packaged in the SPSS ver. 17.0.

2.3. Analysis by scanning electron microscopy (SEM)

For examination of epidermal wax crystals, samples at the 4-leaf and maturing stages were taken from fully-expanded leaves of WT and *wsl3* plants and dried in the air respectively (Jenks 1992). The material was mounted on aluminum stubs and sputter-coated with gold palladium in six 30 s bursts (E-1010 sputter-coater, Hitachi, Tokyo, Japan) in preparation for scanning electron microscopy (SEM) (Hitachi S 3400N, Japan).

2.4. DNA extraction and marker analysis

Genomic DNA was isolated from fresh-frozen leaves of each plant using the hexadecyl trimethyl ammonium bromide (CTAB) method. The PCR reaction mixture and procedure was conducted according to that described by McCouch *et al*. (1988). The PCR products were separated on 6% polyacrylamide gels, and the separated DNA fragments were then silver-stained for visualization.

2.5. Gene mapping of *wsl3*

Two DNA pools, based on the phenotype, were constructed for the bulk segregant analysis using the method described before (Michelmore *et al*. 1991). Simple sequence repeat (SSR) markers were employed to detect the polymorphism between the mutant and each of the two parents, ZD88 and WYJ3. The polymorphic markers were further used to detect the polymorphism between these two pools. Based on the principle of the bulk segregant analysis method, the markers that showed polymorphism between the two DNA pools were putatively considered linked to the *wsl3* gene, which was further confirmed by analyzing the F_2 segregation population.

The primer sequences of SSR markers were downloaded from http://www.gramene.org/. To fine map the *WSL3* gene, the insertion-deletion (InDel) and cleaved amplified polymorphic sequence (CAPS) markers were developed according to the public genome sequences of the *japonica* variety Nipponbare (http://www.rgp.dna.affrc.go.jp/) and the *indica* variety 93-11 (http://www.genomics.org.cn/). The primers were designed by Primer Premier 5.0 according to the criterion described before (Yan *et al*. 2009).

2.6. q-PCR analysis

Total RNA was extracted from leaves of mutant and WT