

Genetic Analysis and Mapping of a Novel Short Root Hair Gene *OsSRH3* in Rice

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Abstract: Root hair is an important organ for uptaking nutrients and water in plant. A rice (*Oryza sativa* L.) mutant, *ossrh3*, with short root hairs was isolated from a T-DNA insertion mutant library of rice on Zhonghua 11 background. The elongation of root hairs in the mutant was severely impaired. Beside, some other traits were also affected, such as plant height, primary root length, lateral root length, and number of lateral roots. Genetic analysis indicated that the mutated phenotype was controlled by a single recessive nuclear gene. An F₂ population was developed by crossing the mutant *ossrh3* with an *indica* cultivar, Kasalath, to map the gene. Using simple sequence repeat (SSR) markers and newly designed sequence-tagged site (STS) markers, the target gene, *OsSRH3*, was located to a 37.7 kb region between markers S38978 and S39016 on chromosome 1. This marker interval contained 8 predicted genes.

Keywords: *Oryza sativa* L.; short root hair mutant; *OsSHR3*, genetic analysis; gene mapping

Root hair is an important component in plant root system to increase root surface area and diameter, facilitate root stabilization in soil, interact with microorganisms, and uptake nutrients [1]. Root hairs are long tubular outgrowths that are specialized root epidermis cells, called trichoblasts [2]. Study on development mechanism of the root hair is helpful to identify genetic materials for an improvement of nutrient uptake, and establish a good model to characterize single cell development and apoptosis in crops.

The mechanism of root hair development has been extensively studied lately. In *Arabidopsis*, many root hair mutants have been identified, and breakthrough progresses have been made in cloning of the responsible genes [3, 4]. Root hairs are developed in 3 steps: cell specification, initiation, and elongation [1]. The mechanism of root hair formation in cereal crops is different from that in *Arabidopsis*, but has been poorly characterized due to the shortage of material and research method. So far, a few root hair mutants have been identified in barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.), and some genes corresponding to these mutants have been characterized on the molecular level. In rice, 5 genes, *OsRHL1*, *OsCSLD1*, *OsAPY*, *OsEXPB5*,

and *OsEXPA17*, are involved in the development of root hairs [5–9]. *OsRHL1* locates on chromosome 6 and encodes a novel bHLH (basic/Helix-Loop-Helix) transcription factor, which is an important regulator for tip growth of root hairs. In two allelic mutants of *OsRHL1*, the initiation of root hairs is not affected but the tip growth is severely impaired [5]. *OsCSLD1* on chromosome 10 is homologous to *KOJAK/AtCSLD3* in *Arabidopsis* and encodes a cellulose synthase. The mutation caused by *OsCSLD1* affected not only the tip growth of root hairs but also the morphology, where the root hairs showed kinks and swellings along their length [6]. *OsAPY* on chromosome 7 encodes an enzyme apyrase to catalyze the hydrolysis of NTPs, where root hair initiation proceeds normally but subsequent elongation of bulges is inhibited, resulting in very short projections in mutants [7]. Two recently identified expansin genes *OsEXPB5* and *OsEXPA17* (on chromosomes 4 and 6, respectively) are directly involved in the change of cell wall during root hair development [8, 9]. Furthermore, 2 mutant genes for short root hair of rice, *Ossrh1* and *Ossrh2*, are mapped on chromosomes 6 and 10, respectively [10, 11]. However, they have not been cloned yet. Identifying more mutants associated with rice root hair will be

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helpful for clarifying the molecular mechanism and regulation pathway of root hair development in the grass family.

In this study, a short root hair mutant, *ossrh3*, was isolated from a T-DNA insertion mutant library with the Zhonghua 11 background. The results of phenotypic, genetic, and gene mapping analyses may provide a basis to clone this gene and characterizes its functions in root hair development of rice.

1 Materials and methods

1.1 Rice materials and population construction

A short root hair mutant, *ossrh3*, was isolated from a T-DNA insertion mutant library with the genetic background of rice Zhonghua 11 (*Oryza sativa* L. subsp. *japonica*). This mutant exhibited short root hair stably through 3 generations of self-pollination. The wild type Zhonghua 11 and Kasalath (*O. sativa* L. subsp. *indica*) plants were used as controls.

The F₂ generations derived from reciprocal crosses between *ossrh3* and wild type Zhonghua 11 were used for genetic analysis. The mapping population was the F₂ generation from the cross between *ossrh3* and Kasalathi.

1.2 Phenotyping

Germinated seeds of *ossrh3* and wild type Zhonghua 11 were hydroponically cultured in nutrient solution described by Yoshida et al.^[12]. The 7-day old seedlings were sampled. The whole plant and roots were photographed using a Nikon DC70s camera (Nikon, Japan), and the root hair zone on seminal roots was observed under a stereomicroscope (Leica MZ95, Germany). Plant height, seminal root length, and adventitious root length were measured with a ruler. Number of adventitious roots was visually counted. The number and length of lateral roots on seminal roots were analyzed using WinRhizo software (Regent Instruments, Quebec, Canada) after scanned with a transmission scanner (Epson STD1600, Japan). Thirty plants were examined for each genotype.

1.3 Cosegregation of mutant phenotype and T-DNA fragment

To confirm that the short root hair phenotype in *ossrh3* was caused by T-DNA insertion, we tested the cosegregation of the

phenotype with hygromycin transgene (HPT). DNA samples of 30 T₂ plants were used as templates to amplify the HPT gene (HPT-F: 5'-CGGTCGCGGAGGCTATGGATG-3'; HPT-R: 5'-GCTTCTGCGGGCGATTTGTGTA-3') through PCR. The 20 µL reaction system contained 1 µL of DNA sample (100 ng µL⁻¹), 1.2 µL of MgCl₂ (25 mmol L⁻¹), 0.3 µL of dNTPs (2.5 mmol L⁻¹), 1 µL of each primer (10 µmol L⁻¹), 2.0 µL of 10× PCR buffer, and 0.2 µL of *Taq* DNA polymerase (5 U µL⁻¹). The PCR cycling program included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 30 s and extension at 72 °C for 30 s, and terminated with a final extension at 72 °C for 5 min. The PCR product was electrophoresed in a 0.8% agarose gel and imaged with a gel imager (Gel Doc XR, Bio-Rad).

1.4 Mapping of mutant gene

Based on genetic analysis, a regressive gene was predicted to control the short root hair phenotype. We primarily mapped this gene using bulked segregant analysis (BSA)^[13]. From the F₂ population of *ossrh3* × Kasalathi, 30 plants showing the mutant or normal phenotype were randomly selected to construct the mutant and wild type pools. Genomic DNA was extracted from leaves of 7-day-old seedlings using the simple TPS method described by Zhang et al.^[14]. DNA samples of individuals were pooled in equal amounts to produce DNA pools. The mutant and wild type pools, both parents, and the F₁ generation were used for primary mapping. A total of 98 SSR markers were selected from the Gramene database (<http://www.gramene.org/markers/microsat/ssr.html>), which were polymorphic between Kasalath and Zhonghua 11 and evenly distributed in rice genome.

To fine map the target gene, we designed 6 pairs of STS primers (Table 1) according to the nucleotide difference of genomic sequence between *indica* rice 9311 and *japonica* rice Nipponbare (<http://rgp.dna.affrc.go.jp/E/toppage.html>). The SSR markers were evenly distributed in rice genome and polymorphic between. The STS primers flanked the different regions between *indica* and *japonica* rice. Parents Kasalath and Zhonghua 11 were used for screening the polymorphic STS primers.

Table 1 Polymorphic STS markers between *ossrh3* mutant and Kasalath for fine mapping in this study

Primer	Forward sequence (5'–3')	Reverse sequence (5'–3')	Product size in Nipponbare (bp)
S38595	CTCGGCTCGAGTTTGTATC	ATGCATGCATGATTGTCGAC	112
S38809	TTACGGTGTGGAATCCACAC	ACATCGGCTCGCTGAACC	140
S38920	TAGGGATGGAGTGATCCCAT	CGTGGTGCTAACATCACTCT	123
S38978	TCTAGTTGCTTGATGGACTG	AATGGGAAAATCTAAGGCC	101
S38996	GGTACCTCTAGAACCTTAT	TCTTCCCTAAATCAGATTCCG	119
S39016	TGTAATGCAAGCACATTGG	TCGATCATCCAACATCATC	103

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