Fine-mapping of SRT7 for short roots and identification of its candidate in rice

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Three allelic short root mutants were identified by screening mutants with defective root elongation of the rice japonica cultivar Nipponbare mutant library generated via 60Co γ-ray irradiation mutagenesis. These mutants, designated srt7-1 (short root 7-1), srt7-2 and srt7-3, respectively, had an extremely short seminal root, adventitious roots and lateral roots. Histological observation revealed the cell length of srt7 mutant roots was significantly shorter than that of wild-type roots. Genetic analysis indicated the short root phenotype was controlled by a single recessive nuclear gene. The SRT7 gene was mapped to a 20-kb interval between the markers STS6 and STS7 on chromosome 4 by a map-based cloning method. Sequencing of the six predicted genes in this region found that all of the three allelic mutants contained a 1-bp or 2-bp deletion in the same gene encoding a putative membrane-bound endo-1,4-β-glucanase. The SRT7 gene was expressed ubiquitously, with higher levels of transcript accumulation in roots at different developmental stages. However, no difference was found in the SRT7 transcription level between the mutant and wild type. Collectively, these results indicate the endo-1,4-β-glucanase encoding gene (LOC_Os04g41970) is likely the candidate for SRT7 that functions posttranscriptionally in rice root elongation.

Article

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at a low temperature (25°C). The OsGNA1 gene encodes a glucosamine-6-P acetyltransferase involved in UDPN-acetylglucosamine biosynthesis. UDPN-acetylglucosamine is essential for glycosylation of many secreted and membrane-associated proteins in eukaryotes. Loss of function of OsGNA1 disrupts microtubule orientation and blocks processes involved in root elongation [11]. GLR3.1, another rice T-DNA insertion mutant showing a short seminal root, adventitious roots and lateral roots at the seedling stage, can produce roots of normal length at later developmental stages [12]. OsCyt-inv1, a rice short-root mutant generated by EMS mutagenesis, has a seminal root, adventitious roots and lateral roots only 15%–20% of those of the wild type. The OsCyt-inv1 gene encodes an alkaline/neutral invertase that cleaves sucrose into glucose and fructose irreversibly. Exogenously supplied glucose rescues the root growth defects of the OsCyt-inv1 mutant. These results show normal sugar metabolism is necessary for root development [13]. The rice short-root mutant ossp1 is derived from the indica cultivar Kasalath. Elongation of the seminal root, adventitious roots and lateral roots of the ossp1 mutant is inhibited sharply, especially for lateral roots. The length of lateral roots in 7-d-old seedlings of the ossp1 mutant is only 12% of that of the wild type. The OsSPR1 gene encodes a novel mitochondrial protein with an Armadillo-like repeat domain involved in iron homeostasis in rice [14].

To better understand the genetic control of root elongation in rice, in this study three allelic mutants that showed defective root elongation were identified, fine-mapping of the causal gene was conducted, and the candidate gene was predicted.

1 Materials and methods

1.1 Plant materials and growth conditions

Three rice short-root mutants (srt7-1, srt7-2 and srt7-3) were screened previously from the japonica cultivar Nipponbare mutant library generated via 60Co γ-ray irradiation mutagenesis. For phenotype analysis, plants of the wild type and srt7-1 mutant were planted on floating nets in a black plastic container in nutrient solution and grown in QHX-350BS-III growth chambers at 25/22°C (day/night) with a 12-h photoperiod for 10 d.

1.2 Microscopic analysis

Lateral roots and root hairs were observed and photographed using a Nikon SMZ1000 stereomicroscope with a color CCD camera. For microscopic analysis, the base of the seminal root was treated with clearing reagent (60 mL H2O, 160 g choral hydrate and 20 mL glycerol) for 5 min. The samples were observed and photographed with a Nikon Eclipse Ti-S microscope with a color CCD camera.

1.3 Genetic analysis and construction of F2 mapping populations

The short-root mutants srt7-1, srt7-2 and srt7-3, as the female parents, were crossed to the japonica cultivar Nipponbare and an indica cultivar Kasalath. The phenotype of F1 and F2 seedlings was investigated and F2 mapping populations were generated by crossing srt7-1, srt7-2 and srt7-3 with Kasalath.

1.4 Gene mapping experiments and molecular marker development

Twenty F2 individuals that showed the mutated phenotype of srt7-1 were used for primary mapping and 2756 F2 mutated individuals were analyzed for fine-mapping of the srt7-1 locus. PCR-based sequence-tagged site (STS) markers were developed from an alignment of genomic sequences of japonica and indica rice (NCBI).

1.5 Sequence analysis and candidate gene identification

Candidate genes were predicted in the target region based on information in the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/). The PCR products of these genes were amplified from srt7-1 genomic DNA by PCR using KOD-plus Polymerase, ligated to the Promega T-easy Cloning Vector, and sequenced (Shanghai Genomics Company). Sequence data were compared with the Nipponbare sequence database (NCBI) using DNAstar software (http://www.dnastar.com).

1.6 Semiquantitative RT-PCR analysis

Total RNA was isolated from rice tissues using the RNAgent total RNA isolation system (Takara, Dalian, China). RNA samples treated with DNase I were reverse-transcribed by M-MLV reverse transcriptase (Promega). The following gene-specific primers were used for cDNA amplification: 5′-GCATCTCCTCAGCACATTCCA-3′ and 5′-CTGGTACCCCTCATCAGG-3′ for OsActin (an internal control), and 5′-AGGCCCTCCCTCTTCTCAAAC-3′ and 5′-GCTGAGCATGTCATGGAGA-3′ for SRT7.

2 Results

2.1 Characterization of the srt7 mutants and genetic analysis

Three allelic mutants were identified from screening of the japonica cultivar Nipponbare mutant library. These mutants had similar phenotypes, such as dwarfism and extremely short seminal roots, adventitious roots and lateral roots (Figure 1), and were designated temporarily short root7-1
(srt7-1), short root7-2 (srt7-2) and short root7-3 (srt7-3). The plant height (6.34 ± 0.31 cm) of 10-d-old srt7-1 mutant seedlings was significantly shorter than that of the wild type (7.83 ± 0.23 cm). The length of the seminal root (1.23 ± 0.13 cm), the average length of the 4 longest adventitious roots (0.97 ± 0.07 cm), and the average length of the 4 longest lateral roots on the seminal root (0.23 ± 0.02 cm) were significantly shorter in comparison with those of the wild type (7.47 ± 0.33, 5.28 ± 0.52 and 1.47 ± 0.19 cm, respectively) (Table 1). The number of lateral roots in the srt7 mutants was reduced, but the number of adventitious roots in the mutants was slightly increased, compared to those of wild-type plants (Table 1). In addition, root hairs were not obvious in the srt7 mutants, whereas many root hairs were present on the seminal root of wild-type plants (Figure 1(b) and (c)).

Histological analysis showed that the cell length in the maturation zone of seminal roots of srt7 mutants was reduced and the cell shape was irregular (Figure 1(d) and (e)). These results indicated the embryonic and postembryonic roots and root hair development were arrested in the mutants. The srt7 mutants showed a short-root phenotype throughout the plant life cycle but survived to maturity and produced seeds.

To investigate inheritance of the short-root phenotype, the srt7-1 mutant, as the female parent, was crossed with the japonica cultivar Nipponbare and indica cultivar Kasalath. The root length was normal in one-week-old F1 seedlings from both crosses. The F2 population of srt7-1 × Nipponbare showed segregation of the WT and short root phenotypes (WT: mutant, 159:38), which fitted well a ratio of 3:1 (χ² = 3.128596 < χ² 0.05 = 3.84, P > 0.05). A similar segregation ratio was observed in the F2 population of srt7-1 × Kasalath (WT: mutant, 257:84; χ² = 0.0088 < χ² 0.05 = 3.84, P > 0.05), which indicated the short-root phenotype was controlled by a single recessive nuclear gene.

### 2.2 Fine-mapping of the SRT7 gene

Genetic mapping of the SRT7 gene was performed using the F2 population generated from a cross between srt7-1 and Kasalath. For primary mapping, 20 F2 individuals showing the mutant phenotype were analyzed using 120 SSR markers distributed with 10–15 cM intervals on 12 chromosomes. The SRT7 gene was mapped in the region between the RM241 and RM564 markers on chromosome 4. Seven STS markers were developed by comparison of the genomic sequences of indica and japonica rice (Table 2). The genotypes of 2756 F2 short-root mutants were analyzed using these polymorphic markers. The genomic region that harbored the srt7-1 locus was narrowed down to a 20-kb interval between the STS6 and STS7 markers in AL606627 (Rice Genome Research Program (http://rgp.dna.affrc.go.jp/), Figure 2(a)).

### 2.3 Prediction of the candidate gene

Six hypothetical or expressed genes were located in the 20-kb interval identified by the Rice Genome Research Program, which comprised an endo-1,4-β-glucanase,
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Figure 2  Fine-mapping and gene structure of the SRT7 gene. (a) Fine-mapping of the SRT7 gene on chromosome 4. The number of recombinants from 2756 F2 mutant plants and the chromosomal positions of all markers are indicated. The triangle indicates the position of the SRT7 gene. (b) Structure of the SRT7 gene and the deletion mutations of three allelic mutants. Exons are indicated by a black box, whereas introns are indicated with a black line. Rec, recombinant.

Table 2  Polymorphic sequence-tagged site (STS) markers between the two parentsa)

| Marker name | Primer sequence forward (5′→3′) | Primer sequence reverse (5′→3′) | BAC  |
|-------------|---------------------------------|---------------------------------|------|
| STS1        | TTCCTGCTTTCCGTCTTGTT             | ACTTTCGTCCGTACCGATT             | AL606629 |
| STS2        | CAAGTGTAGCCTAGTGGTT             | AGATGAAGAAGAGGGAGAT             | AL606594 |
| STS3        | GCATACCTGTGCTTCATT             | CAGCCTTTCTCTTCTTGT             | AL606627 |
| STS4        | TTTGAAATGTCAGACAGTT             | GCGCTATCGTGGTTCGTT             | AL606627 |
| STS5        | GGGACGCAATTCGTGTCTG             | TTTTCCTTGCGGTTTGGT             | AL606627 |
| STS6        | GACATTGTGACACCCCCCAAC           | TGGGTCCCACAGTATTTG             | AL606627 |
| STS7        | CAGCTTCTGGAAGAAGTGGA           | ACACCGCTGCGTACGTG             | AL606627 |

a) The two parents are the japonica cultivar Nipponbare and indica cultivar Kasalath.

2.4 Expression pattern of SRT7 in the wild type and srt7 mutants

The expression pattern of SRT7 was investigated in different tissues at different developmental stages of wild-type plants using semiquantitative RT-PCR. The SRT7 gene was expressed constitutively in the root, stem, leaf and panicle, but higher levels of transcript accumulation were always detected in the roots of wild-type plants at different developmental stages (Figure 3(a)). No significant difference was observed between SRT7 mRNA levels in mutants compared to that of wild-type plants (Figure 3(b)). These results indicated that the deletion in srt7 does not affect its mRNA transcription.

Figure 3  Expression of SRT7 in the wild type (WT) and srt7 mutants. (a) Expression pattern of SRT7 in WT. cDNA was amplified from the root (R), stem (S), leaf (L) and panicle (P) of 2-, 4- and 10-week-old plants. (b) SRT7 expression in the root, stem and leaf of 10-d-old WT and srt7 mutants. Semiquantitative RT-PCR was performed for SRT7 (30 cycles) using OsActin as an internal control (27 cycles).

3  Discussion

Among known rice short-root mutants, the srt7 mutants show a similar phenotype to that of rt, ksr1 and Oscyt-inv1 mutants [9,10,13]. The SRT7, RT and KSR1 genes should be allelic because not only do srt7-1, rt and ksr1 share a similar phenotype but the three genes are located in the same region.
of the genome. The only difference between these mutants is their genetic background; ksr1 is derived from the indica cultivar Kasalath and srt7 from the japonica cultivar Nipponbare. Elongations of all srt7 mutant roots, including the seminal root, adventitious roots and lateral roots, were inhibited severely throughout the entire plant life cycle. This differed from other rice short-root mutants, such as the GLR3.1 mutant, in which the short-root phenotype was only observed at the seedling stage [12].

The prediction of an endo-1,4-β-glucanase (OsGLU3) gene as the candidate for SRT7 was supported by sequence data for two other allelic root mutants (Figure 2(b)). Endo-β-1,4-D-glucanase, which functions to hydrolyze β-1, 4-linkages on unsubstituted glucose residues, is involved in the formation of cell wall structure [15]. Many studies have showed that endo-1,4-β-glucanase plays an important role in cell elongation. In Arabidopsis thaliana, mutation of KOR, a membrane-bound endo-β-1,4-glucanase, causes dwarfism [16,17]. CEL1, a gene that encodes an endo-1,4-β-glucanase, is suggested to be associated with elongation of the root and shoot in A. thaliana [18,19]. At least 15 endo-1,4-β-glucanases are present in the rice genome [20]. OsGLU1, the first functionally known endo-1,4-β-glucanase, plays an important role in internode elongation and assembly of cell wall components in rice [21]. Loss of function of OsGLU1 causes a reduction in cell elongation and plant height, a decrease in cellulose content, and an increase in pectin content. Previous studies showed that endo-1,4-β-glucanase is essential for cell elongation in both dicotyledons and monocotyledons and allows the confident prediction of OsGLU3, a homolog of OsGLU1, as the candidate gene for SRT7. The finding that three allelic root mutants with different positional mutations in the coding sequence had almost identical allelic mutations in the coding sequence had almost identical finding that three allelic root mutants with different positional mutations in the coding sequence had almost identical

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