

11. Mapping of Small and Round Seed 2 (*SRS2*) gene in rice

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To understand the mechanisms regulating seed size in rice, we have embarked on the identification of the causal genes for small and round seed mutants. Previously, five genes regulating seed size were reported, namely the *D1* gene (also named *RG1*) encoding the heterotrimeric G protein α subunit (Ashikari et al., 1999; Fujisawa et al., 1999), *D11* gene encoding a cytochrome P450 involved in brassinosteroid (BR) biosynthesis (Tanabe et al., 2005), *D2* gene encoding an another type of cytochrome P450 involved in BR biosynthesis (Hong et al., 2003), *SRS1* gene located on chromosome 5 (Tanabe et al., 2007a), whose function is unknown (Abe, unpublished data) and *SRS3* gene located on chromosome 7 (Tanabe et al., 2007b), which encoded a kinesin-like protein (Kitagawa, unpublished data). Here, we report the mapping of the causal gene of a new small and round seed mutant of rice, *srs2*.

A small and round seed mutant, TCM192 was obtained from Taichung 65 (T65) mutagenized by γ ray irradiation and renamed small and round seed 2 mutant, *srs2*. The seed of *srs2* was compared with those of *srs1*, *srs3*, *d1*, *d2*, *d11* and T65 as the recurrent parent (Fig. 1). To identify the causal gene in *srs2*, we used F_2 plants that were obtained from a cross between *srs2* (*japonica*) and Kasalath (*indica*). We first screened 42 F_2 plants that bore small and round seeds in 2007. Genomic DNA was extracted from fresh leaves of F_2 plants using the CTAB method. The genetic linkage between the *srs2* locus and molecular markers was determined by PCR with the sequence tagged site (STS) markers reported by the Rice Genome Program and the microsatellite markers (McCouch et al., 2002). We then screened 612 F_3 plants for further mapping of the *SRS2* locus. The candidate region in *srs2* was defined by the two markers, 10-21600 (SNPs) and 10-22067 (STS) on chromosome 10 (Fig. 2 A). Chromosomal locations of *SRS1*, *SRS2*, *SRS3*, *D1*, *D2*, and *D11* genes are summarized in Fig. 2 B. We are constructing a more precise linkage map of *SRS2* locus between 10-21600 and 10-22067.

Table1 Primer sequences for mapping of the *SRS2* gene

Maker name	Sequence (5'→3')	
10-212	Fw: AGCTACCTAAAAGTACAGGCTG	Rv: TACTCATCATCATCCTCTCG
10-21600	Fw: GGGTGATCAATGATTGAAGGG	Rv: AAGCTTCAGCCATCGACCAA
10-22067	Fw: GAAGCAACAAACCCAGGAGG	Rv: GCACCACCAAACTGGAGCT
10-22360	Fw: AACGTTGCTCGCCGTAGCTG	Rv: GTGGGTGATGTAGACCGATG
10-end	Fw: TTTTTCGCGCCATCGGGCTTG	Rv: GGGCGCAGGAAATAGCTCAG

10-212, 10-22067, 10-22360 and 10-end are the sequence tagged site (STS) makers. 10-21600 is the single nucleotide polymorphisms (SNPs) maker.



Fig. 1 Grain morphology of small-round mutants. T65 is the original variety of the small-round seed mutants, *d1*, *d2*, *d11*, *srs1*, *srs2* and *srs3*. Bar = 0.5 cm.

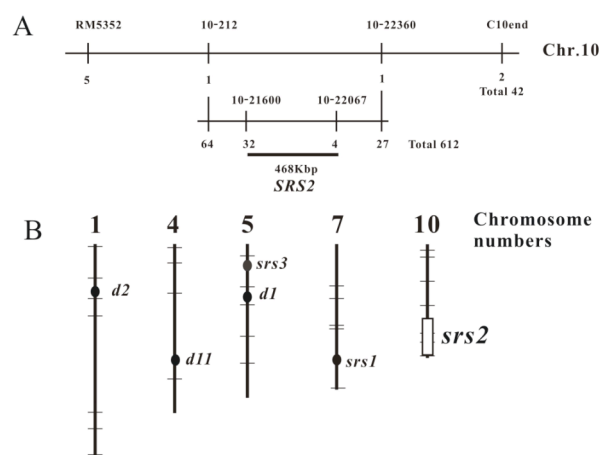


Fig. 2 Linkage map and chromosomal location of *SRS2* gene. (A) Linkage map showing the location of *SRS2* on rice chromosome 10. Vertical lines represent the positions of markers and the numbers of recombinants are indicated below the markers. (B) Chromosomal locations of the causal genes of small-round seed mutants, *srs1*, *srs2*, *srs3*, *d1*, *d2* and *d11*.

References

- Ashikari M., J. Wu, M. Yano, T. Sasaki and A. Yoshimura, 1999. Rice gibberellin-insensitive dwarf mutant gene *Dwarf1* encodes the α -subunit of GTP-binding protein. *Proc. Natl. Acad. Sci. USA.* 96: 10284-10289.
- Fujisawa Y., T. Kato, S. Ohki, A. Ishikawa, H. Kitano, T. Sasaki, T. Asahi and Y. Iwasaki, 1999. Suppression of the heterotrimeric G protein cause abnormal morphology, including dwarfism, in rice. *Proc. Natl. Acad. Sci. USA.* 96: 7575-7580.
- Hong Z., M. Ueguchi-Tanaka, K. Umemura, S. Uozu, S. Fujioka, S. Takatsuto, S. Yoshida, M. Ashikari, H. Kitano, and M. Matsuoka, 2003. A rice brassinosteroid-deficient mutant, *ebisu dwarf (d2)*, is caused by a loss of function of a new member of cytochrome P450. *Plant Cell.* 15: 2900-2910.
- McCouch S. R., L. Teytelman, Y. Xu, K. B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein, 2002a. Development and mapping of 2240 new SSR makers for rice (*Oryza sativa* L.). *DNA Res.* 9: 199-207.
- McCouch S. R., L. Teytelman, Y. Xu, K. B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein, 2002b. Development and mapping of 2240 new SSR makers for rice (*Oryza sativa* L.) (supplement). *DNA Res.* 9: 257-279.
- Tanabe S., M. Ashikari, S. Fujioka, S. Taketsuto, S. Yoshida, M. Yano, A. Yoshimura, H. Kitano, M. Matsuoka, Y. Fujisawa, H. Kato and Y. Iwasaki, 2005. A novel cytochrome P450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, *dwarf11*, with reduced seed length. *Plant cell.* 17: 776-790.
- Tanabe S., K. Mieda, M. Ashikari, H. Kitano, and Y. Iwasaki, 2007. Mapping of Small and Round Seed 1 gene in rice. *Rice Genetics Newsletter.* 23: 44-47.
- Tanabe S., S. Kurinami, M. Ashikari, H. Kitano, and Y. Iwasaki, 2007. Mapping of Small and Round Seed 3 gene in rice. *Rice Genetics Newsletter.* 23: 56-58.
- Ueguchi-Tanaka M., Y. Fujisawa, M. Kobayashi, M. Ashikari, Y. Iwasaki, H. Kitano and M. Matsuoka, 2000. Rice dwarf mutant *d1*, which is defective in the α subunit of the heterotrimeric G protein, affects gibberellin signal transduction. *Proc. Natl. Acad. Sci. USA.* 97: 11638-11643.