

3. Linkage analysis of *SPS6*, *SPS9* and *SPS12*, the sporophytic rice genes acting in pollen development

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To dissect genetic regulation of pollen formation, we have performed gamma-ray mutagenesis to a Japonica rice variety, Taichung 65 (T65), and identified 12 sporophytic and 12 gametophytic mutants showing pollen sterility. The 12 sporophytic mutants were under recessive monogenic control. These genes were designated as *SPOROPHYTIC POLLEN STERILITY 1* (*SPS1*) to *SPS12* (Yamagata et al. 2005). In this report, linkage analysis of *SPS6*, *SPS9* and *SPS12* was described.

The recessive homozygotes of *sps6*, *sps9* and *sps12* showed pollen fertility of 0%, 28.5% and 58.5%, respectively (Fig. 1). These mutants were pollinated with Hinohikari to obtain the F₂ populations used for linkage analysis. The F₂ populations for the mapping of *SPS6*, *SPS9* and *SPS12* consisted of 83, 83 and 82 plants, respectively. To observe pollen fertility in the F₂ population, panicles in the flowering stage were stored in 70% ethanol. Pollen just before anthesis was stained by 1% I₂-KI solution and observed under a microscope. SSR markers (McCouch et al. 2002) were used for the linkage mapping. In the F₂ population of *SPS6*, monogenic segregation of sterile plants was observed (normal: sterile = 66: 17, $\chi^2=0.90$ for 3:1, $P>0.05$). Linkage analysis revealed that *SPS6* was located between the SSR markers *RM4108* and *RM3372* on chromosome 3, at the map distances of 1.0 cM and 2.1 cM, respectively (Fig. 2A). *SPS9* was linked to *RM1553* on chromosome 9 at the map distance of 9.3 cM (Fig. 2B), and *SPS12* was located between *RM7237* and *RM1306* on chromosome 7 at the map distances of 12.5 cM and 19.5 cM, respectively (Fig. 2C).

The rice orthologous genes of *Arabidopsis MS2* (Aarts et al. 1997) and *MS5* (Glover et al. 1998) located in the genomic region where *SPS9* was mapped. In contrast, no homolog of known gene for sterility was found near *SPS6* and *SPS12*. Confirmation whether the *SPS9* identified in this study is identical to *Arabidopsis MS2* or *MS5* or not is underway.

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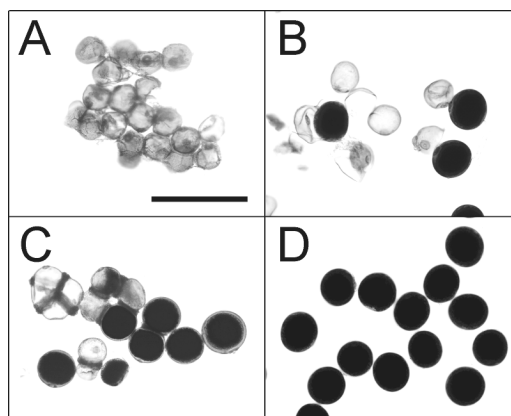


Fig. 1. Pollen morphology of the *sps6* (A), *sps9* (B), *sps12* (C) mutants and T65 (D) stained by I₂-KI solution. Bar = 100 μ m.

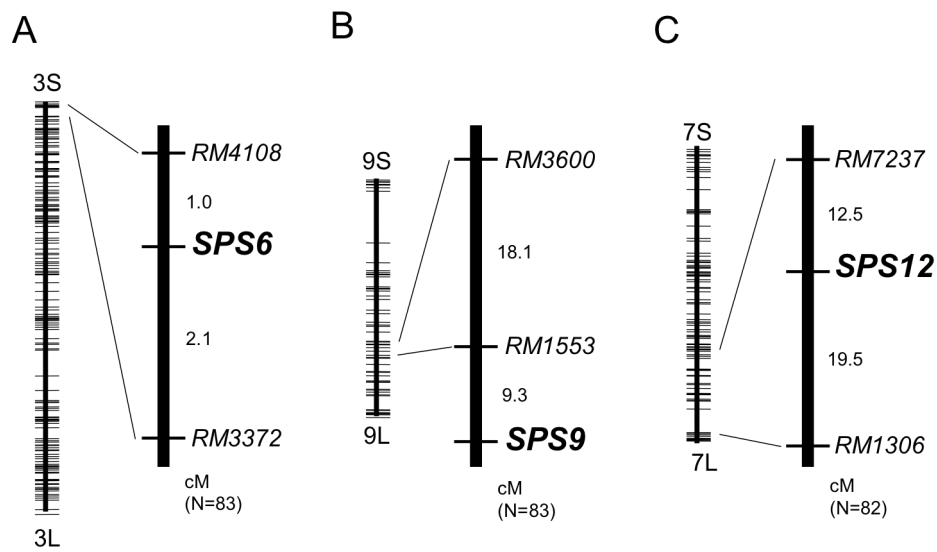


Fig. 2. Linkage maps showing the locations of *SPS6* (A), *SPS9* (B), and *SPS12* (C). Framework maps were quoted from Harushima et al. (1998).

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