

19. Mapping of the *TEMPERATURE SENSITIVE* gene, *TS*

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A chloroplast is an important organelle in the plant cell, which fixes solar energy, supplies a photosynthesis product, and releases oxygen into the atmosphere by photosynthesis including photosynthetic pigments, such as chlorophyll and carotenoid. Chlorophyll-deficient mutant develops chlorotic leaf including immature chloroplasts because of poor accumulation of chlorophyll, and many of them cannot be grown. In order to avoid it, relatively mild chlorophyll mutants, such as *virescent-2* and *zebra* (Sugimoto *et al.* 2004, Kusumi *et al.* 2000), have been analysed for chloroplast differentiation mechanism, but the cause genes are still unknown. To investigate the genetic mechanism for chloroplast differentiation, we are isolating the rice *TEMPERATURE SENSITIVE* gene, *TS*, by positional cloning. *ts* mutant was a temperature sensitive chlorophyll mutant and generated by gamma-ray irradiation to *japonica* rice cv. Nipponbare. Under low-temperature condition, *ts* mutant fails to form normal thylakoid membrane systems (Fig. 1) and develops chlorotic leaves independent of its growth stage (Fig. 2). The F₁ population crossed *ts* mutant and Nipponbare showed normal phenotype (WT) and F₂ population segregated 3:1 (WT: *ts*) following to the Mendelian law; indicating that *ts* mutant is controlled by a single recessive nuclear gene. For mapping, we crossed the *ts* mutant with *indica* rice variety, Kasarath, homozygous plants for the wild-allele (*TS/TS*). Then, F₁ plants were cultivated and self-pollinated to obtain F₂ seeds. In the F₂ seedlings, we screened homozygous plants for *ts* by low-temperature treatment and isolated the genomic DNAs for PCR analysis. We used 50 F₂ *ts* homozygous plants for mapping. The *TS* locus was roughly mapped on the long arm of chromosome 1. For further analysis, we screened about 300 F₂ *ts* homozygous plants, and investigated the linkage between *TS* locus and two markers, S13528 and S10581, located near the *TS* locus. Linkage analysis revealed that *TS* was located between S13528 and S10581, with the genetic distance between them is 14.7cM (Fig. 3). We are determining more precise location of *TS* for cloning.

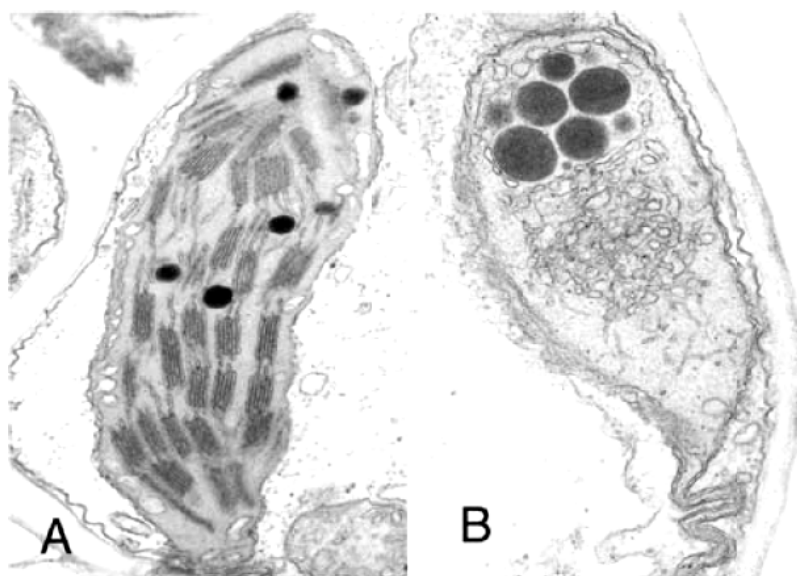


Fig. 1 Electron micrograph of *ts* mutant chlorophyll. A: high-temperature (30°C) condition. B: low-temperature (16°C) condition.



Fig. 2 Phenotype of *ts* mutant under low-temperature condition. A: seedling stage. B: vegetative stage. C: heading stage.

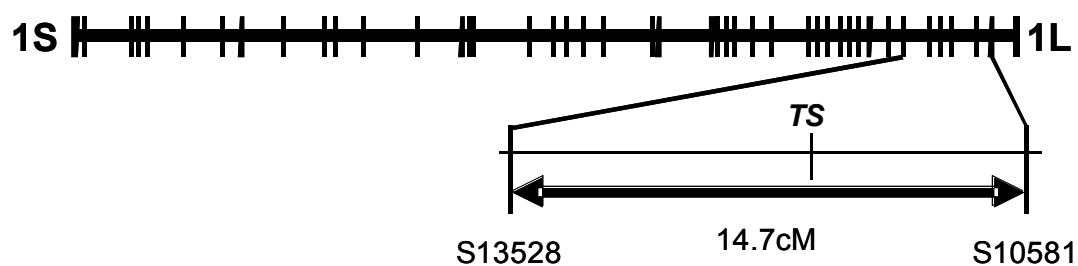


Fig. 3. Map position of the *TS* locus.

References

- Kusumi, K., H. Komori, H. Satoh and K. Iba, 2000. Characterization of a *zebra* mutant of rice with increased susceptibility to light stress. *Plant Cell Physiol.* 41: 158-164.
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