

## 7. Identification and mapping of a *Golden Endosperm* gene in rice

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Rice (*Oryza sativa*) is one of the most important staple food in the world, and is the major source of starch and protein for many people in Asia. However, many essential nutrients, such as flavonoid that are present in low levels in the pericarp, are inevitably lost in the process of polishing and milling. Flavonoid is of much interest for both its importance in metabolism (Shirley 2001), and its nutritional value: it can serve as a powerful antioxidant to reduce the risk of cardiovascular disease, and even as an efficient therapy for cancer (Ness & Powles 1997, Hu 2006).

In *Arabidopsis*, the flavonoid pathway has been well characterized using a series of *transparent testa* (*tt*) mutants, and more than 20 loci have been identified, which correspond to structural enzymes, regulatory factors, and transporters (Lepiniec et al. 2006). In contrast, in rice, there are few studies on the formation and regulation of flavonoid in the pericarp.

We identified a spontaneous mutant of rice known as *golden endosperm* (*GES*). The mutant is isolated from a *Japonica* rice variety, which shows yellow endosperm. The mutant plant morphology was indistinguishable from the wild type plant (Fig. 1). We investigated the structure of rice endosperm using a scanning electron microscope (SEM). No detectable difference was observed between the mutant and the wild type. Furthermore, through a spectrophotometric-based assay method (Chinese Pharmacopoeia 2005), an 80% increase of total flavonoid content was found in mutant seeds compared to the wild type. The endosperm part of the mutant includes little  $\beta$ -Carotene, thus it is significantly different from the previously reported “golden rice” by transgenic experiments (Ye et al. 2000).

Genetic analysis showed that all reciprocal  $F_1$  hybrids of *golden endosperm* mutant crossing to wild-type plants exhibited a wild-type phenotype. Approximately three-quarters of the  $F_1$  progeny of the self-crossed  $F_1$  plants displayed a wild-type phenotype. These results indicated that the *golden endosperm* is a recessive mutation of a single nuclear gene.

Using the methods of bulk segregation analysis, we located the gene on chromosome 9 of the rice genome. In addition, we selected a population including 187 recessive  $F_2$

individuals with golden endosperm phenotype derived from the cross PA64/*GES* and used these for mapping. The locus was mapped in the interval between two newly developed SSR markers, X-16 and S-6 (Fig. 2).

The *golden endosperm* mutant is a valuable resource for studying the flavonoid pathway in rice seeds.

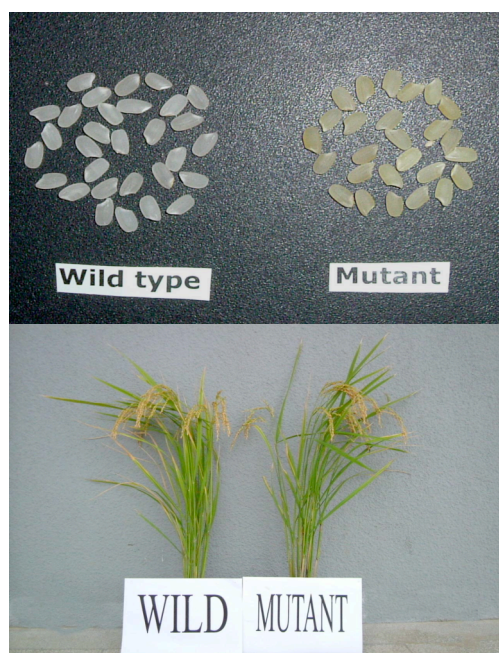


Fig. 1. Phenotypes of milled rice grain and plant morphology of the mutant and wild type.

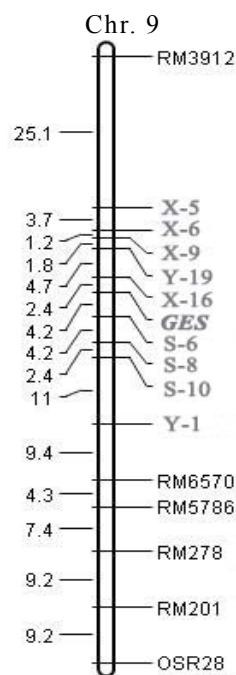


Fig. 2. Location of the *GES* locus on the molecular linkage map of chromosome 9.

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