



Effects of *Se1* gene on basic vegetative growth of rice

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The *Se1* gene, located on the sixth chromosome of rice (*Oryza sativa*), plays an important role in determining the heading time in many rice varieties (Yokoo et al 1980). Through backcrossing, one of the alleles on the *Se1* locus, *Se1-u*, was introgressed from Malaysian rice variety Morak Sepilaito to a genetic background of Fujisaka 5, which has *Se1-e* (Yokoo and Fujimaki 1971). This occurrence suggested that *Se1-u* brought late heading by increasing photoperiod sensitivity. Yokoo and Kikuchi (1982) proposed that *Se1-u* controlled basic vegetative growth.

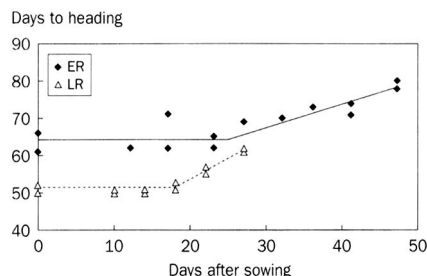
Our objectives in this study were to clarify the effects of *Se1-e* on vegetative growth and determine whether those effects varied with photoperiod.

Two near-isogenic lines of rice with the genetic background for Fujisaka 5 and different alleles on the *Se1* locus, ER (*Se1-e*) and LR (*Se1-u*), were exposed to various photoperiod regimes in two series of experiments.

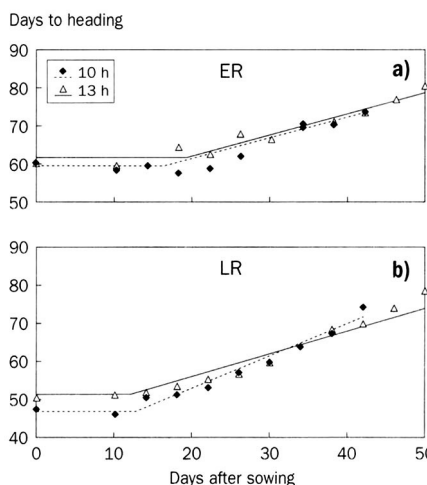
In the first series, the plants were seeded and grown in a phytotron at 28°C for 10 d under a 24-h, long-day (LD) photoperiod. Then some of the plants were shifted to a 12-h, short-day (SD) photoperiod at 4-d intervals (LD-SD shifting). In the second series, LD-SD shifting was conducted, using a 10- or 13-h photoperiod as SD and a

24-h photoperiod as LD in the open air chamber with a mean temperature of 32°C.

Three plants were grown per pot; two pots were used per treatment. White fluorescent light (600 lx at the soil surface) was supplemented with sunlight to prolong photoperiod as needed.



1. Days after sowing to heading of isogenic lines ER and LR, as affected by days after sowing when the plants were alternately shifted from 12 to 24 h photoperiod every 4th day.



2. Days from sowing to heading of isogenic lines ER (a) and LR (b) as affected by days from sowing when the plants were shifted from a 10- or 13-h to a 24-h photoperiod.

In the first series, the horizontal parts of the line graphs were determined by averaging days to heading (DTH), which did not differ significantly from those of the control plants grown under 12 h SD from sowing to heading (Fig. 1). Increase in DTH with days from sowing to shifting was approximated by linear regression using significantly larger DTH than those of the control.

Consequently, the turning points of the graphs project estimated days after sowing during which SD did not accelerate DTH. These days are the photoperiod-insensitive phase (PIP) for each isolate. The estimated length of PIP was 24.8 d for ER and 18.0 d for LR.

In the second series, the estimated length of PIP for each isolate and for each photoperiod regime was 16.2 d for ER-10 SD, 18.8 d for ER-13 SD, 12.7 d for LR-10 SD, and 10.7 d for LR-13 SD (Fig.2). Here, ER also exhibited longer PIP than LR. PIP did not differ significantly between regimes within the same isolate.

We concluded that *Se1* affected not only photoperiod sensitivity but also duration of PIP, and that duration of PIP did not vary with photoperiod but rather with temperature.

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Genetic and cytochemical analysis of high 57-kD polypeptide mutants in rice

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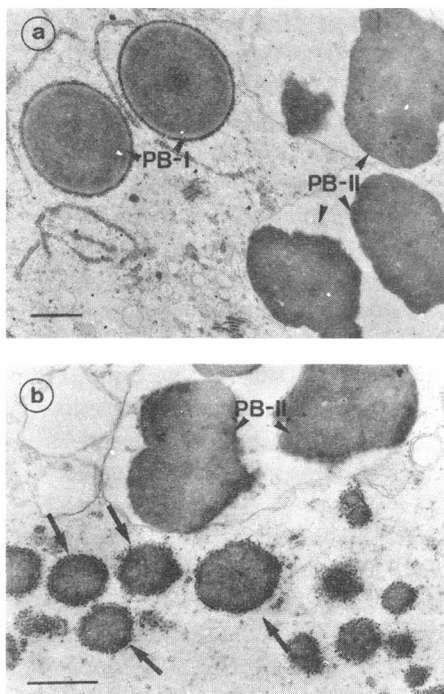
High 57-kD polypeptide mutants (57H mutants) were induced by mutagen N-

methyl-N-nitrosourea (MNU) treatment (Kumamaru et al 1988), while the 57H spontaneous mutants were found in northern Asian varieties. Four 57H mutant loci (*esp2*, *Glup1*, *glup2*, and *glup3*) have been located on chromosomes 11, 9, 9, and 4, respectively (Satoh et al 1994).

The 57-kD polypeptides of these mutants reacted with anti-glutelin β subunit antibody, indicating that 57H mutants accumulate 57 kD glutelin precursors. Glutelins in wild-type Kinmaze were extracted using 1% lactic acid solution (acid solution). The presence of prolamins did not affect extraction of glutelins at all. In

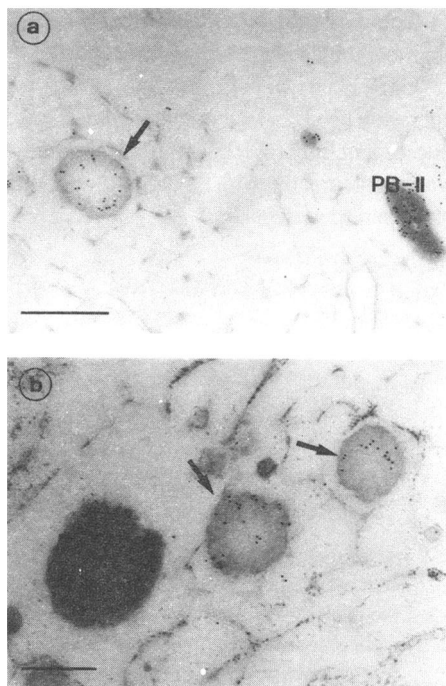
esp2 mutant CM1787, the glutelin precursors could not be extracted without first removing the prolamins. In *Glup1* mutant EM61 and *glup2* mutant EM305, the glutelin precursors were extracted to a slight extent by acid solution, whereas removing the prolamins promoted extensive extraction of glutelin precursors.

These results indicate that glutelin precursors in these mutants coexist with prolamins. In *glup3* mutant HO1274, the glutelin precursors were extracted as easily as the glutelins of Kinmaze, suggesting that the deposition site of the glutelin precursors is different from that of the prolamins.



1. Electron micrographs of the developing endosperm of a) Kinmaze and b) CM1787 (*esp2* mutant). Arrow denotes new type of protein bodies. Bar = 0.5 μ m.

Two types of protein bodies (PB) (PB-I and PB-II) were observed in the endosperm of Kinmaze, as reported by Tanaka et al (1980). In CM1787, although new types of PB and PB-II were observed, PB-I was absent. Many ribosomes were observed on the surface of the new type of PB (Fig. 1 b), suggesting that these PBs, such as PB-I, were derived from endoplasmic reticulum. Immunogold labeling (Fig. 2) showed that the glutelin precursors of *esp2* mutants were deposited in the new type of PB together with prolamin, suggesting that the presence of glutelin precursor in PB-I leads to the formation of the new PB type. In three other types of mutants, both PB-I and PB-II were observed; the new type of PB was not found. The localization of glutelin precursors in these mutants was examined. These observations indicate that the different mutations in the processing pathway of glutelin precursors can cause PB variations among mutants.



2. Electron micrographs of the developing endosperm of *esp2* mutant showing the specificities of a) anti-b subunit antibody and b) anti-13b prolamin polypeptide antibody for a new type of protein body (denoted by arrow). Bar = 0.5 μ m.

We conclude that these mutations are concerned with the genes controlling the processing of glutelin precursors. The study confirmed that *esp2* mutation can result in the deposition of 57-kD glutelin precursors in PB-I, and that *Esp2* is a gene controlling the proteins responsible for targeting glutelin precursors toward the protein vacuole.

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Molecular mapping of genes for F₁ pollen sterility in rice

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F₁ hybrid sterility, probably caused mainly by pollen sterility constrains the use of heterosis in the subspecific hybrid between indica (Hsein) and japonica (Keng). Zhang and Lu (1993) proposed that at least six loci are involved in controlling F₁ hybrid pollen sterility.

To map *S-c*, one of the F₁ pollen sterility genes, Taichung 65 (a japonica variety, denoted as E₁) and E₅, its isogenic F₁ pollen sterile line carrying this gene, were used as parents. E₅ was developed using indica variety Pehku as the donor. It was then backcrossed with E₁ for 11 generations. One hundred and four F₂ plants from E₅/E₁ were used for the segregation analysis.

Pollen fertility was 98.5% for E₁ and 98.6% for E₅; however, pollen fertility for F₁ plants from the cross E₁/E₅ was 53.2%. Segregation of fertile and sterile plants in the F₂ population from this cross fit well to a 1-1 ratio ($\chi^2=0$).

One hundred and seventy restriction fragment length polymorphism (RFLP) markers were used to survey polymorphism between E₁ and E₅, and only three markers located on the same chromosome detected different RFLP patterns. The three positive markers (RG227, RG166, RG369A) were tightly linked to each other and were mapped on chromosome 3 (Causse et al 1994). Polymorphism was low between E₁ and E₅, which could be explained by the short length of introgressed segments in the isogenic line, because it was developed through repeated backcrossing.

The polymorphic RFLP markers were then used to survey the filters with DNA from 104 F₂ plants, and cosegregation of fertility and RFLP patterns were analyzed. The genetic distances between the *S-c* locus and markers RG227 and RG369 were 0.5 and 2.5 cM, respectively (see figure).