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# Isolation, characterization, and mapping of the stay green mutant in rice

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**Abstract** Leaf color turns yellow during senescence due to the degradation of chlorophylls and photosynthetic proteins. A stay green mutant was isolated from the glutinous *japonica* rice Hwacheong-*wx* through N-methyl-N-nitrosourea mutagenesis. Leaves of the mutant remained green, while turning yellow in those of the wild-type rice during senescence. The stay green phenotype was controlled by a single recessive nuclear gene, tentatively symbolized as *sgr*(t). All the phenotypic characteristics of the mutant were the same as those of the wild-type lines except for the stay green trait. The leaf chlorophyll concentration of the mutant was similar to that of the wild-type before heading, but decreased steeply in the wild-type during grain filling, while very slowly in the mutant. However, no difference in photosynthetic activity was observed between the stay green mutant and the yellowing wild-type leaves, indicating that senescence is proceeding normally in the mutant leaves and that the mutation affects the rate of chlorophyll degradation during the leaf senescence. Using phenotypic and molecular markers, we mapped the *sgr*(t) locus to the long arm of chromosome 9 between RFLP markers RG662 and C985 at 1.8- and 2.1-cM intervals, respectively.

**Keywords** *Oryza sativa* L. · Leaf senescence · Stay green mutant · Chlorophyll concentration · Photosynthetic activity · Phenotypic characteristics · Genetic mapping

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## Introduction

Senescence is the final stage of growth and development in plants. Leaf yellowing due to chlorophyll degradation is widely used as a phenotypic marker of plant senescence, although a series of other biochemical and physiological changes are also involved (Noodén 1988; Matile 1992). Leaf senescence is induced by a number of environmental and developmental factors, and timing of leaf senescence is controlled by the genetic background rather than a passive degenerative process (Buchanan-Wollaston 1997; Wingler et al. 1998). Many senescence-related mutants have been found in crop plants that maintain leaf greenness after the grain-ripening stage, and which are referred to as stay green or non-yellowing (Thomas and Smart 1993).

On the basis of the behavior, the stay green phenotype was classified into five types (Thomas and Howarth 2000). With type A stay green, the initiation of senescence is much delayed, but then proceeds at the same rate as the wild-type. Type B stay green initiates senescence at the same time as the wild-type, but leaf yellowing and the decrease in photosynthetic rate are slower. The above two types are regarded as functional stay green due to the prolonged photosynthetic activity during seed filling. On the other hand, type C stay green retains chlorophyll almost indefinitely due to the malfunction of chlorophyll degradation mechanisms. However, as the physiological function reveals, senescence proceeds normally in plant tissues, so called ‘cosmetic’ stay green. Type D stay green results in the leaf death by abrupt freezing or drying. Finally, type E stay green accumulates a high chlorophyll content but without increasing the photosynthesis. Thus, the functional stay green (types A and B) retains both chlorophylls and photosynthetic competence in leaves during seed filling, while leaves of the nonfunctional stay green (types C, D and E) appear green but lack the photosynthetic competence.

Physiological, cytological, biochemical and genetic features of the non-yellowing mutant arising from *Festuca pratensis* have been reported in detail (Thomas

1977, 1982, 1987; Thomas and Matile 1988). The stay green character was induced spontaneously and regulated by a single recessive allele of the nuclear locus *sid* (Thomas 1987), and two linked AFLP markers were identified (Thomas 1997). In soybean, three genotypes of the stay green mutation have been found in leaves and cotyledons. These mutations were controlled by nuclear genes (*G*, *d1d2*) and a cytoplasmic gene (*cytG*) (Guiamet et al. 1990). A dominant gene, *G*, retains the green color in the seed coats. A cytoplasmic gene, *cytG*, two recessive alleles, *d1d1d2d2*, and *G_d1d1d2d2*, regulate the greenness in leaves, pod walls, seed coats, and embryos (Guiamet et a l. 1990). The *cytG* mutation renders the chlorophyll *b* of senescing soybean leaves more stable than chlorophyll *a* (Guiamet et al. 1991), and the *d1d2* homozygous mutation shows a significant delay of soluble protein degradation during senescence (Guiamet and Giannibelli 1996). The amount of leaf chlorophylls decreased in the yellowing kidney bean cultivar, Red Mexican, while the stay green cultivar, Alamo, retained the chlorophylls in the senescent leaves (Bachmann et al. 1994). In *Phaselous vulgaris*, chlorophyllase activity of the non-yellowing mutant leaves was not altered. Phaeophorbide *a* was detected neither in the senescent leaves of the wild-type nor the mutant. However, accumulation of chlorophyllide *a* and *b* was detected in the mutant, while no detectable amount was present in the wild-type, indicating that chlorophyllides are not quickly catalyzed during senescence and, thus, the mutant may be deficient in Mg-dechelatase activity (Fang et al. 1998). The stay green phenotypes of pea, kidney bean, and the grass *F. pratensis* are due to the reduced activity of phaeophobide *a* oxygenase during chlorophyll catabolism (Bachmann et al. 1994; Thomas 1987; Vicentini et al. 1995; Thomas et al. 1996). Though several reports have described the phenotypic and physiological characteristics with respect to different types of stay green plants induced spontaneously or artificially, the exact functions or the map positions of the stay green genes have not yet been revealed.

In this study, a stay green mutant induced from rice using a chemical mutagen was characterized by investigating the inheritance mode as well as the phenotypic and physiological differences from the wild-type rice lines. The stay green locus was then mapped using phenotypic and molecular markers.

## Materials and methods

### Induction of the stay green mutant in rice

The stay green mutation was induced by the treatment of a chemical mutagen N-methyl-N-nitrosourea (MNU) on the fertilized egg cells (Kim et al. 1991) of a glutinous *japonica* rice Hwacheong-*wx* derived from a *japonica* rice cultivar Hwacheongbyeo through MNU mutagenesis.

### Genetic and linkage analyses

The stay green mutant was crossed with the wild-type varieties to determine the inheritance mode of the mutation. To determine the chromosomal location of the stay green mutation, the mutant was crossed with 18 phenotypic linkage testers on chromosome 1 (*eg, spl-6*), 2 (*bl, spl-2*), 3 (*dl*), 4 (*lg*), 5 (*gl, nl*), 6 (*ws, st, dp-1*), 7 (*rfs*), 8 (*su, v-8*), 9 (*Dn-1*), 10 (*fgl, rk*) and 11 (*la*). The stay green phenotype was verified initially by detaching the flag leaves of $F_1$ and $F_2$ individuals at heading and placing them in a moistened plastic bag at 30°C for 9 days in the dark, and finally by observing the leaf color of each plant 2 weeks before harvesting.

### Phenotypic and physiological characterizations of the stay green mutant

Phenotypic traits such as heading date, culm and panicle lengths, panicle number per hill, spikelet number per panicle, fertility, 1,000 grain weight, grain dimension, and yield per hill were measured for comparison between the parental lines, Hwacheongbyeo and Hwacheong-*wx*, and the stay green mutant. To determine the physiological characteristics of the stay green mutation, chlorophyll concentration and photosynthetic activity of the upper leaves were measured from the beginning of heading. The seeds were sown on May 3, 1997, and the seedlings were transplanted on June 1, 1997, with a single plant per hill spaced at 30×15 cm. Fertilizer was applied at a rate of N-$P_2O_5$-$K_2O$=100–80–80 kg per hectare. Leaf chlorophylls were extracted with 80% acetone and the concentration was measured using a UV/VIS spectrophotometer (Sinco, Korea). The photosynthetic rate was measured using LI-6400 (Li-Cor, USA) under a fixed LED light source (1,000 μmol $m^{-2}$ $s^{-1}$) at 25°C as described by the manufacturer's instruction. Means and standard deviations were obtained from at least three replicates and compared using Fisher's LSD at the 0.05 probability level using the SAS program (SAS Institute 1988).

### Mapping population and genotyping of $F_2$ plants

To create the $F_2$ mapping population, the stay green mutant was crossed with a tongil rice cultivar Milyang23 which was bred from an *indica*×*japonica* hybridization and had a genetic makeup close to *indica*. Using a modified CTAB method (Causse et al. 1994) developed by Murray and Thompson (1980), genomic DNA was extracted from the mature leaves of 305 $F_2$ plants and the parents. To identify the genotype of each $F_2$ individual, 20 $F_3$ seeds produced were sown in a greenhouse. Leaves of $F_3$ plants grown for 1 month in a long-day (16 h light/8 h dark) condition were detached and incubated in the moistened plastic bag in the dark at 30°C for 9 days. The genotyping of each $F_2$ individual was performed twice.

### Analyses of simple sequence repeats (SSRs) and sequence tagged sites (STSs) polymorphism

The SSR (RM160 and RM189) and STS (T4) markers were selected from the web database of rice chromosome 9 at RiceGenes, USA (http://genome.cornell.edu/rice), and the Rice Genome Research Program (RGP), Japan (http://rgp.dna.affrc.go.jp). The protocol included 5 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and finally extended by 15 min at 72°C using a thermocycler (MJ Research, USA). PCR was performed with 50 ng of genomic DNA, 0.8 μM of each primer and 1 unit of *Taq* DNA polymerase (Promega, USA) in a 25-μl reaction volume. The SSR polymorphic bands were separated on the sequencing gel containing 6% polyacrylamide and 7 M urea. After electrophoresis, the amplified DNA bands were detected using the silver staining method (Bassam et al. 1991). The STS polymorphism was identified by ethidium bromide-stained bands on 1.5% TBE agarose gel.

### Analysis of restriction fragment length polymorphism (RFLP) and genetic mapping

Eight RFLP markers (RG662, RG570, RZ596, C1263, R3330, G1085, C985 and C570) on chromosome 9 in rice were kindly pro-

vided from RiceGenes at Cornell University, USA, and RGP, Japan. Genomic DNAs (8 µg per lane) from the parents, the stay green mutant and Milyang23, were digested with the restriction enzymes *Eco*RI, *Eco*RV, *Dra*I, *Hind*III, *Bam*HI, *Sca*I, *Xba*I and *Bgl*II (Promega, USA), and used to prepare the parental survey filters for polymorphism analysis (McCouch et al. 1988). Genomic DNAs from 305 $F_2$ progenies were subsequently digested with the restriction enzymes based on the results of the survey filters. Electrophoresis was performed on a 0.8% agarose gel, and Southern blotting and hybridization procedures were accomplished as described in the protocol of the Zeta-Probe GT Blotting Membrane (BioRad, USA). RFLP probes were labeled with α[$^{32}$P]dCTP (Amersham, USA) according to the protocol of Prime-a-Gene Labeling System (Promega, USA). Filters were exposed on X-ray films (Kodak XOMAT, USA) with an intensifying screen at –70°C for 3 to 5 days.

The linkage map and map distance (cM) were analyzed with MAPMAKER/EXP 3.0 (Kosambi 1944; Lander et al. 1987).

## Results

### Isolation and genetic characterization of the stay green mutant

Among the total 30,000 $M_2$ plants derived from 1,500 MNU (N-methyl-N-nitrosourea)-mutagenized $M_1$ seeds of the glutinous *japonica* rice Hwacheong-*wx*, a stay green mutant, with a green leaf phenotype at the end of grain filling, was detected and isolated in the paddy field. During vegetative growth, no phenotypic difference was observed between the wild-type and the mutant plants (Fig. 1A). After heading, leaf color of the wild-type rice turned yellow during grain filling, while the mutant leaves remained green thereafter (Fig. 1B). We found that the leaf green color of the stay green mutant was not changed even in dark-induced senescing treatment for 2 weeks, while the wild-type turned yellow completely. The color change of the detached leaves at any growth stage after 9-days dark incubation (Fig. 1C) was consistent with that of naturally senesced leaves in the stay green mutant and the wild-type rice.

The stay green mutant was genetically fixed through advancement to the $M_7$ generation, and crossed with the wild-type varieties to determine the genetic mode of the mutation (Table 1). The stay green mutation was identified initially by detaching the flag leaves at the heading date and incubating them in the dark for 9 days (dark-induced senescence), and then verified by checking each plant phenotype after the grain-filling stage (natural senescence). The phenotypic results of leaf color were consistent between dark-induced senescence and naturally induced senescence in $F_1$ plants and $F_2$ populations of all crosses. The stay green phenotype was not expressed in $F_1$ plants, and $F_2$ populations of all crosses showed a segregation ratio of three wild-type to one stay green phenotype, indicating that the mutation is controlled by a single recessive nuclear gene, henceforth tentatively symbolized as *sgr*(t).

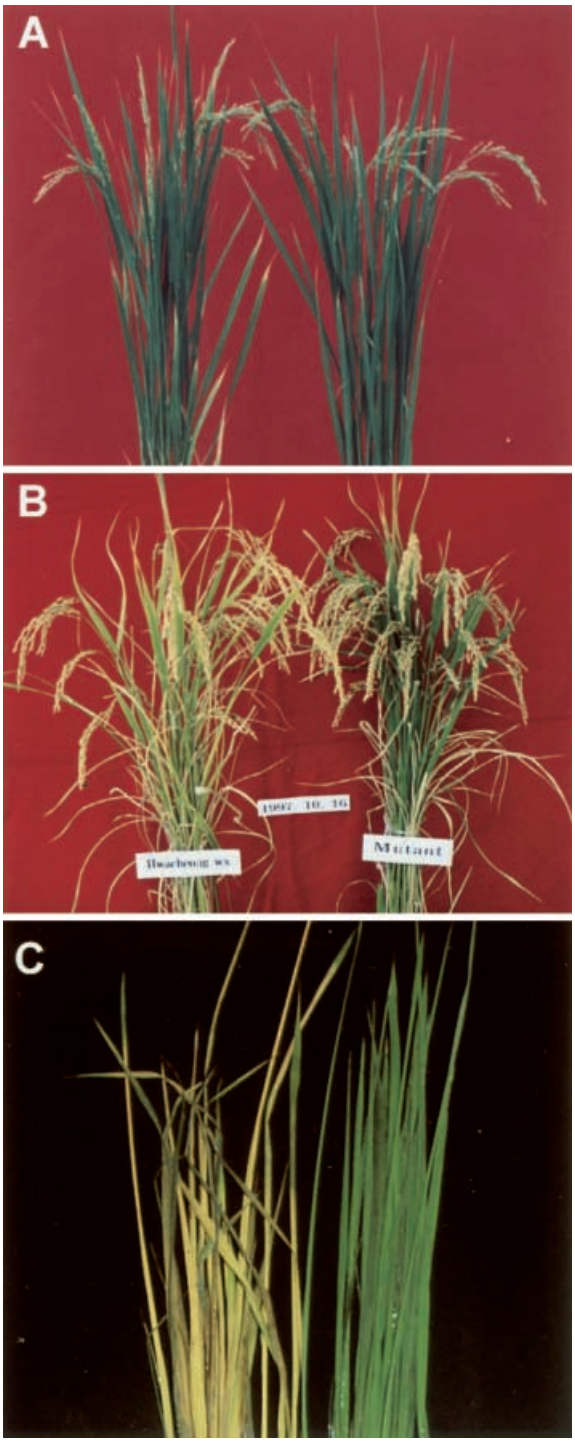


**Fig. 1A–C** Phenotypic difference between Hwacheong-*wx* and the stay green mutant. Field-grown Hwacheong-*wx* (left) and the stay green mutant (right) were photographed at 2 days (**A**) and 40 days (**B**) after heading. Leaf color difference between Hwacheong-*wx* (left) and the stay green mutant (right) became significant when the leaves of 1-month-old plants were detached and incubated in moistened-bags in the dark at 30°C for 9 days (C)

### Phenotypic and physiological characteristics

In heading date, the stay green mutant was not significantly different from the parental lines, nor in other phenotypic traits such as culm and panicle lengths, panicle and spikelet numbers, fertility, 1,000 grain weight, grain dimension, and yield (Table 2). Except for the stay green trait, yield and yield components of the mutant were equal to the parental lines.

**Table 1** Phenotypes of $F_1$ plants and the segregation of $F_2$ populations from the crosses between the stay green mutant and the wild-type rice varieties

| Cross | $F_1$ plants | | | $F_2$ plants | | | $\chi^2$ (3:1) | *P* |
|---|---|---|---|---|---|---|---|---|
| | WT[a] | Mutant[b] | Total | WT | Mutant | Total | | |
| Mutant /Hwacheongbyeo | 9 | 0 | 9 | 250 | 94 | 344 | 0.99 | 0.5–0.1 |
| Mutant/Ilpumbyeo | 9 | 0 | 9 | 192 | 57 | 249 | 0.59 | 0.5–0.9 |
| Mutant/Milyang23 | 9 | 0 | 9 | 129 | 35 | 164 | 1.17 | 0.5–0.1 |
| Mutant/Dasanbyeo | 9 | 0 | 9 | 179 | 59 | 238 | 0.01 | >0.9 |
| Mutant/IR8 | 9 | 0 | 9 | 211 | 61 | 272 | 0.96 | 0.5–0.1 |
| Mutant/CP-SLO | 9 | 0 | 9 | 153 | 42 | 195 | 1.25 | 0.5–0.9 |

[a] Wild-type
[b] Stay green mutant phenotype

**Table 2** Comparison of phenotypic characteristics between the parental lines and the stay green mutant

| Line[a] | Heading date | Culm length (cm) | Panicle length (cm) | Panicles/ hill (No.) | Spikelets/ panicle (No.) | Fertility (%) | 1,000 Grain weight (g) | Grain | | | Yield/ hill (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Length (mm) | Width (mm) | Thickness (mm) | |
| Hwacheongbyeo | 8/17 | 89.0 | 18.7 | 17.3 | 129.0 | 92.9 | 21.7 | 4.7 | 2.7 | 1.9 | 45.0 |
| Hwacheong-*wx* | 8/18 | 93.7 | 21.4 | 17.0 | 126.7 | 91.2 | 20.3 | 4.7 | 2.7 | 1.9 | 42.6 |
| Stay green mutant | 8/18 | 93.3 | 20.8 | 17.5 | 129.5 | 92.7 | 20.5 | 4.7 | 2.7 | 1.9 | 43.0 |
| Difference | NS[b] | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a] Hwacheong-*wx* is a glutinous endosperm mutant line derived from a *japonica* rice cultivar Hwacheongbyeo, and the stay green mutant has also a glutinous endosperm derived from Hwacheong-*wx*

[b] Statistically not significant at the 0.05 level as determined by ANOVA (SAS Institute 1988)

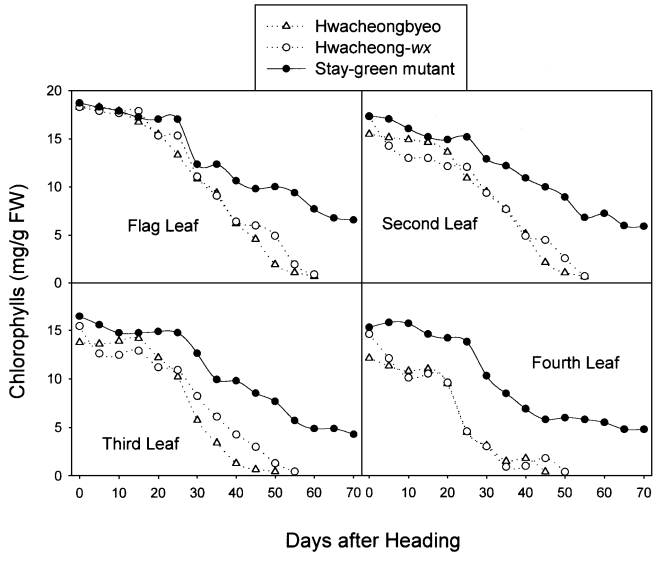


**Fig. 2** Time-course changes in the chlorophyll concentration of the upper leaves of the parental lines and the stay green mutant after heading

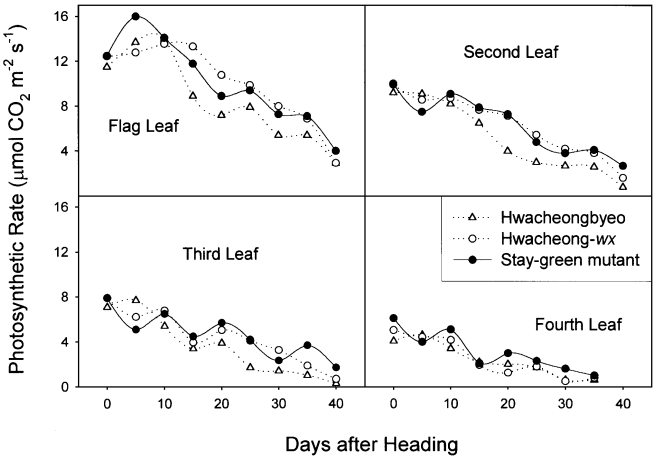


**Fig. 3** Time-course changes in the photosynthetic rate of the upper leaves of the parental lines and the stay green mutant after heading

The time-course changes in the chlorophyll concentration of the upper leaves were compared between the wild-type parental lines and the stay green mutant after heading (Fig. 2). The chlorophyll concentration of the stay green mutant leaves was not significantly different from the parental lines at flowering (0 days after heading). With the progress of grain filling, the phenotypic difference between the mutant and the parental lines became clearer in that the leaves of the parental lines turned yellow, while those of the mutant remained green until the onset of the chilling temperature, which began at 50 days after heading. The reduction rate of chlorophyll concentration was much slower in the mutant leaves than in the parental lines. However, the photosynthetic rate of the mutant green leaves was not significantly different from that of the parental yellowing leaves after heading (Fig. 3).

**Table 3** Linkage analysis of the *sgr*(t) locus using phenotypic linkage testers

| Chromosome no. | Marker | Segregation in $F_2$ plants | | | | Total | Expected ratio | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|---|---|---|
| | | +/+[a] | Maker/+ | +/*sgr*(t) | Double[b] | | | | |
| 1 | *eg* | 75 | 32 | 26 | 8 | 141 | 9:3:3:1 | 1.487 | 0.9–0.5 |
| 1 | *spl-6* | 80 | 27 | 27 | 7 | 141 | 9:3:3:1 | 0.403 | 0.9–0.5 |
| 2 | *spl-2* | 182 | 61 | 47 | 27 | 317 | 9:3:3:1 | 5.327 | 0.5–0.1 |
| 2 | *bl* | 161 | 65 | 68 | 23 | 317 | 9:3:3:1 | 3.948 | 0.5–0.1 |
| 3 | *dl* | 177 | 50 | 69 | 16 | 312 | 9:3:3:1 | 3.761 | 0.5–0.1 |
| 4 | *lg* | 174 | 58 | 65 | 14 | 311 | 9:3:3:1 | 2.295 | 0.9–0.5 |
| 5 | *gl* | 183 | 51 | 66 | 24 | 324 | 9:3:3:1 | 2.716 | 0.5–0.1 |
| 5 | *nl* | 193 | 56 | 51 | 24 | 324 | 9:3:3:1 | 3.265 | 0.5–0.1 |
| 6 | *ws* | 206 | 54 | 48 | 23 | 331 | 9:3:3:1 | 6.601 | 0.1–0.05 |
| 6 | *st* | 147 | 56 | 48 | 11 | 262 | 9:3:3:1 | 2.753 | 0.5–0.1 |
| 6 | *dp-1* | 191 | 60 | 63 | 17 | 331 | 9:3:3:1 | 0.864 | 0.9–0.5 |
| 7 | *rfs* | 182 | 59 | 50 | 20 | 311 | 9:3:3:1 | 1.494 | 0.9–0.5 |
| 8 | *su* | 204 | 48 | 68 | 16 | 336 | 9:3:3:1 | 3.413 | 0.5–0.1 |
| 8 | *v-8* | 180 | 71 | 71 | 14 | 336 | 9:3:3:1 | 4.794 | 0.5-0.1 |
| 9 | *Dn-1* | 26 | 143 | 27 | 21 | 217 | 3:9:1:3 | 31.73 | <0.01[c] |
| 10 | *fgl* | 165 | 48 | 81 | 18 | 312 | 9:3:3:1 | 3.761 | 0.5–0.1 |
| 10 | *rk* | 193 | 55 | 61 | 28 | 337 | 9:3:3:1 | 3.484 | 0.5–0.1 |
| 11 | *la* | 155 | 48 | 51 | 8 | 262 | 9:3:3:1 | 4.775 | 0.5–0.1 |

[a] +: wild-type
[b] Plants showing both morphological marker and stay green phenotypes
[c] Statistically significant at the 0.01 probability level as determined by the $\chi^2$ test, indicating that *sgr*(t) is linked to *Dn-1* on chromosome 9

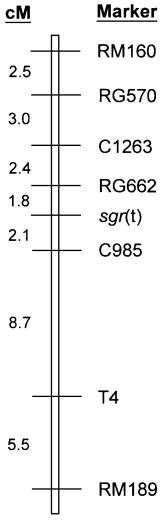


**Fig. 4** Molecular genetic mapping of the stay green *sgr*(t) gene on chromosome 9 in rice

### Genetic mapping using phenotypic and molecular markers

Linkage analysis using phenotypic markers showed that the *sgr*(t) locus was linked to *Dn*–1 (*Dense panicle*–1) on chromosome 9 with approximately 25% recombination value (Table 3). We then surveyed the web database of molecular markers on chromosome 9 and chose the candidate markers presumably around the *sgr*(t) locus. Between the stay green mutant and Milyang23 as the parents, two SSR markers, RM160 and RM189, showed polymorphic bands in approximately 0.1- and 0.13-kb regions, respectively. One STS marker, T4, was useful for detecting polymorphism through the presence of a single 0.6-kb band in the stay green mutant and the absence in Milyang23. Among the eight RFLP markers analyzed on the survey filters, RG662, RG570, C1263 and C985 showed polymorphism between the parents when treated with *Dra*I, *Eco*RV, *Dra*I and *Eco*RI, respectively. Subsequently, seven molecular markers on chromosome 9 were used to map the *sgr*(t) locus with 305 $F_2$ progenies from a cross of the stay green mutant×Miyang23, whose genotypes were identified by $F_3$ plant phenotypes showing all green leaves for the homozygous stay green mutant, all yellow for the homozygous Milyang23, and segregation for the heterozygous type.

Using MAPMAKER/EXP 3.0 (Lander et al. 1987), the *sgr*(t) gene was mapped between RFLP markers RG662 and C985, with distances of 1.8- and 2.1-cM, respectively, on the long arm of chromosome 9 (Fig. 4).

## Discussion

Plant productivity can be determined by net assimilation, that is the difference between the gross amount of resources captured by photosynthesis and those dissipated by growth and maintenance. To increase the productivity, one of the possible ways is to increase or retain the photosynthetic activity during grain filling. Therefore, the unusual non-yellowing phenotype during grain filling has been focused on as a desirable character in agriculture (Hauck et al. 1997). The stay green or non-yellowing mutants have been found naturally or induced by mutagenesis from a few plant species such as *F. pratensis*, *P. vulgaris*, soybean, sorghum, pea and maize. The stay green character has been investigated from the viewpoint of increasing agronomic potentials in crops such as the yield of grains and biomass, the resistance to abiotic

stress, and the forage quality (Honma et al. 1968; Duncan et al. 1981; Tollenaar and Daynard 1982; Guiamet and Giannibelli 1996; Guiamet et al. 1990, 1991; Tollenaar and Aguilera 1992; Phillips et al. 1984; Pierce et al. 1984; Noodén 1988; Thomas and Smart 1993).

Our study shows that a new stay green mutant induced from a glutinous *japonica* rice is controlled by a single recessive nuclear gene (Fig. 1 and Table 1). The locus of the stay green mutation was mapped to the long arm of chromosome 9 between two RFLP makers at a 3.9-cM interval (Fig. 4). To determine the agronomic characteristics of the stay greenness in rice, the phenotypic characteristics between the wild-type parental lines and the mutant were compared. The stay green mutation does not increase the yield or yield components in rice (Table 2). Although the stay green mutant does not synthesize more chlorophylls than the wild-type parental lines, it retains the chlorophylls much longer in plants (Fig. 2). However, the photosynthetic rate of the mutant leaves was not higher than that of the wild-type leaves during grain filling (Fig. 3), indicating that the persistence of greenness is not associated with the extension of photosynthetic activity. Conclusively, even though the mutant leaves appear green, leaf senescence is proceeding normally, but without the normal rate of chlorophyll degradation in the plant tissues. Thus, this mutation is similar, but not identical, to the nonfunctional type C stay green (Thomas and Howarth 2000). Although the persistence of chlorophylls in leaves is not associated with the activity of photosynthesis in the type C stay greens, their cellular and physiological phenotypes are more complex. Loss-of-function of genes related to active chlorophyll degradation or senescence metabolisms has been considered in the monogenic and recessive stay green mutants (Nam 1997). Genes associated with leaf senescence, SAGs (senescence-associated genes), have been cloned from several plant species and their homologues have also been identified (Smart 1994; King and O'Donoghue 1995; Buchanan-Wollaston 1997; Medina-Suárez et al. 1997; Nam 1997). However, neither altered leaf senescence nor the stay green phenotype has been reported based on a loss-of-function by specific mutagenic (e.g. transposon or T-DNA insertion) targeting of any SAGs (Buchanan-Wollaston 1997).

Drought during grain filling speeds up the leaf senescence, resulting in a premature termination of grain filling and finally death. However, under limited water condition, the stay green genotype of sorghum retains more green leaf areas than the yellowing wild-types and also continue to fill the grain normally (Rosenow and Clark 1981; Rosenow et al. 1983). Stay green sorghum also reduces lodging and is resistant to stem rots (Rosenow 1984). This is important in sorghum where tolerance to post-flowering drought stress uses stay green as a key trait in selection. Therefore, the possibility that the stay green mutant in rice might possess the resistance to abiotic stresses is worth examining, though the mutation has no beneficial effect on the increase in photosynthesis or grain yield under normal growth conditions.

The nonfunctional stay green mutant is also useful for studying the biochemical pathway of chlorophyll degradation in plants. In addition, information on the stay green gene helps increase the agricultural potentials of crops; not only will the cloning of the stay green gene(s) increase our understanding on leaf senescence, but, via the gene transfer, we could also breed or manipulate leaf vegetables, forage crops, and turf grasses to retain greenness much longer, or food crops to render more durable photosynthetic activity under unfavorable environmental conditions. Since the stay green gene has not yet been mapped nor cloned in plants, we are currently performing the map-based cloning of the *sgr*(t) gene using the current information on the rice physical map. The success of the gene cloning and the understanding of the gene function in rice will promise new opportunities for increasing the agronomic potentials and economic values of other green plants.

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