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Identification of a mutable *slender glume* gene in rice (*Oryza sativa* L.)

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Abstract The segregation pattern and chromosomal location of a slender glume mutation, induced by gamma-ray irradiation, was investigated. The mutation is genetically unstable: in the selfed progenies of slender glumed plants, not only plants with normal glumes but also plants that are chimeric for glume shape almost always appear at low frequency. The results showed that the mutation is controlled by a single recessive, mutable mutant gene *slg*. The frequency of reversion of *slg* to its wild-type state was little affected by crossing, backcrossing, genetic background or cytoplasmic factors. Conventional trisomic and linkage analyses revealed that the *slg* locus was located close to the *rfs* (rolled fine stripe leaf) locus on chromosome 7. In a subsequent RFLP analysis, *slg* was found to be located between the two RFLP loci *XNpb20* and *XNpb33,* with recombination values of 3.0 and 3.2%, respectively. Southern analysis indicated that the mutability of *slg* is caused by none of the known transposable elements in rice. From these results, we infer that *slg* has a novel transposable DNA insert in its vicinity, which was possibly activated by gamma-ray irradiation.

Key words Rice · Mutable gene · Chromosomal location · *Slender glume* mutation

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Introduction

A slender glume mutant was induced by gamma-ray irradiation of seeds of the *japonica* rice variety Gimbozu (Fig. 1A). This mutant character is probably controlled by a single recessive mutant gene. But the mutation has never been fixed genetically in spite of repeated self-propagation: in successive generations, not only normal plants but also plants chimeric for glume shape almost always appear with low frequency. The chimeras can be classified into three types: between-panicle chimeras, within-panicle (between primary branch) chimeras (Fig. 1B), and the mixed type. Such a phenomenon is likely to be due to the mutability of the mutant slender glume gene(s), which occasionally reverts to its wild-type (normal glume) state. In higher plants, there are many reports on mutable traits especially for genes involved in pigmentation (Bonas et al. 1984; Fedoroff et al. 1984; Brown et al. 1989; Inagaki et al. 1994) and endosperm quality (Fedoroff et al. 1983). Recent molecular biological analyses have revealed that many such mutable traits are controlled by transposable elements (Fedoroff et al. 1983; 1984; Bonas et al. 1984; Brown et al. 1989; Inagaki et al. 1994) or result from epigenetic transformation (Vongs et al. 1993; Bender and Fink 1995). This suggests that the mutability of the *slender glume* may also be caused either by a transposable element or by epigenetic transformation. Since reports on mutable morphological traits are few, the identification of genetic factor(s) controlling the mutability of the slender glume mutation could advance our knowledge of transposable elements or epigenetic transformation and of complicated morphogenetic processes in rice.

Our ultimate goal is to understand whether the mutable slender glume mutation is associated with the insertion of a transposable element. In the present study, the genetic factor(s) controlling the slender glume phenotype and its mutability were investigated. Subsequently, the chromosomal location of the mutation was determined by RFLP (restriction fragment length

polymorphism) analysis, following trisomic and conventional linkage analysis. Southern blot analyses with known rice transposable elements were also performed to investigate whether the mutability of the slender glume mutation is caused by one of these elements.

Materials and methods

Inheritance of the slender glume mutation

The slender glume mutant (mutant line IM294) was induced by gamma-ray irradiation of seeds of the *japonica* rice variety Gimbozu (Fig. 1A). The husked grains of this mutant are also slender in shape (Fig. 1A). IM294 was crossed with four *japonica* rice varieties, Gimbozu, Koshihikari, Nipponbare, and Taichung 65. Reciprocal crosses were made with the parental variety. A total of 20 F_2 populations from five different cross combinations were subjected to analysis for glume shape in 1992. Four F_2 populations derived from different parental slender glume plants (SGPs) from line IM294 were used for each cross combination. Each population consisted of 247 to 360 plants. A progeny test was conducted for the cross 'IM294/Gimbozu' in 1993 using 100 F_3 lines, which were derived from randomly selected F_2 normal glume plants (NGPs). Each F_3 line consisted of 30 plants. All the materials were grown in an experimental paddy field at Kyoto University, Kyoto.

Effects of genetic background

The results of the test crosses revealed that the slender glume phenotype was caused by a recessive mutation in a single gene. To examine the effect of genetic background on the mutability of this gene, the reverse mutation frequency (RMF) was calculated using the progenies of F_2, F_3, BC_1F_2, and BC_1F_3 SGPs that were all covered with plastic bags to prevent outcrossing. Backcrossing with parental varieties was carried out for two cross combinations, IM294/Gimbozu and IM294/Nipponbare. The F_3 and BC_1F_3 populations were grown in 1993, and the F_4 and BC_1F_4 populations in 1994. IM294 was grown as control in both years.

Trisomic and conventional linkage analyses

The primary trisomic series of the *japonica* rice variety Nipponbare, which was kindly provided by Dr. Iwata at Kyushu University, Japan, was crossed with IM294. Not all cross combinations could be obtained, and out of 11 kinds of cross combinations that produced F_1 trisomic plants, three for extra chromosomes 1, 2, and 3 were not suitable for analysis due to the extremely low seed fertility. Consequently, eight kinds of F_2 population, each derived from several F_1 trisomic plants, were subjected to analysis for glume shape. Since the results of the trisomic analysis suggested that the mutant gene was unlikely to be located on chromosomes 4, 5, 6, 8, 9, 10, 11, or 12. IM294 was crossed with seven conventional genetic marker lines for chromosomes 1, 2, 3, and 7 (Table 3). The F_2 populations used in the trisomic and conventional linkage analyses were grown in 1993 and 1995, respectively. The recombination value was estimated by the maximum likelihood method (Immer 1934; Allard 1956).

RFLP analysis

In the RFLP analysis, 75 F_2 plants from the cross IM294/ML17 were used. ML17 was derived from the cross Nipponbare/Kasalath (an *indica* cultivar)//Nipponbare, and has a Nipponbare-derived normal glume (*japonica*-type round glume) and Kasalath-derived RFLP alleles in the homozygous form at four loci that are tightly linked to the *rfs* (rolled fine stripe leaf) locus on chromosome 7. Total gnomic DNAs were extracted from leaves sampled before flowering time using the CTAB method (Murray and Thompson 1980) with slight modifications. Extracted DNAs were digested with four restriction enzymes, *Bam*HI, *Bgl*II, *Eco*RV, and *Hin*dIII. After electrophoresis, the DNAs were blotted onto a positively charged membrane (Hybond N+; Amersham) and were subjected to Southern hybridization. Four probes on chromosome 7, *XNpb91*, *20*, *33*, and *152*, which were kindly provided by the Rice Genome Research Group at the National Institute of Agrobiological Resources, Japan, were used. Labeling of probes and Southern hybridization procedures were performed using the DIG DNA labeling kit and the DIG luminescent detection kit (Boehringer Mannheim), respectively. Linkage relationships were estimated with the MAPL program of Ukai et al. (1990).

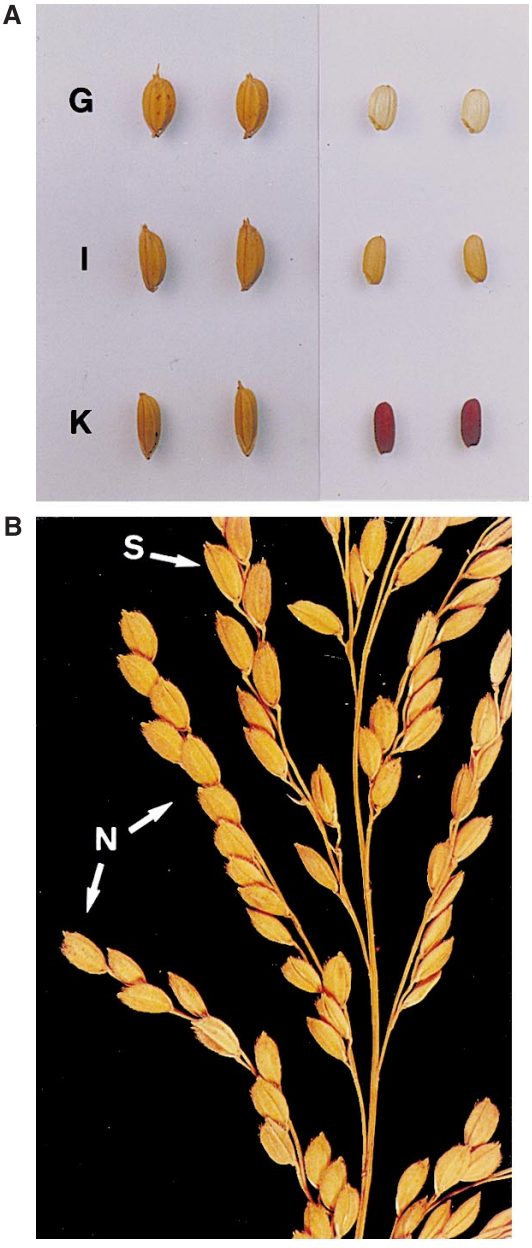

Fig. 1A Glume shapes in the *japonica* rice variety Gimbozu (G), its slender glume mutant line IM294 (I), and the *indica* rice variety Kasalath (K). Most *japonica* and *indica* varieties have such characteristic glume shapes. **B** An example of a within-panicle chimera that appeared in the slender glume mutant line IM294. S and N indicate the slender and normal glume branches, respectively

Southern analyses with known rice transposable elements

Southern blot analyses were performed to investigate whether *slg* was associated with the insertion of known rice transposable elements, retrotransposons of the *Tos* family or the DNA element *RAc* (*Ac*-like element in rice) (Hirochika and Fukuchi 1992; Hirochika et al. 1992). Total genomic DNAs extracted from seedling leaves of IM294 and its parental variety with the CTAB method (Murray and Thompson 1980) were digested with the restriction enzymes *Bam*HI, *Bgl*II, *Eco*RV, *Hin*dIII and *Xba*I, and were subjected to electrophoresis and capillary blotting to nylon membranes. Radioactive labeling of probes (*Tos 1*, *2*, *3*, *5*, *6*, *7*, *8*, *9*, *11*, *12*, *13*, *14*, *15*, *17* and *RAc*) and Southern hybridization procedures were carried out according to the method of Sambrook et al. (1989).

Results

Inheritance of the slender glume mutation

In all the F_2 populations, SGPs and NGPs were found to segregate. The proportion of SGPs varied considerably among F_2 populations (range: 10.5–21.9%, average: 15.0%) (Table 1). All the F_2 populations except two showed ratios significantly lower than the 25% expected if the slender glume phenotype is governed by one recessive mutant gene. Preliminary experiments, however, suggested that SGPs were inferior to NGPs in germination ability and seedling viability. The slender glumes of IM294 and normal glumes of Gimbozu were mixed in a 1:3 ratio. A total of 3000 seeds were sown in nursery beds with field soil, and 1000 seedlings were transplanted to a paddy at the four- to five-leaf stage. After maturation, the proportion of slender glume plants was determined. The ratio of 14.2% did not significantly differ from the overall mean (13.8%) of two F_2 populations, IM294/Gimbozu and Gimbozu/IM294. Among the 100 F_3 lines derived from F_2 NGPs from the cross IM294/Gimbozu, 65 exhibited almost the same segregation pattern as observed in the F_2 population, and 35 consisted of only NGPs. The ratio of 65:35 fits the 1:2 ratio expected for one-locus segregation ($\chi^2 = 0.125$, $P = 0.723$). Since the RMF from slender glume to normal glume was at most 1% (Table 2), reverse mutation was not regarded as the major factor determining the low frequency of F_2 SGPs. Thus, the reduced yield of SGPs in F_2 could be attributed to weak germination ability and/or weak seedling viability; this, in turn, supports the idea that the slender glume character is controlled by a single recessive mutant gene. The cross IM294/Koshihikari showed a significantly larger mean segregation ratio than others, all of which did not significantly differ from each other. This suggests that the germination ability and/or seedling viability might be influenced somewhat by genetic background, though the inheritance of the slender glume mutation is not markedly affected by genetic background or cytoplasmic

Table 1 Segregation ratios of slender-glume plants in F_2 populations from crosses between the slender-glume mutant line IM294 and four varieties

Cross	No. of populations	No. of slender glume plants	No. of non-slender glume plants	No. of chimeric plants	Total	Proportion of slender glume plants (%)[a]	χ^2 value[b]
Gimbozu/IM294	4	37	281	0	318	11.6	30.29($P < 0.001$)
		50	275	0	325	15.4	16.03($P < 0.001$)
		32	248	0	280	11.4	27.50($P < 0.001$)
		40	247	0	287	13.9	18.73($P < 0.001$)
	Total	159	1051	0	1210	13.1[x]	90.80($P < 0.001$)
IM294/Gimbozu	4	48	273	0	321	15.0	17.28($P < 0.001$)
		43	236	0	279	15.4	13.68($P < 0.001$)
		46	229	0	275	16.7	10.04($P = 0.002$)
		27	229	0	256	10.5	28.52($P < 0.001$)
	Total	164	967	0	1131	14.5[x]	66.50($P < 0.001$)
IM294/Koshihikari	4	48	230	1	279	17.2	8.84($P = 0.003$)
		53	284	0	337	15.7	15.45($P < 0.001$)
		54	193	0	247	21.9	1.29($P = 0.255$)
		71	266	1	338	21.0	2.77($P = 0.096$)
	Total	226	973	2	1201	18.8[y]	24.16($P < 0.001$)
IM294/Nipponbare	4	51	309	0	360	14.2	22.53($P < 0.001$)
		42	234	2	278	15.1	14.00($P < 0.001$)
		35	286	0	321	10.9	34.02($P < 0.001$)
		35	239	0	274	12.8	21.84($P < 0.001$)
	Total	163	1068	2	1233	13.2[x]	90.63($P < 0.001$)
IM294/Taichung65	4	31	246	0	277	11.2	28.17($P < 0.001$)
		47	229	0	276	17.0	9.35($P = 0.002$)
		46	230	0	276	16.7	10.22($P = 0.001$)
		54	270	0	324	16.7	12.00($P < 0.001$)
	Total	178	975	0	1153	15.4[x]	56.22($P < 0.001$)

[a] Different letters indicate a significant difference at the 5% level in pair-tests
[b] Test of one-locus segregation (1:3)

factors. In conformity with the rules of gene nomenclature in rice, the mutant gene was designated *slg*.

Effects of genetic background on the mutability of *slg*

The proportions of NGPs and chimeric plants (CPs) in progenies (F_3 and F_4) of F_2 SGPs are shown in Table 2. The proportions of NGPs and CPs in IM294 were also determined as a control. The frequencies of NGPs and CPs in IM294 in 1994 were 0.84 and 0.31%, respectively, while in 1995 the values were 0.48 and 0.40, respectively. In all the single cross combinations, NGPs and/or CPs appeared with low frequency, suggesting the occurrence of reversion from *slg* to its wild-type state in all the genetic backgrounds tested. The exact RMF could not be estimated due to the small number of plants examined, but the results indicated that the RMF was little affected by crossing or genetic background. The cross IM294/Gimbozu showed a slightly higher RMF than the reciprocal cross, and backcrossing to the original variety seem to reduce the RMF. Although a further analysis of this aspect will be needed, it is likely that backcrossing and cytoplasmic factors do not have noticeable effects on RMF and thus on the mutability of *slg*.

Linkage analysis

All the F_2 populations used for the trisomic analysis showed disomic segregation for glume shape (data not shown), suggesting that *slg* was unlikely to be located on chromosome 4, 5, 6, 8, 9, 10, 11, or 12. Based on these results, linkage analysis of *slg* was conducted using conventional marker genes assigned to chromosomes 1, 2, 3, and 7. In all the F_2 populations from crosses between IM294 and these marker lines, the observed segregation ratio of SGPs was less than 25% for the reasons described above. But this does not bias the values for recombination between *slg* and marker genes. The linkage analysis showed that *slg* is linked to *Rc* (brown pericarp and seed coat), *v-11* (virescent-11), *rfs*, and *E1* (heading date-1) on chromosome 7, with recombination values of 20.4, 18.9, 0.0 and 9.1%, respectively (Table 3). Thus, *slg* is tightly linked to, or represents the same locus as *rfs* on chromosome 7, though the latter possibility appears unlikely.

Based on the conventional linkage analysis, RFLP analysis using probes for the chromosomal regions near the *rfs* locus appeared feasible. But first we had to overcome the following difficulties. (1) Large numbers of RFLPs between *indica* and *japonica* rices are known (McCouch et al. 1988; Saito et al. 1991; Kurata et al. 1994), but they are rarely seen among *japonica* varieties (Zhang et al. 1992); hence, an *indica* variety must be used in the analysis. (2) Most *indica* varieties have slender glumes like the SGP, while most *japonica* varieties have normal (round) glumes (cf. Fig. 1A); therefore, the segregation of glume shape in F_2 populations from crosses between SGP and such *indica* varieties are too complicated for analysis. To overcome such problems, we attempted to construct some *japonica*-type (round) glume lines having several homozygous *indica*-derived DNA segments near the *rfs* locus (Kishimoto et al. 1992). In the BC_1F_2 population from the cross 'Nipponbare/Kasalath//Nipponbare, we fortunately found a favorable line, ML17, having Kasalath-derived alleles in homozygous form at all the four known RFLP loci linked to the *rfs* locus.

The results showed that *slg* was located between *XNpb20* and *XNpb33*, with recombination values of 3.0 ± 2.1 and 3.2 ± 2.3%, respectively (Table 4). The location of *slg* on the RFLP map of Saito et al. (1991) is shown in Fig. 2.

Southern blot analyses with known rice transposable elements

The band patterns obtained by Southern analysis with known rice transposable elements, retrotransposons of

Table 2 Reverse mutation frequency (RMF) for slender glume to non-slender glume in different genetic backgrounds

Cross (line)	F_3		Total	F_4		Total
	RMF (%)[a]			RMF (%)[a]		
	Non-slender glume plants	Chimeric plants		Non-slender glume plants	Chimeric plants	
Gimbozu/IM294	0.23(1)	0.00(0)	439	0.53(3)	0.71(4)	561
IM294/Gimbozu	2.30(10)	0.23(1)	435	0.63(7)	0.54(6)	1115
IM294/Koshihikari	0.07(2)	0.00(0)	2609	0.13(2)	0.00(0)	1581
IM294/Nipponbare	0.41(7)	0.94(16)	1695	0.53(8)	0.40(6)	1518
IM294/Gimbozu//Gimbozu	0.00(0)	0.00(0)	40	1.33(11)	0.72(6)	828
IM294/Nipponbare//Nipponbare	0.16(0)	0.00(0)	631	0.06(1)	0.00(0)	1661
		1993(year)			1994(year)	
IM294	0.84(11)	0.31(4)	1312	0.48(6)	0.40(5)	1249

Figures in parentheses show the numbers of plants

Table 3 Linkage relationships between *slg* and conventional marker genes

Marker line	Locus[a]	Chromosome	Normal glume		Slender glume		χ^2 value	Recombination fraction (%)
			Wild type	Marker type	Wild type	Marker type		
T41	*lax*	1	281	90	28	13	1.09	–
KL806	*gh-2*	2	288	81	48	15	0.11	–
TD12	*bc-1*	3	289	85	52	9	1.97	–
T46	*lg*	4	273	90	54	21	0.34	–
KL1002	*g-1*	7	250	124	46	15	1.77	41.0 ± 3.9
KL1003	*Rc*	7	40	314	49	32	98.03[b]	20.4 ± 2.2
KL1008	*v-11(t)*	7	369	143	86	2	26.97[b]	18.9 ± 3.9
KL1005	*rfs*	7	51	24	26	0	10.91[b]	0.0 ± 10.0
EG2	*e1*	7	213	134	92	1	48.60[b]	9.1 ± 4.7

[a] *lax*, lax panicle; *gh-2*, gold hull-2; *bc-1*, brittle culm-1; *g-1*, long sterile lemmas-1; *lg*, liguleless; *Rc*, brown pericarp and seed coat; *v-11(t)*, virescent-11; *rfs*, rolled fine striped leaf; *e1*, heading date-1
[b] Significant at the 0.1% level

Table 4 RFLP mapping of *slg*

RFLP probe	F_2 segregation[a]						Recombination fraction (%)
	Normal glume			Slender glume			
	AA	*Aa*	*aa*	*AA*	*Aa*	*aa*	
XNpb152	22	35	7	1	3	7	20.0 ± 5.7
XNpb91	22	37	5	0	4	7	14.9 ± 4.9
XNpb20	22	42	0	0	2	9	3.2 ± 2.3
XNpb33	18	44	2	0	0	11	3.0 ± 2.1

[a] *A*, Kasalath-type RFLP band; *a*, IM294-type RFLP band

the *Tos* family and the DNA element *RAc* were compared between IM294 and its parental variety Gimbozu. Various band patterns, from a single band to multiple bands, were observed (Fig. 3). If a known transposable element is inserted in *slg*, polymorphism in band should be observed between IM294 and Gimbozu. In most cases, however, such polymorphism was not detected, and none of the polymorphic bands observed corresponded to the segregation of the *slg* locus. This implies that the mutability of *slg* is caused by none of the known rice transposable elements tested.

Discussion

The present study revealed that the induced slender glume mutation is caused by a single recessive, mutable gene *slg*, which occasionally reverts to its wild-type state. The reverse mutation frequency was little affected by crossing, backcrossing, genetic background, or cytoplasmic factors. The appearance of chimeric plants is a clear indication of the occurrence of reverse mutation during mitosis as well as meiosis: *slg* is capable of re-

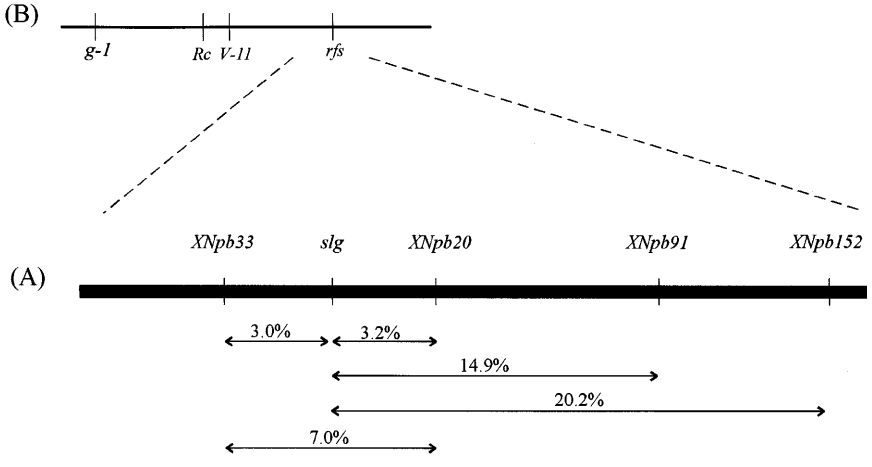

Fig. 2A, B Estimated location of the *slg* locus on the RFLP linkage map of Saito et al. (1991). The relationship between the RFLP map (**A**) and the conventional linkage map (**B**) is shown. According to Kishimoto et al. (1992), the *rfs* locus is located near the RFLP locus *XNpb20*

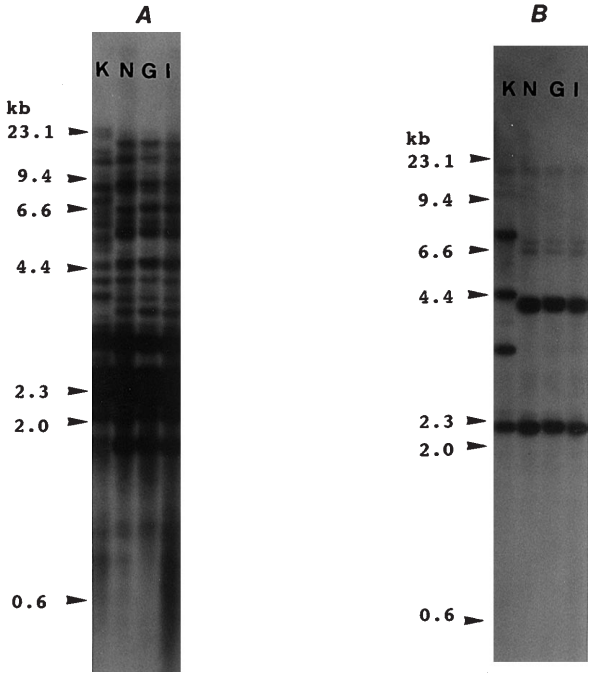

Fig. 3 Southern analysis using the retrotransposons *Tos* 1(**A**) and *Tos* 17(**B**), as probes. DNAs were digested with *Hin*dIII(**A**) and *Xba*I (**B**). I, G, N, and K indicate IM294, Gimbozu, Nipponbare, Kasalath, respectively

verting to its wild-type state throughout the whole growth stage of rice plants.

In maize (*Zea mays*), morning glory (*Pharbitis nil*), and *Antirrhinum majus*, there are many reports on mutable genes (unstable alleles) and most of them were found to be caused by transposable elements (Fedoroff et al. 1983; Bonas et al. 1984; Sommer et al. 1985; Pereira et al. 1986; Inagaki et al. 1994). Therefore, it is very probable that the mutability of *slg* is also caused by a transposable element. Transposable elements are divided into two groups according to their transposition mechanism and mode of propagation: retrotransposons (class I elements) transpose via an RNA intermediate, while the DNA transposable elements (class II elements) move by excision and reintegration (Kunze et al. 1997). Based on the structures of DNA copies, elements of the former type are classified into long-terminal repeat (LTR) retrotransposons, non-LTR retrotransposons or long interspersed element (*LINE*)-like retrotransposons, short interespersed element (*SINE*)-like retrogenes, and others (Kunze et al. 1997). To date, more than 30 retroelements have been reported in plants. As many as 40 elements of Class II have been at least partially analysed at the molecular level, and those of the *Ac* superfamily (Müller-Neumann et al. 1984; Pohlman et al. 1984), the *En*/*Spm* superfamily (Pereira et al. 1986; Gierl et al. 1985), and the *Mutator* family (Chomet et al. 1991; Hershberger et al. 1991) have been especially well investigated.

Although there are few reports of mutable characters in rice, many transposable elements are found in its genome (Hirochika et al. 1992; Mochizuki et al. 1992; Bureau and Wessler 1994; Motohashi et al. 1996). Most of them, however, transpose very infrequently in the intact plant. One of the retrotransposons, *Tos 17*, often transposes during callus induction in vitro (Hirochika et al. 1996), but there are no other effective ways to stimulate the activity of transposable elements in rice. Transposable elements are useful for genetic engineering techniques in plants, such as transposon tagging (McLaughlin and Walbot 1987; Schmidt et al. 1987; Balcells et al. 1991; Gierl and Saedler 1992; Chuck et al. 1993; Biezen et al. 1996) and reverse genetics (Koes et al. 1995; Bensen et al. 1995), and for investigating the evolution of plant species (Mochizuki et al. 1993; Peterson 1993; Thompson et al. 1994; Huttley et al. 1995; Thatiparthi et al. 1995; Kunze et al. 1997). With the aim of applying the transposon tagging technique in rice, many researchers have attempted to introduce maize transposable elements, such as *Ac-Ds*, into the rice genome, but the efficiency of mobilization is still quite low (Izawa et al. 1991; Sugimoto et al. 1994). Concerning the roles of transposable elements in the evolution of plant species, there are two different views (Lönnig and Saedler 1997): some researchers regard transposable elements as selfish DNA without any phenotype function in the host organism (Doolittle and Sapienza 1980; Charlesworth and Langley 1989), others see them as a major source of variability in plant species (Alberts et al. 1994; Lewin 1994). It is thus still not clear whether transposable elements are deployed in eukaryotic organisms because of their selective advantage for their host organisms (Kunze et al. 1997). If so, successful cloning of the transposable element that presumably confers the mutability of *slg* will advance our knowledge of rice genome evolution as well as facilitating the efficient genetic engineering of the rice genome.

In the selfed progenies of NGPs, reversion in SGPs, and various kinds of morphological and physiological mutants, such as early- or late-maturing, dwarf, and rolled leaf mutants, are often observed (data not shown). This fact supports the idea that the mutability of *slg* might be caused by a DNA transposable element(s). In this case, reversion could be caused by the excision of the transposable element inserted in the *slg* locus, while the novel mutations associated with the reversion may be induced by the insertion of the excised transposable element into other chromosomal regions. The change from the wild-type allele to *slg* could have been caused by the insertion of a transposable element that was activated and excised from some other chromosome region following the application of gamma-ray radiation. Such an effect of gamma-ray irradiation is similar to that of the in vitro culture procedures employed by Hirochika et al. (1996).

The Southern analysis revealed that the element that confers the mutability of *slg* differs from the DNA transposable element *RAc* and from all the retro-

transposons of the *Tos* family tested. So far, two LTR retrotransposon families, *Tos* and *RIRE1*, and the *SINE*-like element *p-SINE*, have been characterized in rice (Motohashi et al. 1997; Noma et al. 1997). Among the retrotransposons of the three families, only *Tos 17* demonstrates transpositional activity, which is enhanced under in vitro culture stress, but *Tos 17* never induces reverse mutations (Hirochika et al. 1996). Thus, the transposable element inserted in *slg* differs essentially from retrotransposons of the *Tos* family, and possibly those of the *RIRE1* and *p-SINE* families. In addition to *RAc,* tested in the present study, *Tnr* and *MITE* families are known as DNA transposable elements in rice, but they are quite stable and rarely transpose in the genome of intact plants (Ohtsubo and Ohtsubo 1994; Tenzen et al. 1994; Bureau et al. 1996; Motohashi et al. 1996). They do not differ in chromosomal localization between wild rice and cultivated rice, and thus were not influenced by the divergence of species in the genus *Oryza*. According to Hirochika and Fukuchi (1992), polymorphic hybridization band patterns were observed even among closely related rice varieties when *RAc-1* was used as a probe. Although mutable traits caused by *RAc* have not been found yet, this suggests that *RAc* could be activated frequently in the rice genome. Compared with *RAc*, however, the element inserted in *slg* appears to transpose frequently in the genome. This appears to be the first report of the possible presence of a DNA transposable element related to the mutability of a Mendelian gene in rice.

Two RFLP markers, *XNpb20 and XNpb33,* were found to be linked to *slg*. But the genetic distances between *slg* and these markers are too large to permit the map-based cloning of *slg*. We attempted to find RAPD markers more closely linked to *slg*. In spite of using 400 random primers, however, no favorable RAPDs could be found. We are now undertaking detailed molecular mapping of *slg* using a YAC clone library provided by the Japanese Ministry of Agriculture, Forestry and Fisheries. We have already identified several YAC clones corresponding to *XNpb20* and *XNpb33,* which are closely linked to *slg*. According to our preliminary results, the frequency of repeated sequences in the chromosomal region near the *slg* locus is low enough to allow chromosome walking toward the *slg* locus.

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