Theor Appl Genet (2001) 102:1136–1141

Y. Nakagawa · C. Machida · Y. Machida
K. Toriyama

A system to induce the deletion of gemomic sequences using R/*RS* site-specific recombination and the *Ac* transposon in transgenic rice plants

Received: 30 June 2000 / Accepted: 16 October 2000

Abstract Recombinase encoded by the *R* gene of *Zygosaccharomyces rouxii* mediates reciprocal recombination between two specific recombination sites (*RS*s) to induce deletion or inversion of the DNA segment that is flanked by the *RS*s. The *R* gene under the control of the CaMV 35 S promoter was introduced into rice (*Oryza sativa* L.). R/*RS*-specific deletion was first demonstrated in transgenic rice callus carrying the *R* gene by transient introduction of a cryptic reporter gene that was designed to confer β-glucuronidase (GUS) expression once deletion between two *RS*s took place. The rice containing the *R* gene was subsequently crossed with transgenic rice carrying (I-RS/dAc-I-RS) T-DNA that contained *RS* sequences within the T-DNA and another *RS* in a modified *Ac* element that had been transposed to a new locus by *Ac* transposase. Deletion of the gemomic sequences flanked by the two *RS*s was detected by PCR analysis in somatic cells of F_2 plants. These results demonstrate a technical advance in that the R/*RS* recombination system, in combination with the *Ac* transposable element, can be used to generate deletion in rice chromosomes.

Keywords Deletion · *Oryza sativa* L. · R/*RS* recombination system · Site-specific recombination · Transposon *Ac*

Communicated by K. Oono

Y. Nakagawa · K. Toriyama (✉)
Laboratory of Plant Breeding and Genetics,
Graduate School of Agricultural Science, Tohoku University,
Aoba-ku, Sendai 981-8555, Japan
e-mail: torikin@bios.tohoku.ac.jp
Fax: +81-22-7178654

C. Machida · Y. Machida
Laboratory of Developmental Biology,
Division of Biological Science, Graduate School of Science,
Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan

Introduction

The development of recombinant DNA technology for the manipulation of large segments of DNA is of importance for the structural and functional analysis of eukaryotic chromosomes. For the development of such a technology in plants, the use of site-specific recombination systems, such as the Cre-*lox* system (Qin et al. 1994; Vergunst et al. 1998), the R-*RS* system (Onouchi et al. 1991, 1995) and the FLP-FRT system (Kilby et al. 1995; Lyznik et al. 1993), has been proposed. Chromosomal deletions and inversions have been demonstrated in Arabidopsis, tobacco and tomato plants using these systems (Medberry et al. 1995; Osbone et al. 1995; Stuurman et al. 1996).

Recombinase encoded by the *R* gene of *Zygosaccharomyces rouxii* mediates reciprocal recombination between two specific recombination sites (*RS*s) that are placed in the same orientation (Araki et al. 1985, 1992). In plants, deletions and inversions using the R/*RS* recombination system have been demonstrated in tobacco suspension cells and Arabidopsis plants (Onouchi et al. 1991, 1995). To monitor R/*RS* recombination events in these plant cells, a cryptic β-glucuronidase (GUS) reporter gene has been designed in such a way that expression of the GUS gene is induced by *R* gene-mediated recombination.

With the aim of developing a system for site-specific chromosomal rearrangement, employment of a set of specific recombination sites, such as *RS* and *lox*, in combination with the transposable element *Ac* of maize, has been proposed. In this system, two site-specific recombination sites are included in the T-DNA construct, one of which is placed within the defective *Ac* (dAc) or *Ds* element. Transposition of the *Ds* element mediated by *Ac* transposase should carry one site-specific recombination site to a new genomic locus, while the other site-specific recombination site should remain at the locus of the original T-DNA integration site. Plants containing transposed *Ds* and T-DNA, in which a genomic sequence is flanked by two site-specific recombination sites, have been

crossed to plants expressing recombinase, which catalyzes recombination between two recombination sites and induces chromosomal rearrangement. The successful demonstration of such a chromosomal rearrangement has been reported in Arabidopsis, tobacco and tomato using the Cre-*lox* system (Medberry et al. 1995; Osbone et al. 1995; Stuurman et al. 1996). The successful employment of the R/*RS* system, however, has not yet been reported.

In order to develop a similar system using R/*RS* recombination in combination with the *Ac*/*Ds* elements, we have constructed the (I-RS/dAc-I-RS)T-DNA, which contains an *RS* site in the T-DNA and two *RS*s within the defective *Ac* element (Machida et al. 1997). The two *RS*s within the dAc-I-RS are placed in opposite orientation so that, after transposition of dAc-I-RS, one of the *RS*s has the same orientation as that of the *RS* in the T-DNA region outside the dAc-I-RS element. The construct also contains sites for cleavage by endonuclease I-*Sce*I. The (I-RS/dAc-I-RS)T-DNA was used in Arabidopsis for determining the physical distance of transposition of the dAc-I-RS element (Machida et al. 1997). We introduced the same construct into rice and characterized several plants in which the dAc-I-RS element was transposed by *Ac* transposase (Nakagawa et al. 2000).

In the study reported here, the rice plant carrying the transposed dAc-I-RS was crossed with the plants carrying the *R* gene to mediate the R/*RS* site-specific recombination. The deletion between the *RS* in the original T-DNA locus and the *RS* in the transposed dAc-I-RS was detected in somatic cells of F_2 plants. Preliminary to this crossing experiment, we carried out a transient assay to confirm the recombinase activity in plant materials used for the crossing experiment by bombardment delivery of the plasmid of the cryptic GUS reporter gene into rice calli.

Materials and methods

Plants and constructs

Rice plant #d5 has a single copy of (I-RS/dAc-I-RS)T-DNA (Nakagawa et al. 2000). The T-DNA contains three *RS*s as shown in Fig. 1. Plant #d5 was crossed with a plant carrying the gene for *Ac* transposase under the regulation of P35S. Several F_2 plants carrying the transposed dAc-I-RS element at an independent position were selected and characterized (Nakagawa et al. 2000). The position of the transposed dAc-I-RS element was determined by sequencing the flanking region of dAc-I-RS which was isolated by thermal asymmetric interlaced-polymerase chain reaction (TAIL-PCR, Liu et al. 1995).

A rice plant expressing *R* recombinase was produced by introducing pGAHdNR, which contains the *R* gene under the control of P35S (Onouchi et al. 1995) by means of *Agrobacterium*-mediated gene transfer (Nakagawa et al. 2000). Plant #Re-2 contained a single copy of the *R* gene, while plant #Re-3 had two copies, as determined by Southern blot analysis and segregation analysis in the selfed progeny.

The plant carrying the transposed dAc-I-RS element was crossed as a female parent with plant #Re-2 having the *R* gene. F_1 plants carrying both a transposed dAc-I-RS element and the *R* gene were selected by PCR analysis and allowed to set F_2 seeds. The F_2 seedlings were used for the detection of deletion between the two *RS*s.

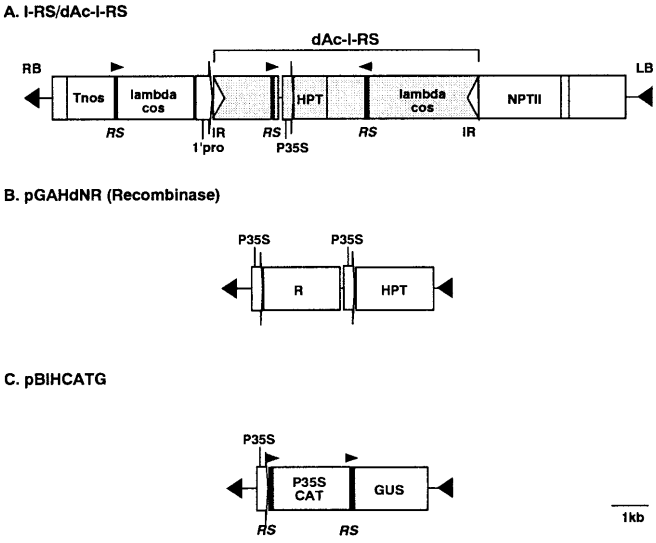

Fig. 1A–C Schematic diagram of the T-DNA region of I-RS/dAc-I-RS (**A**), pGAHdNR (Recombinase) (**B**) and pBIHCATG (**C**). *RB* Right border, *LB* left border, *P35S* CaMV 35S promoter, *IR* inverted repeat, *HPT* hygromycin phosphotransferase, *RS* recombination site recognized by R protein, *NPTII* neomycin phosphotransferase, 1'pro promoter on T-DNA, *lambda cos* fragment of lambda DNA containing the "cos"-region of lambda phage, *Tnos* 3' signal of nopaline synthase, *CAT* chloramphenicol acetyltransferase, *GUS*, β-glucuronidase. Direction of *RS*s are indicated by *arrowheads*

Particle bombardment-mediated transformation of rice callus for transient assay

Calli were induced from scutella of seeds of plants #Re-2 and #Re-3, which were homozygous for the *R* gene. They were bombarded with the plasmids of pBIHCATG (Onouchi et al. 1995) or pBI221 (Jefferson 1987) with the PDS-1000/He Particle Delivery System (BioRad) employing the methods described by Wakita et al. (1998). Between 20 and 50 calli were used for each of the three bombardments. Calli were used for the detection of deletion between *RS*s 2 days after bombardment.

pBIHCATG contains a cryptic GUS gene that was placed between the 35S promoter and the chloramphenicol acetyltransferase (CAT) gene bounded by two *RS*s so that the recombination between the two *RS*s would excise the CAT gene and allow activation of the GUS gene (Fig. 2). GUS activity was detected by the histochemical GUS assay using X-glucuronide as a substrate (Jefferson 1987).

PCR analysis

Genomic DNA was isolated from young leaves of seedlings, and PCR was carried out as described by Nakagawa et al. (2000). The dAc-I-RS element was detected using the AcG4178F (5′-GTGTCATGTGTGCTAGTAGA-3′) and AcG4542R (5′-GGATG-AAAACGGTCGGTAAC -3′) primers. The *R* gene was detected using the R-fow (5′-CGTGTGCATGTATCGAGCCT-3′) and R-rev (5′-ATTTGTTGTGGGGGTACACG -3′) primers.

To increase the specificity and sensitivity of the detection of deletion between the *RS*s, we carried out two-step PCR using nested primers and Z-taq polymerase (Takara). The first PCR consisted of 30 cycles of denaturation at 98°C for 5 s, and annealing and extension at 68°C for 2 min. The second PCR consisted of 30 cycles of denaturation at 98°C for 5 s, and annealing and extension at 68°C for 20 s using $^1/_{25}$ of the first PCR products. To estimate the percentage of leaf cells in which the deletion was induced, we carried out PCR using the inner primers used in the second PCR.

The resulting band corresponding to *RS* deletion was detected by Southern blot analysis using the second PCR product as a probe. A one-copy marker was reconstructed from the second PCR product. The intensity of the band was compared between leaf cells and the series of a dilution of the one-copy marker. The 5′ to 3′ sequences of primers are as follows: a, CATGGAACAAGCGGATTTCG in the *Ac* genome; b, ACCTGCGTGCAATCCATCTT in the neomycin phosphotransferase (NPTII) gene; c, GTTAGCCTAAAGAAGCTCTAGG in the junction between the *Ac* genome and *RS*; d, CCGCCACCAATTCCCGATCT in the junction between the lambda sequence and nopaline synthase (nos) terminator; e, CTATCGCCTTCTTGACGAGT in the NPTII gene; f, CTCATGATTTGTTGCAGCAG in the *Ac* genome; g, TCTTGCGTTGATGAAGCTCGGG in the junction between the nos terminator and the *RS*; h, TTGACGGATCTCTAGGACGCG in cauliflower mosaic virus 35S promoter (P35S). The position of each primer is indicated in Fig. 3.

Results

Transient GUS assay for the detection of R/*RS* recombination in rice callus

To examine recombinase activity in transgenic rice with the *R* gene (plants #Re-2 and #Re-3), we used a cryptic GUS reporter gene (pBIHCATG) that was designed in such a way that recombinase mediates the excision of the CAT gene flanked by *RS*s and generates a P35S-GUS structure resulting in GUS expression. When the calli induced from seeds of #Re-2, and #Re-3 were bombarded with a cryptic reporter gene, GUS activity was observed as a blue spot (Fig. 2). Five such blue spots were observed in the 170 calli of #Re-2, and only one was observed in the 130 calli of #Re-3. The calli of the wild type did not show any blue spots after bombardment with the cryptic GUS gene. The unbombarded calli and the calli bombarded with only gold particles did not show any GUS activity (Fig. 2). These results indicated that plants #Re-2 and #Re-3 had recombinase activity and that the deletion mediated by the R/*RS* recombination took place in rice cells.

When the wild-type calli were bombarded with pBI221, which contained P35S-GUS, 44 blue spots were detected in 120 calli (Fig. 2). Assuming similar efficiencies of gene transfer, it is likely that the deletion between two *RS*s occurred in approximately 5% of the rice cells into which the cryptic GUS gene had been introduced.

We decided to use plant #Re-2 for further crossing experiments because #Re-2 contained a single copy of the *R* gene and yielded more blue spots than #Re-3 which contained two copies of the *R* gene.

Induction of somatic R/*RS* recombination through the crossing of transgenic rice plants

We crossed the transgenic rice plants that carried the *R* gene (#Re-2) with each line that carried the transposed dAc-I-RS (k2–1, k4–1, k-4–4 and k2–2). The position of each transposed dAc-I-RS had been determined by se-

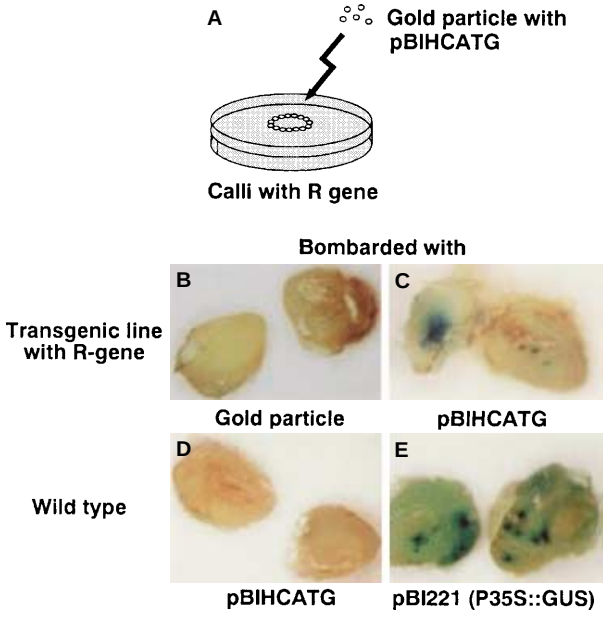

Fig. 2A–E Transient GUS assay for detection of R/*RS* recombination in rice calli. **A** Schematic diagram of plasmid delivery into rice calli by particle bombardment. **B** Transgenic calli integrated with the *R* gene (#Re-2) were bombarded with gold particles only and showed no blue spots. **C** Transgenic callus integrated with the *R* gene (#Re-2) was bombarded with pBIHCATG (cryptic GUS gene), with blue spots of GUS activity appearing. **D** Wild-type callus was bombarded with pBIHCATG and showed no blue spots. **E** Wild-type callus bombarded with pBI221(P35S::GUS), with many blue spots appearing

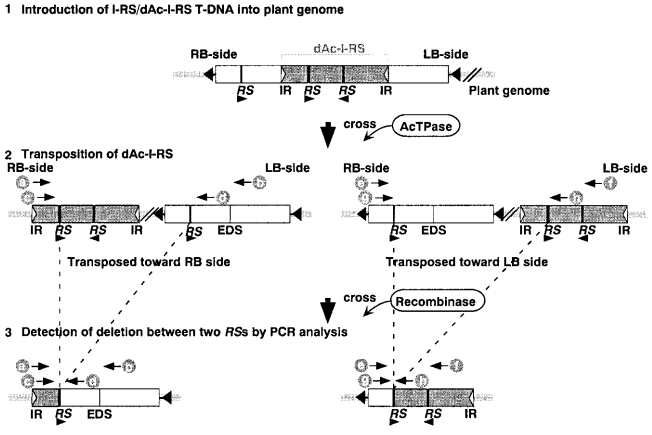

Fig. 3 Flow chart for the induction of the R/*RS* deletion using I-RS/dAc-I-RS T-DNA and the strategy used to detect deletion between two *RS*s by PCR analysis. The dAc-I-RS element was transposed by crossing with the transgenic plants expressing AcTPase. The R/*RS* deletion was expected to take place between the *RS* in the T-DNA region and one of the *RS*s within the dAc-I-RS element in the same orientation. Two-step PCR was carried out to detect deletion between the two *RS*s. When the dAc-I-RS element transposed toward the RB side, the first PCR used primers a and b, and second PCR used primers c and d, resulting in amplification of the 300-bp band. When the dAc-I-RS element transposed toward the LB side, the first PCR used primers e and f and the second PCR used primers g and h, resulting in amplification of the 200-bp band. *Arrowheads* indicate the orientation of *RS*, and *arrows* with *letters* represent the positions of the primers. Other abbreviations are the same as those in Fig. 1

Table 1 Distance between two *RS*s in the transposed dAc-I-RS lines and the number of F_2 plants showing *RS* deletion in the F_2 progeny of each F_1 plant resulting from crosses between designated transposed dAc-I-RS (♀) lines and the recombinase line (#Re-2)

dAc-I-RS line	Direction of transposition	Distance between two *RS*s (kbp)	F_2 plants tested	Number of F_2 plants showing *RS* deletion
k2–2	LB side	7.4	82	3
k2–1	RB side	10.1	308	5
k4–1		8.3	151	16
k4–4		7.3	32	1
Total			573	25

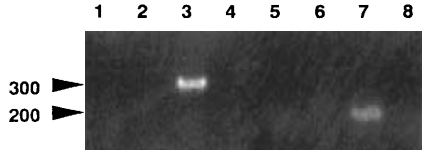

Fig. 4 Detection of the R/*RS* deletion in rice leaves by PCR analysis. A 300-bp band indicates that the R/*RS* deletion when dAc-I-RS has transposed toward the RB side, while a 200-bp band indicates deletion when dAc-I-RS has transposed toward the LB side. *Lane 1* k2–1 (the dAc-I-RS transposed toward the RB side), *lane 2*, #Re-2 containing the *R* gene, *lane 3* an F_2 individual of k2–1 × #Re-2; a 300-bp band was detected indicating R/*RS* deletion, *lane 4* an F_2 individual in which no deletion was detected, *lane 5* k2–2 (the dAc-I-RS transposed toward the LB side), *lane 6* #Re-2, *lane 7* an F_2 progeny of #d5-k2–2 × #Re-2; a 200-bp band was detected indicating R/*RS* deletion, *lane 8* an F_2 progeny in which no deletion was detected. The bands were visualized by staining the gel with ethidium bromide

quencing the flanking region of dAc-I-RS. The physical distance of transposition in these plants varied from 2.6 to 7.4 kbp. The dAc-I-RS in plant k2–2 transposed toward the LB side, while that in other plants transposed toward the RB side. The orientation of dAc-I-RS was the same as that of the original I-RS/dAc-I-RS. The R/*RS*-mediated deletion was expected to take place between two *RS*s in the same orientation. The distance between the two *RS*s in the same orientation was 7.3–10.1 kbp in the transposed dAc-I-RS lines (Table 1). Figure 3 shows a flow chart with a schematic diagram of the expected R/*RS* recombination and strategy for detecting deletion by PCR analysis. Amplification of a 300-bp band is expected once R/*RS* deletion takes place in the case where dAc-I-RS has transposed toward the RB side, while a 200-bp band is expected when dAc-I-RS has transposed toward the LB side.

Out of 82 F_2 plants of k2–2 (dAc-I-RS transposed toward LB side) × #Re-2, three plants showed an expected band of 200 bp, as shown in lane 7 in Fig. 4, while most of the plants did not yield any bands (lane 8 in Fig. 4). No bands were amplified in either parent (Fig. 4, lanes 5 and 6). On the other hand, a band of 300 bp was detected in some plants of F_2 derived from k2–1, k4–1 and k4–4 (dAc-I-RS transposed toward RB side). Figure 4 shows the representative result of two individual F_2 plants derived from k2–1, one of which showed a 300-bp band (lanes 3 and 4). None of the parental lines yielded any bands (Fig. 4, lanes 1 and 2).

Analysis of the nucleotide sequences of the 200-bp and 300-bp fragments showed that recombination took place precisely within the two *RS*s as expected. These results indicate that deletion was induced between *RS*s in transposed dAc-I-RS and *RS* in the original T-DNA. Plants k2–1, k4–1 and k4–4 contained genomic sequences of 2.8 kbp, 1.0 kbp and 0.1 kbp, respectively. Thus, the deletion of gemomic sequences flanked by two *RS*s did occur.

The number of F_2 plants showing *RS* deletion is summarized in Table 1. In total, 25 out of the 573 F_2 plants tested were found to show the *RS* deletion. The presence of the *R* gene in these F_2 plants was examined by PCR analysis. All of the plants showing the *RS* deletion were revealed to contain the *R* gene (data not shown).

It is possible that the *RS* deletion detected in this study originates in the somatic cells of the F_2 plants or in the germinal cells of the F_1 plants. However, in the Southern blot analysis of F_2 plants in which *RS* deletion was detected by PCR, we did not observe the band pattern expected from germinal deletion (data not shown). Therefore, the deletion detected herein took place in somatic cells but was not derived from the germinal recombination of F_1 plants.

In order to estimate the percentage of somatic cells in which the deletion was induced, we compared the amount of the PCR product, the 300-bp fragment, from leaf cells of k4–1 F_2 plants with that from the reconstructed templates by Southern blot analysis. The intensity of the band of the PCR product varied among plants, with the most intense band showing almost the same intensity of signals as that obtained from a dilution of 1:10000 of the one-copy marker (data not shown). It is estimated, therefore, that the *RS* deletion took place in at most 0.01% of the leaf cells of the F_2 plants tested.

Discussion

Recombinase encoded by the *R* gene of *Z. rouxii* mediates reciprocal recombination between two *RS*s to induce excision or inversion of the DNA segment that is flanked by the *RS*s. Such R/*RS* recombination has been successfully induced in tobacco and *A. thaliana* (Onouchi et al. 1995). In the present study, we demonstrated that R/*RS* recombination can be also induced in rice. A transient assay using the cryptic GUS gene was effective in determining the recombinase activity in rice cells.

The same cryptic GUS reporter gene has been used in tobacco and *A. thaliana* for the visualization of R/*RS* re-

combination (Onouchi et al. 1991, 1995). Tobacco BY-2 cells transformed with the cryptic GUS genes have been used as target cells to deliver plasmid carrying P35S and the *R* gene (pGAHdNR). Onouchi et al. (1991) observed the *RS* deletion in approximately 40% of the plant cells into which the *R* gene had been incorporated. In the present study in rice, we estimate that the deletion between two *RS*s occurred in approximately 5% of the rice cells integrated with the *R* gene following the introduction of the cryptic GUS gene by particle bombardment. This frequency is lower in rice than that found in the previous study in tobacco, although the transgene in the target cells and gene transiently delivered is opposite. Plant #Re-2 showed a higher activity of recombinase than #Re-3 based on the number of blue spots. It is possible that R/*RS* recombination in rice cells would be further enhanced by a stronger expression of the *R* gene.

The cryptic reporter gene has been also introduced into *A. thaliana*, with subsequent crossing of the transgenic Arabidopsis line that carried the *R* gene (Onouchi et al. 1995). Both somatic and germinal recombination was obtained in the F_2 plants. The percentage of F_2 plants showing somatic and germinal recombination out of the total number of F_2 plants tested varied from 0 to 62.5% and 0 to 2.4%, respectively, depending on the transgenic line. In the transgenic line with the highest frequency, the ratio of the number of plants with germinal recombination to those with somatic recombination was 38/745, i.e. 5%. In the present study, we observed R/*RS* recombination in 25 out of 573 F_2 plants derived from the cross between the transgenic line with transposed dAc-I-RS and that with the *R* gene. It is likely that the *RS* deletion detected in this study took place in the somatic cells of rice leaves. Assuming the ratio of germinal recombination to somatic recombination of the previous study of Arabidopsis, several more investigations of the number of F_2 plants might be necessary to identify the plants with germinal recombination. Investigations are now in progress to find out which plants underwent germinal R/*RS* deletion by screening the F_3 population.

R/*RS* recombination induces excision or inversion. In the present study we focused on excision because our aim was to induce a large deletion in rice chromosomes. We did not carry out the experiment to detect recombinase-mediated inversion that might occur in the rice cells.

The strategy to induce chromosomal rearrangement using a combination of site-specific recombination and the *Ac*/*Ds* systems has been reported using the Cre-*lox* as a site-specific recombination system. Somatic and germinal inversion of a 130-kbp fragment in tomato (Stuurman et al. 1996), up to a 16.5-cM fragment in Arabidopsis (Osbone et al. 1995) and a nearly 10-cM fragment in tobacco (Medberry et al. 1995) has been observed. However, deletion mutants have not yet been reported using these systems. Our results indicate that the R/*RS* recombination system, in combination with the *Ac* transposable element, can be used to generate deletion in rice chromosomes.

The original T-DNA integration locus of plant #d5 has been mapped on the restriction fragment length polymorphism (RFLP) linkage map (Nakagawa et al. 2000). Most lines of the transposed dAc-I-RS were characterized as being closely linked to the original T-DNA locus, as was expected based the previous reports that the *Ac* element efficiently transposes to a nearby site (Machida et al. 1997). The physical distance between two *RS*s was 10.1 kbp at most in this study. We have found some lines in which dAc-I-RS transposed to a new locus that was weakly linked to or not linked to the original T-DNA locus (Nakagawa et al. 2000). We are now attempting to detect the deletion of a large chromosomal segment between two distantly separated *RS*s. The (I-RS/dAc-I-RS)T-DNA used in this study contained I-*Sce*I sites so that the physical distance between two *RS* sequences on the chromosome could be precisely determined (Machida et al. 1997). The rice plants in which the dAc-I-RS transposed will therefore be useful for the systematic examination of chromosomal deletions mediated by the R/*RS* system.

Acknowledgments This research was supported by the "Research for the Future" Program of The Japan Society for the Promotion of Science (JSPS=RFTF97L00601). Y. Nakagawa is the recipient of a JSPS Fellowship for Japanese Junior Scientists.

References

Araki H, Jearnpipatkul A, Tatsumi H, Sakurai T, Usio K, Muta T, Oshima Y (1985) Molecular and functional organization of yeast plasmid pSR1. J Mol Biol 182:191–203

Araki H, Nakanishi N, Evans BR, Matsuzaki H, Jayaram M, Oshima Y (1992) Site-specific Recombinase, R, encoded by yeast plasmid pSR1. J Mol Biol 225:25–37

Jefferson RA (1987) Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep 5:387–405

Kilby NJ, Davies GJ, Snaith MR, Murray JAH (1995) FLP recombinase in transgenic plants: constitutive activity in stably transformed tobacco and generation of marked cell clones in *Arabidopsis*. Plant J 8:637–652

Liu YG, Mitsukawa N, Oosumi T, Whittier R (1995) Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J 8:457–463

Lyznik LA, Mitchell JC, Hirayama L, Hodges TK (1993) Activity of yeast FLP recombinase in maize and rice protoplasts. Nucleic Acids Res 21:969–975

Machida C, Onouchi H, Koizumi J, Hamada S, Semiarti E, Torikai S, Machida Y (1997) Characterization of the transposition pattern of the *Ac* element in *Arabidopsis thaliana* using endonuclease I-*Sce*I. Proc Natl Acad Sci USA 94:8675–8680

Medberry SL, Dale E, Qin M, Ow DW (1995) Intra-chromosomal rearrangements generated by Cre-*lox* site-specific recombination. Nucleic Acids Res 23:485–490

Nakagawa Y, Machida C, Machida Y, Toriyama K (2000) Frequency and pattern of transposition of the maize transposable element *Ds* in transgenic rice plants. Plant Cell Physiol 41:733–742

Onouchi H, Yokoi K, Machida C, Matsuzaki H, Oshima Y, Matsuoka K, Nakamura K, Machida Y (1991) Operation of an efficient site-specific recombination system of *Zygosacharomyces rouxii* in tobacco cells. Nucleic Acids Res 23: 6373–6378

Onouchi H, Nishihama R, Kudo M, Machida Y, Machida C (1995) Visualization of site-specific recombination catalyzed by a recombinase from *Zygosaccharomyces rouxii* in *Arabidopsis thaliana*. Mol Gen Genet 247:653–660

Osbone BI, Wirtz U, Baker B (1995) A system for insertional mutagenesis and chromosomal rearrangement using the *Ds* transposon and Cre-*lox*. Plant J 7:687–701

Qin M, Bayley C, Stockton T, Ow DW (1994) Cre recombinase-mediated site-specific recombination between plant chromosomes. Proc Natl Acad Sci USA 91:1706–1710

Stuurman J, Vroomen MJ, Nijikamp HJJ, van Haaren MJJ (1996) Single-site manipulation of tomato chromosomes *in vitro* and *in vivo* using Cre-*lox* site-specific recombination. Plant Mol Biol 32:901–913

Vergunst AC, Hooykaas PJJ (1998) Cre/*lox*-mediated site-specific integration of *Agrobacterium* T-DNA in *Arabidopsis thaliana* by transient expression of *cre*. Plant Mol Biol 38:393–406

Wakita Y, Otani M, Shimada T (1998) Co-integration, co-expression and co-segregation of an unlinked selectable marker gene and *NtFAD3* gene in transgenic rice plants produced by particle bombardment. Genes Genet Syst 73:219–226