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Repetitive Homologous Sequences in Flanking Region of Gametophytic Self-Incompatibility Allele in *Lycopersicon peruvianum*

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Abstract : *Lycopersicon peruvianum* shows a gametophytic self-incompatibility (GSI). GSI is controlled by a single locus (*S* locus) with multiple alleles. *S* ribonucleases encoded in *S* alleles cosegregate with their phenotypes of GSI in genetic cross. To understand the genetic role of *S* allele in *L. peruvianum*, two large genomic fragments isolated previously were analyzed with total genomic DNAs from several tomato lines generated by cross-pollination. Southern blot analysis with the *S* allele fragments as probes revealed that the flanking region of *S* allele contained the highly homologous regions. It is speculated that they may play an important role to prevent genetic cross by self-pollination.
Key words : gametophytic self-incompatibility, *Lycopersicon peruvianum*, *S* allele.

Many species of flowering plants possess self-incompatibility (SI) mechanism that acts to prevent self-pollination and promotes cross-pollination (de Nettancourt, 1977). There are two general systems in homomorphic SI called as sporophytic SI (SSI) and gametophytic SI (GSI). These two major systems are distinguished by the phenotypes of pollen's *S* alleles; SSI phenotype of pollen is determined by two *S* alleles of the pollen parent, and GSI phenotype of pollen is controlled by one of two *S* alleles of the pollen parent (de Nettancourt, 1977). In the GSI family, rejection for self pollens occurs when *S* allele of haploid pollen is coincident to one of *S* alleles in the diploid style tissue. Self-pollens grow pollen tubes into the style, but they are subsequently arrested within the upper region of the transmitting tissue (for reviews, see Ebert *et al.*, 1989; Haring *et al.*, 1990; Newbigin *et al.*, 1993; Kao and Huang, 1994).

S glycoproteins of many plants in solanaceous family cosegregated with their respective *S* alleles in genetic cross (Anderson *et al.*, 1986; Mau *et al.*, 1986; Chung *et al.*, 1994b). Therefore, *S* glycoprotein has been known as a crucial factor of the SI in the solanaceous family. *S* glycoproteins from *Nicotiana alata* (McClure *et al.*, 1989), *Petunia inflata* (Ai *et al.*, 1990), *Solanum chacoense* (Xu *et al.*, 1990) and *L. peruvianum* (Chung, 1994) had ribonuclease activity which may cause the cytotoxic effect on self-pollen tube during growth in the style tissue (McClure *et al.*, 1990).

L. peruvianum has a gametophytic SI system, which is genetically controlled by a single locus with multiple alleles (*S* alleles) (Tanksley and Loaiza-Figueroa 1985; Rivers *et al.*, 1993). We isolated the three cDNA clones coding for *S* glycoprotein of *L. peruvianum* and showed that they cosegregated with their respective *S* alleles in genetic crosses (Chung *et al.*, 1993; Chung, 1994; Chung *et al.*, 1994). Moreover, two genomic clones, named λGST11 and λGST12, which contain S_{11} and S_{12} RNase coding regions were isolated from the genomic library of two *S* genotypes of *L. peruvianum*, respectively. Three short sequences highly homologous between the two *S* allele genes were found within 360 bp upstream of the ATG initiation codon. It was suggested that these sequences might be involved in the regulation of transcription of the *S* genes in *L. peruvianum* (Chung *et al.*, 1995).

In this report, the flanking region of the *S* allele gene was further investigated with various *S* genotypes using genomic λ fragments as probes in Southern blot. The flanking region of *S* allele of *L. peruvianum* contains the highly homologous regions.

Materials and Methods

Plant materials

Various *S* genotype plants used in this study were

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randomly chosen from the seed-stocks of Kagome Research Institute in Japan. Three tomato plants were self-pollinated to check their self-incompatibility using matured pollens of yellow-bud stage. All of them showed self-incompatibility. Then, these three abundantly flowering plants were used to determine their *S* genotypes by the reciprocal hand pollination and backcross. Each of these parent strains in this study used has different *S* genotype. So, *S* genotypes of these parent strains were named $S_{11}S_a$, $S_{12}S_b$ and $S_{13}S_c$, respectively.

DNA purification and Southern blot analysis

Genomic DNAs of various genotype plants $S_{11}S_a$, $S_{12}S_{12}$, $S_{12}S_b$, and $S_{13}S_c$ used for Southern blot analysis (Fig. 1) were purified using the modified CTAB method followed by standard CsCl gradient centrifugation (Chung *et al.*, 1994). Fifteen μg of genomic DNAs from various *S* genotypes, $S_{11}S_a$, $S_{12}S_{12}$ $S_{12}S_b$, and $S_{13}S_c$, were digested with *Eco*RI, separated by electrophoresis in a 1.0% (W/V) agarose tructions. Hybridization with various fragments from λGST12 as hybridization probes as shown in the upper panel of Fig. 1 and 2 was performed in a hybridization buffer (6× SSC, 0.5% SDS, 5× Denhardt's solution, and 100 μg/ml of denatured salmon sperm DNA) under moderate hybridization conditions (56°C). Filters were washed at a final stringency of 0.1× SSC and 0.1% SDS at 56°C. Autoradiography was performed for two days with an intensifying screen at −80°C.

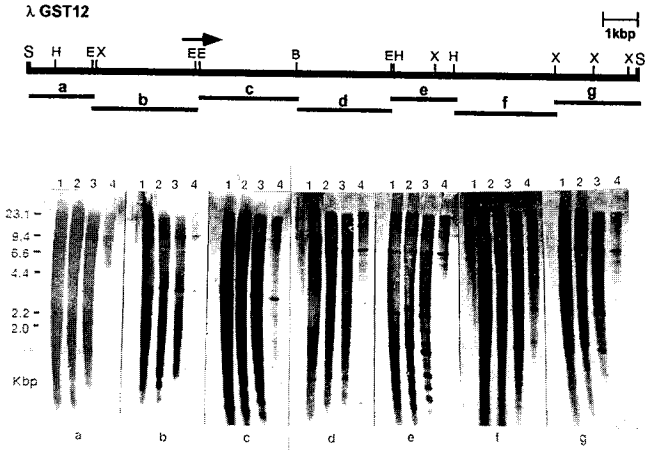

Fig. 1. Southern blot analyses of *L. peruvianum* DNA probed with fragments of genomic insert contained in λGTS12. Arrow on the restriction map indicates the coding region of cDNA for S_{12} RNase (Chung *et al.*, 1994). Abbreviations of the restriction enzymes are as follows: E, *Eco*RI; B, *Bam*HI; H, *Hind*III; S, *Sal*I; and X, *Xba*I. The hybridization probes are indicated at the top. Hybridization patterns of the *Eco*RI-digested genomic DNAs from different *S* genotypes: lane 1, $S_{11}S_a$; lane 2, $S_{12}S_{12}$; lane 3, $S_{12}S_b$; lane 4, $S_{13}S_c$ genotypes. The variation of signal intensity between individuals is likely to be due to variation in amount of DNA loaded. Molecular size markers are indicated at left in kilobase-pair.

Results and Discussion

To characterize the molecular structure of the flanking regions of the *S* genes, total genomic DNAs from the four genotypes and cloned DNA fragments of λGST11 and λGST12 were analyzed using seven fragments (probes a to g in the upper panel of Fig. 1 and 2) of λGST12 genomic insert as probes. As shown in panel A of Fig. 1, each probe hybridized to many bands of *Eco*RI digested DNAs from four different *S* genotypes and the patterns of hybridizing bands were almost similar. This result indicates that repetitive sequences in *S* allele gene are dispersed on the entire genome of *L. peruvianum*.

To identify the presence of homologous regions in the two λ clones (λGST11 and λGST12). *Eco*RI digested fragments from the two λ clones were analyzed by Southern blot hybridization. Hybridizing bands were not detected in λGST11 clone (Fig. 2). Unexpectedly, when the four fragments (fragments a, b, c and g) were used as probes for Southern blot hybridization, two or

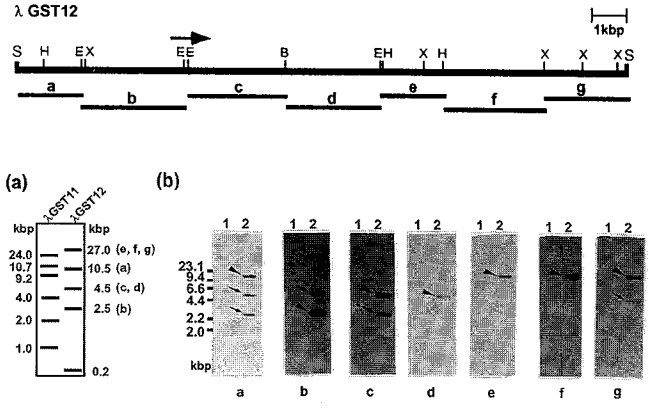

Fig. 2. Hybridization of *Eco*RI-digested genomic clones between two different *S* allele genes. Arrow on the restriction map indicates the coding region of cDNA for S_{12} RNase (Chung *et al.*, 1994). The hybridization probes are indicated at the top. Abbreviations of the restriction enzymes are as follows: E, *Eco*RI; B, *Bam*HI; H, *Hind*III; S, *Sal*I; and X, *Xba*I. (a) The restriction pattern of *Eco*RI-digested two genomic clones. Restriction fragments corresponding to probes in this study used are indicated at right in kilobase-pair. The fragments for probes a, e, f and g are included in the arm of λEMBL3 vector. (b) Hybridization of *Eco*RI-digested genomic clones: lane 1, λGST11; lane 2, λGST12. The expected bands corresponding to individual restriction fragments are indicated by arrowheads. Additional hybridizing bands are shown by arrows. Molecular size markers are indicated at left in kilobase-pair.

three bands, except for the expected site (Fig. 2, arrowheads), were detected in the four fragments from λGST 12 inserts (Fig. 2, arrows). For example, probes a and b detected three bands with *Eco*RI digested λGST12 genomic clones, with less intense bands at the same sites. In case of probe b, it is possible that the 27 kbp hybridizing band contains the arm of λEMBL3 vector. Probes c and g hybridized to the other b and c or d regions. This result indicates that the homologous sequences are scattered in the flanking region of the S_{12} allele gene.

When the fragments derived from the λGST12 (for S_{12} allele) were used as probes for Southern hybridization, highly homologous regions were detected in genomic DNAs from four different genotypes of *L. peruvianum* (Fig. 1). A similar result was obtained when the Southern blot analysis was carried out with various fragments of a genomic clone containing S_{11} allele (data not shown). When the flanking regions derived from the S_{11} and S_{12} allele genes were sequenced, highly repetitive sequences were detected within 360 bp upstream of the ATP initiation codon of two different genomic clones (Chung *et al.*, 1995). Repetitive DNA sequences have also been identified in the flanking regions of *S* allele genes of *N. alata* (Bernatzky *et al.*, 1989) and *P. inflata* (Coleman and Kao, 1992). In general, repeated DNA sequences on the chromosome is associated with generation of chromosome rearrangements (Charlesworth, 1991). However, the chromosomal regions of restricted recombination such as the mammalian Y chromosomes are also found to accumulate repetitive DNA sequences. It is suggested that they may be responsible for inhibition of genetic recombination on the X and Y chromosomes (Charlesworth, 1991). The flanking regions of S_{12} allele of *L. peruvianum* are located in the surround of coding region of S_{12} allele gene as well as further expanded regions in the large S_{12} genomic clone (Fig. 2). When the exposure times were 30 min, instead of overnight used in this experiment (Fig. 2), additional bands could not be detected in the DNA gel blot, and only the arrowhead marked bands were detected (data not shown). Therefore the length of homologous repeated sequences may be relatively small in size in the S_{12} allele of *L. peruvianum*. The accumulation of repetitive sequences of *S* allele are analogous to the mammalian Y chromosomes. However, the significance of the genetic role of repetitive sequences for *S* alleles is not clear yet. The functional role of repetitive sequences of *S* allele of *L. peruvianum* should be investigated.

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