Breakdown of Self-incompatibility in *Brassica* by the Antisense RNA of the *SLG* Gene

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Abstract : The self-incompatibility (SI) system of *Brassica* is sporophytically controlled by multiallenic genes at an *S*-locus. Two genes, *SLG* (*S*-locus glycoprotein) and *SRK*(*S*-receptor protein kinase), on the S-locus are thought to play an important role in recognition of the self-pollen, though there is no direct evidence. We introduced the antisense *SLG* gene to self-incompatible *Brassica* species through *Agrobacterium*-mediated transformation. We found that a transgenic plant of *B. rapa* (syn. *B. campestris*) could set seeds after self-pollination, and that no SLG protein was detectable in a gel blot analysis of the transgenic plant. These observations provide strong evidence that SLG and/or SRK are directly involved in the pollen-stigma recognition of SI.

Key words : Self-incompatibility; *Brassica*; *S*-locus glycoprotein; *S*-receptor protein kinase; transgenic plant; antisense RNA.

The self-incompatibility (SI) system in flowering plants prevents self-fertilization and promotes outcrossing with fertile hermaphrodite seed plants.[1] In *Brassica*, the SI reaction is sporophytically controlled by multialleic genes at the *S*-locus. Molecular analysis showed that a pair of genes, *SLG* and *SRK*, are on the *S*-locus. *SLG* encodes *S*-locus glycoprotein (SLG), and *SRK* the *S*-receptor protein kinase (SRK) that has an extracellular receptor domain with high homology to *SLG*. Various lines of circumstantial evidence suggest that SLG and SRK function in the recognition of self pollens in the papillar cells on the stigma,[2),3)] but, there is no direct evidence from transformation experiments for such a key function. A few attempts have been made to introduce these genes to self-incompatible and self-compatible *Brassica* species,[4)–6)] but no new SI phenotype has been obtained. This suggests that the "gain-of-function" approach for transgenic plants does not provide clear information on the function of SLG and SRK. We therefore used a "loss-of-function" approach to show the involvement of SLG and/or SRK in the SI system by introducing the antisense gene of *SLG* to self-incompatible *Brassica* species through *Agrobacterium*-mediated transformation.

In this paper we describe the transformation of self-incompatible *Brassica* plants with the antisense *SLG* gene resulting in the and breakdown of the SI system in a transformed plant.

We first constructed an antisense *SLG* gene from an SLG^8 cDNA.[7] The construction of this gene is shown in Fig. 1. The antisense gene was introduced into self-incompatible *B. rapa* by *Agrobacterium*-mediated transformation.[9] In this case, a hybrid promoter (Cauliflower Mosaic Virus (CaMV) 35S truncated promoter-SLG^8 promoter[8]) was used to direct the strong, pistil-specific transcription of antisense *SLG* in the transgenic plant.

Only one transgenic plant was obtained from 30 cotyledons, the properties of which were further analyzed. DNA gel blot analysis showed that this transgenic plant retained both the Km^R and SLG^8 genes and contained one copy of the transgene (data not shown).

Self-pollination was used to investigate whether the introduced antisense gene affected the elongation of pollen tubes in the transgenic plant. As is clear from Fig. 2, pollen tubes penetrated the stigma in the

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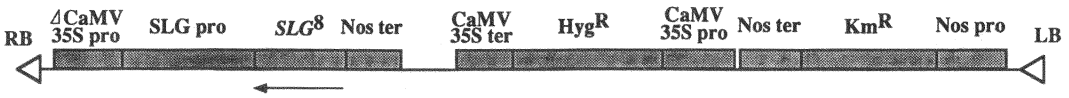

Fig. 1. Construction of the antisense *SLG* gene (pANTI4). Nos pro: nopaline synthase promoter from pBI121; Nos ter: nopaline synthase terminator from pBI121; Km^R: the kanamycin-resistance gene from pBI121; Hyg^R: the hygromycin-resistance gene from pBI303; RB and LB: right and left borders of T-DNA. An arrow under SLG^8 indicates the 5′ to 3′ orientation of the gene.

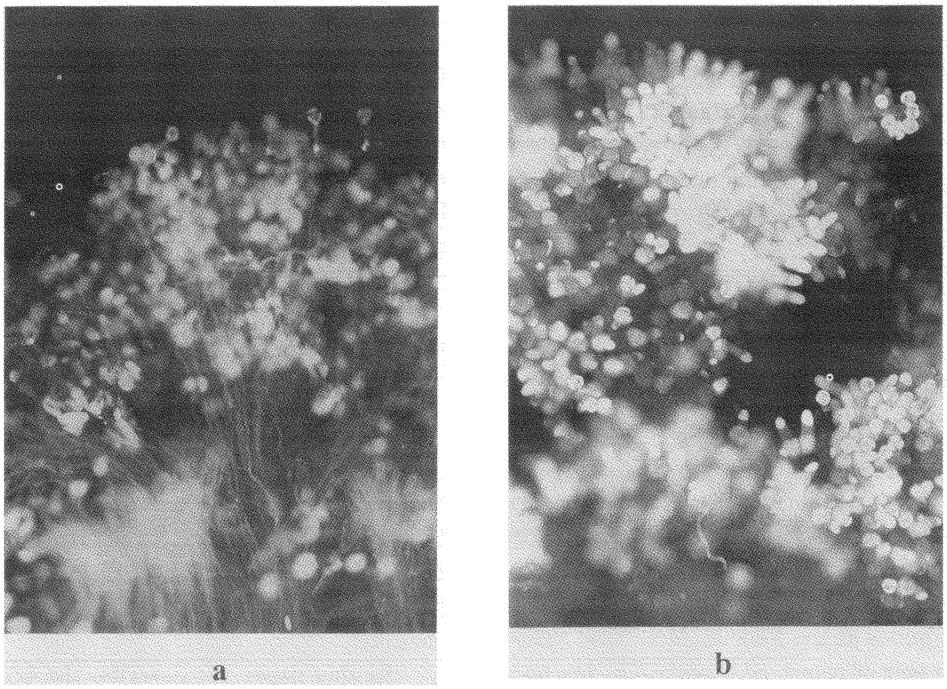

Fig. 2. Pollen behavior at the stigma surface after self-pollination in transgenic and untransformed *Brassica* plants. **a,** transgenic plant; **b,** untransformed control. Bar=40 μm.

transgenic plant, but the control plants had few pollen tubes and many callose plugs on the papillar cells. In addition, the transgenic plant set high levels of seeds by self-pollination (about 80% of the total flowers), whereas the control plants set few seeds (less than 3% of the total flowers). These results clearly show that the antisense gene broke down the SI system in the transgenic plant.

The presence of the SLG protein was examined to determine whether the breakdown of SI was caused by inhibition of the *SLG* gene. Stigma proteins were subjected to IEF-immunoblot analysis in order to estimate the quantity of SLG in the transgenic plant. As the result, no bands were shown in the range of pI 3.5 to 9.5, even though the control plants had a high level of SLG, approximately pI 8.15 as shown in Fig. 3.

We used the SLG^8 cDNA isolated from self-incompatible *B. campestris* S^8 homozygotes[10] as an antisense SLG gene to transform plants because it

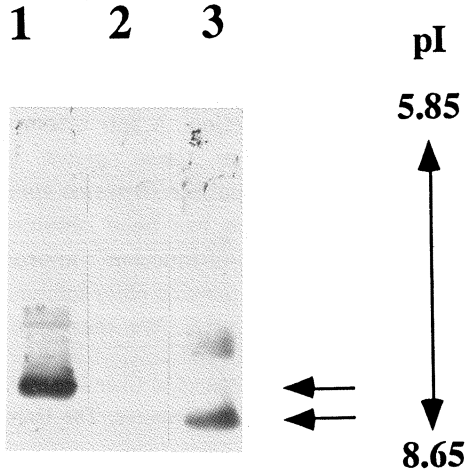

Fig. 3. IEF-immunoblot analysis of the protein extracts from the untransformed control (lane 1), transgenic *Brassica* (lane 2) and *B. campestris* S^8 homozygote (lane 3). The arrows indicate the SLG bands. The pI gradient is shown at right.

hybridized to the genomic DNA of the recipient *B. rapa* under high stringency hybridization conditions. Multiple bands showing *SLG* and *SLG*-like genes of the recipient plants were detected as was the case for *Brassica* species.[7),11)] Further, the *SLG* shares more than 90% DNA sequence homology with those of the *Brassica* species, except for the class II *S* allele.[12)] Therefore, it is likely that the antisense RNA of SLG^8 inhibits the endogenous *SLG* gene of the recipient. Furthermore, we could assume that the endogenous *SRK* transcript also would be inhibited by antisense SLG^8 mRNA because of the high DNA sequence similarity between the *SLG* gene and the *SLG* homologous region of the *SRK* gene.[4)]

Thus, in this paper we have been able to show that the introduction of the antisense *SLG* gene broke down the SI system of transgenic *Brassica* and that no SLG protein was detectable in the transgenic plant. The introduced antisense gene should inhibit an SLG transcript and probably SRK transcript as well. These observations provide strong evidence that SLG and/or SRK are involved in the pollen-stigma recognition of SI.

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References

1) Nettancourt, D.d. (1977) Incompatibility in Angiosperms. Springer-Verlag, Berlin, pp. 1–230.
2) Hinata, K., Watanabe, M., Triyama, K., and Isogai, A. (1993) International Review of Cytology **143**, 257–296.
3) Nasrallah, J. B., and Nasrallah, M. E. (1993) Plant Cell **5**, 1325–1335.
4) Stein, J. C., Howlett, B., Boyes, D. C., Nasrallah, M. E., and Nasrallah, J. B. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 8816–8820.
5) Sato, T., Thorsness, M. K., Kandasamy, M. K., Nishio, T., Hirai, M., Nasrallah, J. B., and Nasrallah, M. E. (1991) Plant Cell **3**, 867–876.
6) Toriyama, K., Stein, J. C., Nasrallah, M. E., and Nasrallah, J. B. (1991) Theor Appl. Genet **81**, 769–776.
7) Yamakawa, S., Shiba, H., Watanabe, M., Shiozawa, H., Takayama, S., Hinata, K., Isogai, A., and Suzuki, A. (1994) Biosci. Biotech. Biochem. **58**, 921–925.
8) Dwyer, K. G., Balent, M. A., Nasrallah, J. B., and Nasrallah, M. E. (1991) Plant Molecular Biology **16**, 481–486.
9) Randke, S. E., Turner, J. C., and Facciotti, D. (1992) Plant Cell Reports **11**, 499–505.
10) Nou, I. S., Watanabe, M., Isogai, A., Shiozawa, H., Suzuki, A., and Hinata, K. (1991) Jpn. J. Genet **66**, 227–239.
11) Dwyer, K. G., Chao, A., Cheng, B., Chen, C.-H., and Nasrallah, J. B. (1989) Genome **31**, 969–972.
12) Nasrallah, J. B., Nishio, T., and Nasrallah, M. E. (1991) Annu. Rev. Plant Mol. Biol. **42**, 393–422.