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Expression of the *S* receptor kinase in self-compatible *Brassica napus* cv. Westar leads to the allele-specific rejection of self-incompatible *Brassica napus* pollen

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Abstract Expression of an *S* receptor kinase (SRK_{910}) transgene in the self-compatible *Brassica napus cv.* Westar conferred on the transgenic pistil the ability to reject pollen from the self-incompatible *Brassica napus* W1 line, which carries the S_{910} allele. In one of the *SRK* transgenic lines, 1C, virtually no seeds were produced when the transgenic pistils were pollinated with W1 pollen (Mean number of seeds per pod = 1.22). This response was specific to the W1 pollen since pollen from a different self-incompatible *Brassica napus* line (T2) and self-pollinations were fully compatible. Westar plants expressing an *S* locus glycoprotein transgene (SLG_{910}) did not show any self-incompatibility response towards W1 pollen. Transgenic Westar plants resulting from crosses between the 1C *SRK* transgenic line and three SLG_{910} transgenic lines were also tested for rejection of W1 pollen. The additional expression of the SLG_{910} transgene in the SRK_{910} transgenic plants did not cause any significant further reduction in seed production (Mean seeds/pod = 1.04) or have any detectable effects on the number of pollen grains that adhered to the pistil. Thus, while the allele-specific *SLG* gene was previously reported to have an enhancing effect on the self-incompatibility response, no evidence for such a role was found in this study.

Key words *Brassica napus* · *S* locus receptor kinase (SRK) · *S* locus glycoprotein (SLG) · Self-incompatibility · Signal transduction

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Introduction

In many flowering plants the close proximity of the anther and pistil can promote self-pollination and inbreeding. Since genetic variability plays an important role in reproductive success, many flowering plants have developed self-incompatibility systems to enhance out-crossing by preventing self-fertilization through the rejection of self-pollen (for review, see de Nettancourt 1997; Gaude and McCormick 1999). In *Brassica*, self-incompatibility is genetically controlled by a single highly polymorphic *S* locus, and self pollen as well as pollen from a plant sharing a common *S* allele is rejected (for review, see Cock 2000). The *S* locus has turned out to be a complex region composed of many genes (Cui et al. 1999; Suzuki 1999; Casselman et al. 2000). Thus, loss-of-function studies are not sufficient to determine which genes are required for the self-incompatibility phenotype. Recently, the *SCR* gene was shown to encode the male determinant of self-incompatibility through gain-of-function experiments in which it was shown to confer the pollen *S* phenotype (Schopfer et al. 1999; Takayama et al. 2000).

For the pistil, two genes linked to the *S* locus have been implicated in determining the self-incompatibility phenotype, the *S* locus glycoprotein (*SLG*) and the *S* receptor kinase (*SRK*) genes (Nasrallah et al. 1985; Stein et al. 1991). Both genes are predominantly expressed in

the pistil, and the SLG shows a high degree of sequence identity to the putative extracellular domain of SRK (Stein et al. 1991). The SLG is secreted to the stigmatic papillar cell wall at the top of the pistil, while the SRK is a membrane protein in the stigma (Kandasamy et al. 1989; Delorme et al. 1995). Cosupression of the endogenous SLG gene in transgenic SLG plants resulted in a loss of self-incompatibility suggesting a requirement for SLG in this response (Toriyama et al. 1991; Takasaki et al. 1999). However, since the *SLG* and *SRK* genes share a high degree of sequence identity, the expression of both genes was probably affected in these cosuppression studies, as demonstrated by Conner et al. (1997). Support for a requirement for SLG in self-incompatibility also comes from the analysis of a self-compatible *Brassica* line in which a mutation in an unlinked gene was found to cause down-regulation of *SLG*, but not *SRK* expression (Nasrallah et al. 1992). Evidence for the requirement of SRK in self-incompatibility was provided by studies on two self-compatible *Brassica* lines which were found to carry mutations in the *SRK* gene (Goring et al. 1993; Nasrallah et al. 1994). In addition, a breakdown of self-incompatibility in the pistil was observed following the expression of a dominant-negative SRK variant (Stahl et al. 1998). Recently, Takasaki et al. (2000) demonstrated, through transformation experiments with the self-incompatible S_{60} line of *B. campestris*, that SRK_{28} encoded the female determinant of self-incompatibility and that SLG_{28} enhanced the self-incompatibility phenotype in the pistil. We have also recently shown that BAC clones carrying the *S* locus region that included both the SLG_{910} and SRK_{910} genes dominantly conferred the self-incompatibility trait on the pistil when transformed into self-compatible *B. napus cv.* Westar (Cui et al. 2000). The purpose of this study was to determine if SRK_{910} alone could dominantly confer the self-incompatibility trait in the pistil of self-compatible *B. napus cv.* Westar, and if SLG_{910} played either an essential or an auxiliary role in the self-incompatibility response.

Materials and methods

Generation of transgenic plants

The SRK_{910} cDNA and the SLG_{910} cDNA were separately cloned under the control of the *S* locus-related (SLR)-1 promoter, which directs high-level expression in the stigma (Franklin et al. 1996). The transformation vectors were then individually transformed into the self-compatible Westar cultivar using *Agrobacterium*-mediated transformation as previously described (Sulaman et al. 1997). The resulting SRK_{910} transgenic plants and SLG_{910} transgenic plants were selfed to produce the F1 generation of plants for analysis.

Genomic DNA analysis

The presence of the transgene was determined by PCR and genomic Southern analysis. For the PCR, primers specific to the SLG_{910} or SRK_{910} genes were used to amplified the transgene from genomic DNA. For the genomic Southern analysis, genomic DNA was extracted as previously described (Goring et al. 1992b). Then 10-μg aliquots of genomic DNA were digested with *Hin*dIII, which cuts once at the start of the *SLR1* promoter. *Hin*dIII was chosen since potential cross-hybridizing bands have been well characterized (Goring et al. 1992a; Goring and Rothstein 1992; Goring et al. 1993). The digested DNA was fractionated by gel electrophoresis and transferred to a nylon membrane, and hybridized to the SRK_{910} probe or to a mixture of the SRK_{910} and SLG_{910} probes. Membranes were then washed at 68°C in 1 × SSC and 0.1% SDS.

RNA analysis

Expression of the transgenes was analyzed by RT-PCR and RNA blot hybridization. Total RNA was extracted following the procedure described in Cock et al. (1997). For RT-PCR, total RNA was used in a reverse transcription reaction with the M-MLV reverse transcriptase. For each sample, a second reaction containing all components except the reverse transcriptase was also set up as a control. The resulting cDNA was amplified by PCR using SLG_{910}- or SRK_{910}-specific primers for a total of 25 cycles. The products were separated by gel electrophoresis, blotted onto filters and hybridized with the appropriate probes.

For the RNA blot analysis, 40 μg of total RNA, extracted from the top half of pistils (stigma + style), was fractionated on a formaldehyde gel, transferred to a membrane, hybridized and washed as described above.

Pollinations

Pollinations for seed production and for fluorescence microscopy were carried out as described before (Sulaman et al. 1997; Stone et al. 1999). One day prior to anthesis, buds were stripped of anthers and petals and bagged to prevent contaminating pollen grains from landing on the stigma. Pistils were pollinated the following day. To determine seed production levels, pollinated pistils were left bagged for 1 week; then bags were removed to allow seed development. Two to three weeks later pods were dried and seeds were counted. For pollen adhesion studies, pistils were removed 16 h following pollination and fixed in ethanol:glacial acetic acid (3:1) solution for at least 15 min. After rinsing three times with water, the pistils were treated with 1 N NaOH at 60°C for 1 h, followed by another three rinses with water. The pistils were then treated with a solution of 0.1% aniline blue in 0.1 M K_3PO_4 pH 7.0 buffer for approximately 1 h and wet mounted on slides for UV fluorescence microscopy. The number of pollen grains remaining adhered following this treatment was counted.

Results

Expression of SRK_{910} in the self-compatible *B. napus* cultivar Westar

Previous work on a self-incompatible *B. napus* W1 line led to the isolation of the SLG_{910} and SRK_{910} cDNAs which showed linkage to the self-incompatibility phenotype in W1 and were expressed in the pistil (Goring et al. 1992a; Goring and Rothstein 1992). To determine if SRK_{910} was sufficient to confer self-incompatibility on the pistil, the SRK_{910} cDNA, driven by the strong stigma-specific *SLR1* promoter (Franklin et al. 1996), was transformed into the *B. napus* line Westar. Westar does not possess a functional self-incompatibility system, and is self-compatible and fully compatible with W1. If SRK_{910} is sufficient to confer self-incompatibility, then

the transgenic plants should gain the ability to reject W1 pollen and fail to produce seeds. Six primary transformants were generated and self-pollinated to produce the next generation of transgenic plants for analysis.

To test for the rejection of W1 pollen, pistils from the transgenic plants were pollinated with W1 pollen and seed production was analyzed. In two of the six lines (1C and 2A), rejection of W1 pollen occurred only when the *SRK* transgene was present (Fig. 1A, plants 1C-4 to 12 and 2A-6 to 19). All of the siblings which did not carry the transgene were compatible with W1 pollen, and seed production was normal (Fig. 1A, plants 1C-1 to 3 and 2A-1 to 5). Thus, in two independent lines, SRK_{910} alone could confer the self-incompatibility trait in the pistil, leading to the rejection of W1 pollen.

In the 1C line, all of the transgenic plants showed very efficient pollen rejection and little or no seed set (Fig. 1A; plants 1C-4 to 12). The overall mean seed set was 1.3 seeds/pod and, on average, 38% of the pollinations failed to produce any seed. Pollination of 1C transgenic pistils with pollen from a different self-incompatible *B. napus* line, T2 (Goring et al. 1992b; Glavin et al. 1994), resulted in full seed production, indicating that pollen rejection was specific for the S_{910} allele in the W1 line (data not shown). Self-pollinations also resulted in full seed production, indicating that the transgenic plants were fully fertile, and the pollen therefore had not acquired the self-incompatibility trait – as expected (Fig. 1B). Southern analysis showed that the 1C line carries a single transgene on a *Hin*dIII fragment of approximately 8.5 kb (Fig. 2A). Due to cross-hybridization with some closely related sequences in the Westar genome (see Fig. 2A, lanes 1, 3–5), PCR with SRK_{910}-specific primers was also used to confirm the presence or absence of the transgene (data not shown). Expression of the transgene was studied using RT-PCR with SRK_{910}-specific primers (Fig. 3A). All of the transgenic plants expressed the SRK_{910} transgene, and no expression was detected in the non-transgenic siblings (Fig. 3A).

In the 2A line, pollen rejection was not as efficient as in the 1C line and was highly variable, ranging from 2.8 seeds/pod for plant 2A-14 to 17.3 seeds/pod for plant 2A-15 (Fig. 1A). However, all of the 2A transgenic plants showed lower seed production values (Fig. 1A, plants 2A-6 to 19) when compared with siblings which did not carry the transgene (Fig. 1A, plants 2A-1 to 5) or when compared with self-pollinated plants (Fig. 1B).

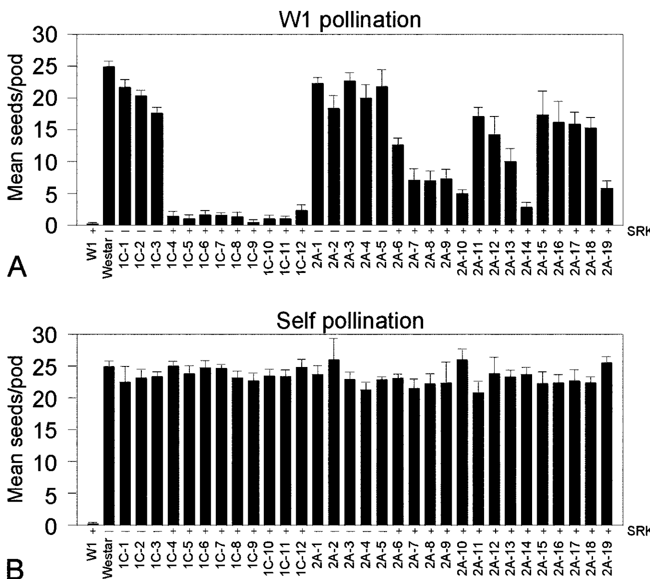


Fig. 1A, B Seed production in the two SRK_{910} transgenic lines, 1C and 2A. **A** Mean number of seeds per pod following pollination of transgenic pistils with incompatible W1 pollen. **B** Mean number of seeds per pod following self-pollination. Westar is the compatible control and produces ample numbers of seeds when self-pollinated and cross-pollinated. W1 is the self-incompatible control and produces little or no seed when self-pollinated. If the SRK_{910} transgene confers the pistil self-incompatibility trait, then the W1 pollen should be rejected while self-pollinations should be compatible. (*Error bars* indicate ± standard error)

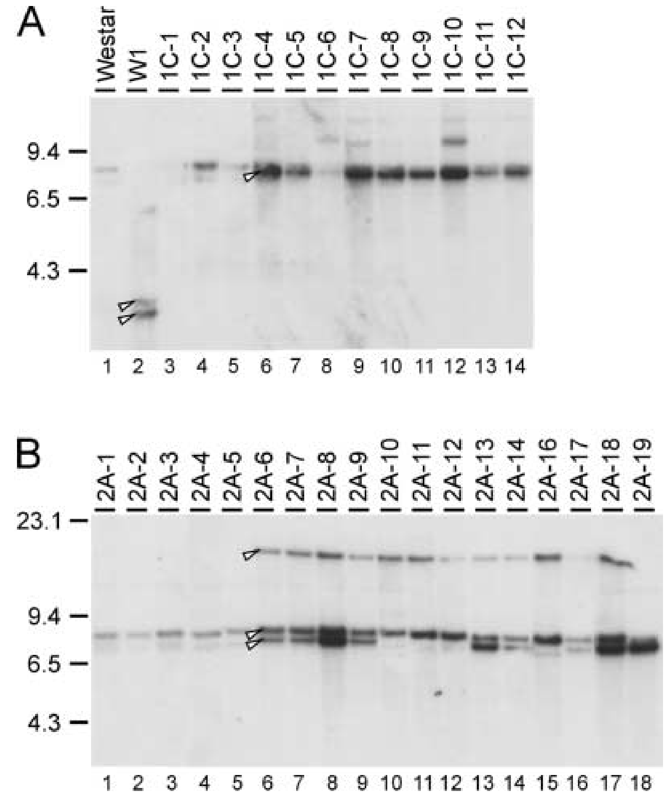


Fig. 2A, B Southern analysis of transgenic plants carrying the SRK_{910} transgene. Approximately 10 μg of genomic DNA was digested with *Hin*dIII (one *Hin*dIII site is present in the transformation vector) and hybridized with ^{32}P-labeled SRK_{910} cDNA. **A** The 1C SRK_{910} transgenic line. **B** The 2A SRK_{910} transgenic line. 2A-15 is not shown on this blot. The *open arrowhead* in lane 1C-4 (**A**) marks the SRK_{910} transgene. Bands in the W1 lane (**A**) represent the endogenous SRK_{910} gene (*open arrowheads*). Faint bands below the 9.4-kb marker in the Westar lane and in the plants that do not carry the transgene (1C-1 to 3; 2A-1 to 5) represent cross-hybridization to the non-functional SLG_{A10} and SRK_{A10} genes as previously described (Goring et al. 1993). All genotypes were confirmed by PCR analysis of genomic DNA with SLG_{910}- and SRK_{910}-specific primers (data not shown)

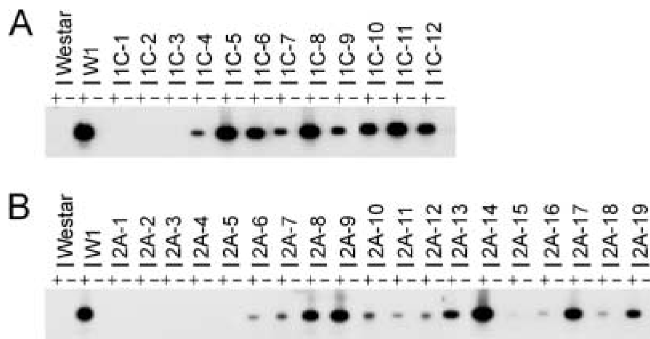


Fig. 3A, B RT-PCR analysis of SRK_{910} transgene expression in the 1C (**A**) and 2A (**B**) lines. The + symbol indicates the lanes where reverse transcriptase was added to the RT-PCR reaction, while the – symbol indicates that reverse transcriptase was not added and the reaction served as a control for potential genomic DNA contamination. All – lanes were negative. The RT-PCR samples were amplified for 25 cycles, and the products were subjected to gel electrophoresis, and then blotted and hybridized with a ^{32}P-labeled SRK_{910} cDNA. The signal for 2A-15 in the (+) lane is faint, but was clearly detected after a longer exposure (data not shown)

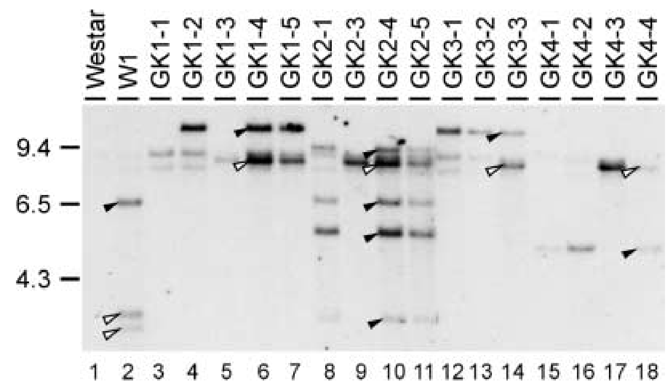


Fig. 4 Southern analysis of transgenic plants carrying the SLG_{910} or SRK_{910} transgene or both transgenes. Approximately 10-μg aliquots of genomic DNA were digested with *Hin*dIII, and hybridized with a mixture of ^{32}P-labeled SLG_{910} cDNA and SRK_{910} cDNA. GK2-2 is not shown on this blot. The *filled arrowheads* mark the SLG_{910} transgene and the *open arrowheads* mark the SRK_{910} transgene. A representative plant for each line is marked. Bands in the W1 lane represent the endogenous SLG_{910} and SRK_{910} genes. Faint bands just below the 9.4 kb marker in some of the lanes containing more genomic DNA (e.g. lanes 3, 4 and 12) represent cross-hybridization to the non-functional SLG_{A10} and SRK_{A10} genes as previously described (Goring et al. 1993). All genotypes were confirmed using PCR with SLG_{910}- and SRK_{910}-specific primers (data not shown). The only discordant result was obtained with GK4-3, which does not show a visible band for SLG_{910}, yet is consistently positive when tested for the presence of the transgene using PCR with SLG_{910}-specific primers and shows SLG_{910} expression both on the RNA blot (Fig. 1B) and with RT-PCR using SLG_{910}-specific primers (data not shown)

The mean seed production for the 2A transgenic plants of 11 seeds/pod was significantly different from the means of 21 seeds/pod for the non-transgenic siblings and 23 seeds/pod for the self-pollinations. In the 2A line, there are three copies of the transgene (Fig. 2B, lanes 6–9, 13, 14, 16, 17) though some of the transgenic plants carried only a subset of these transgenes (Fig. 2B, lanes 10–12, 15, 18). While expression of the transgene could be detected by RT-PCR (Fig. 3B), it appeared to be more variable than in the 1C transgenic plants. Thus, the expression and copy number of the SRK_{910} transgene appeared to be less stable in the 2A line, and this instability could account for the variable seed production observed.

Expression of SRK_{910} and SLG_{910} in the self-compatible *B. napus* cultivar Westar

To investigate if SLG might have an additive effect on SRK, we examined the self-incompatibility response in the 1C line in the presence or absence of SLG_{910}. As in the case of the SRK_{910} transgenic plants, Westar plants were transformed with the *SLR1* promoter driving the SLG_{910} cDNA. The 1C line was crossed to three independent SLG_{910}-expressing lines (the T1 generations were used for the crosses). Four resulting lines (GK1, GK2, GK3, GK4) were analyzed; GK1 and GK3 resulted from crosses to the same *SLG* line, and GK2 and GK4 resulted from crosses to two other independent *SLG* lines. The presence of the transgenes was determined by Southern analysis, and all of the transgenes were present either as a single copy or in low copy number (Fig. 4). The *SRK* transgene from the 1C line migrates at approximately 8.5 kb (Fig. 4). The *SLG* transgene migrates at 11 kb in the GK1 and GK3 lines, at 9, 6.5, 5.4, and 3.4 kb in the GK2 line, and at 4.9 kb in the GK4 line (Fig. 4). The SLG_{910} transgene by itself is present in GK1-2, 2-1, 2-2, 3-1, 3-2, 4-1, and 4-2; the SRK_{910} transgene is present by itself in GK1-3 and 2-3; and both transgenes are present in GK1-4, 1-5, 2-3, 2-4, 3-3, 4-3 and 4-4 (Fig. 4). In the GK1 line, 1-1 is a sibling which does not carry a transgene. Expression of the transgenes was tested by RNA blot analysis (Fig. 5). All transgenic plants showed expression of their respective transgenes at levels similar to the endogenous SLG_{910} and SRK_{910} genes in W1 (Fig. 5A and B, lane 2), with the exception of the SLG_{910} transgene in the GK2 line which was expressed at lower levels (Fig. 5A). SRK_{910} transgene mRNA levels (Fig. 5B) were calculated to represent 56% of that in the W1 line, which is homozygous for S_{910}. Therefore, the SRK_{910} transgene was expressed at levels roughly equivalent to those in an S_{910} heterozygote.

The transgenic plants were tested for their ability to reject W1 pollen by determining seed production following pollination with W1 pollen. Seed counts are shown in Fig. 6 and representative seed pods are shown in Fig. 7. Rejection of W1 pollen leading to little or no seed production was correlated with the presence of the SRK_{910} transgene (Figs 6A and 7). The SLG_{910} transgenic plants were fully compatible with W1 pollen (Fig. 6A; Fig. 7, GK1-2), as was the sibling not carrying a transgene (Fig. 6A; Fig. 7, GK1-1). No noticeable differences were seen between the SRK_{910} transgenic

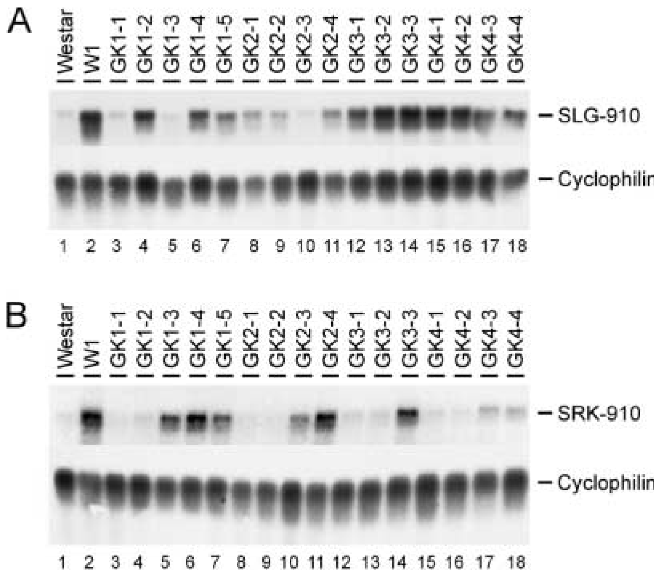


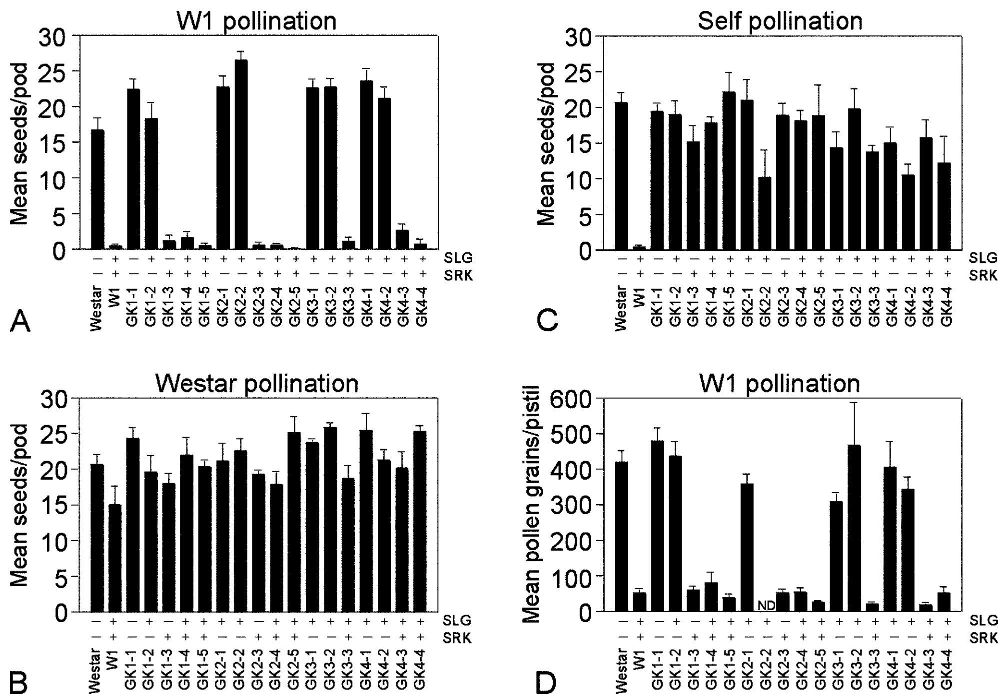
Fig. 5A, B Northern analysis of transgenic plants carrying either the SLG_{910} or SRK_{910} transgene or both. **A** Expression of the SLG_{910} transgene. **B** Expression of the SRK_{910} transgene. Approximately 40 μg of total RNA extracted from the stigma/style was hybridized to either ^{32}P-labeled SLG_{910} cDNA to detect the 1.5-kb SLG_{910} transcript or with ^{32}P-labeled SRK_{910} cDNA to detect the 3-kb SRK_{910} transcript. The blots were subsequently hybridized with a cyclophilin probe as a control for even loading. GK2-5 is not shown on these blots. Faint bands in some lanes (*SLG*, lanes 1, 3, 5 and 10; *SRK*, lanes 1, 3, 4, 8, 9, 12, 13, 15 and 16) represent cross-hybridization to the non-functional SLG_{A10} or SRK_{A10} mRNAs as previously described (Goring et al. 1993). Transgene expression was also confirmed by RT-PCR using SLG_{910}- and SRK_{910}-specific primers (data not shown)

plants and the SLG_{910}/SRK_{910} double transgenic plants (Figs. 6A and 7). Control pollinations with compatible Westar pollen resulted in full seed production, indicating that all the transgenic plants were fertile and ruling out other possible causes for the lack of seed production with the W1 pollen (Figs. 6B and 7). Self-pollinations also resulted in normal seed production, indicating that the pollen from the transgenic plants remained compatible as expected (Fig. 6C).

When the data shown in Figs. 1A and 6A are combined, the mean seed production for the SRK_{910} transgenic plants was 1.22 seeds/pod (11 plants, 60 pistils) and the mean seed production for the SLG_{910}/SRK_{910} double transgenic plants was 1.04 seeds/pod (7 plants, 57 pistils). The number of seeds/pod produced by the SRK_{910} transgenic plants is not significantly different from that produced by the SLG_{910}/SRK_{910} double transgenic plants ($t_{16} = 0.54$, $P \geq 0.59$). Thus, SRK alone caused a strong self-incompatibility reaction towards the W1 pollen and no enhancing effect could be detected in the presence of SLG_{910}.

While any effects resulting from SLG_{910} were not apparent at the level of seed set, there may be more

Fig. 6A–D Seed production in SLG_{910}, SRK_{910}, and SLG_{910}/SRK_{910} transgenic plants. **A–C** Mean numbers of seeds per pod. Means represent at least 6 pods per plant (with the exception of three compatible pollinations where 4–5 pods were used). **A** Incompatible W1 pollen on transgenic pistils. **B** Compatible Westar pollen on transgenic pistils. **C** Self-pollination. **D** Mean numbers of pollen grains adhering to the stigmatic surface after pollination with W1 pollen. Means represent pollen grain counts from 3–10 pistils per plant. ND, not determined. Westar is the compatible control and produces ample numbers of seeds when self-pollinated and cross-pollinated. W1 is the self-incompatible control and produces little or no seed when self-pollinated. If the SLG_{910} and/or the SRK_{910} transgenes confer the pistil self-incompatibility trait, then the W1 pollen should be rejected and Westar pollen should be compatible. (*Error bars* indicate ± standard error)



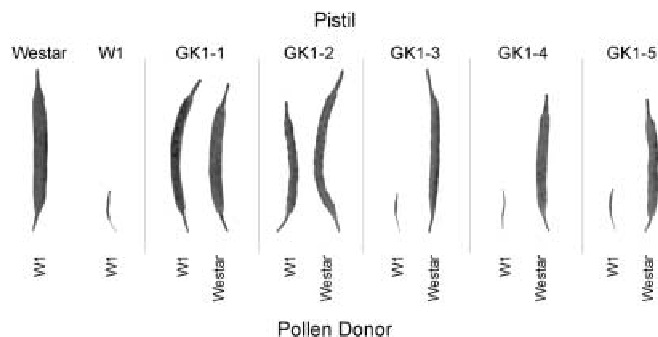


Fig. 7 Seed pods from transgenic pistils pollinated with W1 and Westar pollen. Westar is the compatible control and has a fully developed pod. W1 is the self-incompatible control and the pod is very small owing to the lack of seeds. The pods from the GK1 line are shown as representative samples

subtle effects earlier on. Pollination is followed by a series of steps, starting with pollen adhesion, followed by pollen germination, pollen tube growth, and fertilization. We have previously found that loss of ARC1, a component of the self-incompatibility response, leads to a large increase in the number of pollen grains that adhere to the stigma surface, but only a small fraction of the pollen can successfully fertilize the plant (Stone et al. 1999). To look at these stages in more detail, pistils pollinated with W1 pollen were left for 16 h and then examined by fluorescence microscopy. The number of pollen grains that remained adhered to the stigmatic papillae following fixation and staining were then counted. The transgenic plants transformed with only SLG_{910} showed a large number of pollen grains adhering to the pistil surface, like the self-compatible Westar and non-transgenic GK1-1 controls (Fig. 6D). These pollen grains had germinated to produce pollen tubes which were growing down the pistil (data not shown). The SRK_{910} transgenic plants and the SLG_{910}/SRK_{910} double transgenic plants showed low numbers of pollen grains adhering to the surface, similar to the self-incompatible W1 control (Fig. 6D). Approximately 50% of these pollen grains had adhered to the stigmatic papillae, but had not germinated. The remaining pollen grains had germinated, but pollen tube growth into the stigma was blocked and the pollen tube was curled on the pistil surface (data not shown). Thus, no apparent differences could be detected between the SRK_{910} transgenic plants and the SLG_{910}/SRK_{910} double transgenic plants.

Discussion

In this study, we have shown that only SRK_{910} is required to confer the self-incompatibility trait in the pistils of *B. napus cv.* Westar plants. SLG_{910} has no detectable effects on the self-incompatibility phenotype, either on its own or in the presence of SRK_{910}. This raises the question of the function of SLG, given that it shows a high level of sequence identity to the extracellular domain of SRK and is expressed abundantly in the pistil. SLG has been proposed to be required for the initial stages of pollen adhesion in compatible pollinations (Luu et al. 1999), and for SRK stabilization (Dixit et al. 2000). The results of this study suggest that the allele-specific SLG_{910} is not required for SRK_{910} stabilization. However, one cannot rule out the possibility that other related proteins are fulfilling this role. *B. napus* cv. Westar expresses the SLG_{A10} gene from the non-functional S_{A10} allele (Goring et al. 1993). In addition, other genes such as *SLR1* and *SLR2*, which are more distantly related, are expressed in Westar (Goring et al. 1992a, 1992b). Thus, while there may not be a clear role for the abundant SLG in the *Brassica* self-incompatibility response, this study demonstrates that the allele-specific SLG is not required. In addition, if other related proteins are involved, they are able to fully compensate for the absence of the allele-specific SLG.

Takasaki et al. (2000) have also shown that SRK encodes the female determinant of self-incompatibility in *B. campestris*. This was demonstrated by the transformation of the SRK_{28} gene into a different self-incompatible *B. campestris* line, S_{60}, which resulted in a gain-of-function, allowing the transgenic pistil to reject S_{28} pollen and produce very low levels of seeds. These authors also found that SLG_{28} on its own could not confer the self-incompatibility trait. The Takasaki et al. (2000) study differs from this study in that they found that SLG_{28} could influence the self-incompatibility response in the presence of SRK_{28}. The effects of three different SLGs on SRK_{28}-mediated rejection of S_{28} pollen were studied: (1) SLG_{60}, which shares 65% amino acid sequence identity with the extracellular domain of SRK_{28}; (2) SLG_{52}, which shares 76% identity with the extracellular domain of SRK_{28}; and (3) the allele-specific SLG_{28}, which is 98% identical to the extracellular domain of SRK_{28}. Upon pollination with S_{28} pollen, SRK_{28} transgenic plants in the presence of the endogenous SLG_{60} produced an average of 1.9 seeds/pod; SRK_{28} transgenic plants in the presence of SLG_{60} and transgenic SLG_{52} produced an average of 1.1 seeds/pod; and SRK_{28} transgenic plants in the presence of SLG_{60} and transgenic SLG_{28} produced an average of 0.3 seeds/pod. Thus, Takasaki et al. (2000) concluded that as the degree of sequence identity between the SLG and SRK_{28} increased there was an accompanying enhancement of the self-incompatibility response.

In this present study, the SRK_{910} transgenic plants produced a mean of 1.22 seeds/pod, and the mean seed production for the SLG_{910}/SRK_{910} double transgenic plants was 1.04 seeds/pod. There is no statistically significant difference between these means. The different conclusions regarding the role of SLG in the self-incompatibility response could be due to differences in the systems studied. For example, the levels of the transgenic *SRK* mRNAs differ, with Takasaki et al. (2000) reporting transgenic SRK_{28} mRNA levels equivalent to 32–35% of those in a S_{28} heterozygote, while in this study, the transgenic SRK_{910} mRNA levels were on

average the same as in a W1 heterozygote. Thus, the higher transgenic SRK_{910} mRNA levels may account for the lack of effect following the addition of the SLG_{910} transgene. Other perhaps relevant differences include the *Brassica* species used, the compatibility status of the transformed plant, and the *S* allele used. The degree of identity between the *B. campestris* SLG_{28} and SRK_{28} proteins (98%) is unusually high for an SLG/SRK pair. SLG_{910} shares 84% identity with the extracellular domain of SRK_{910}. Other related proteins which could be compensating for the absense of SLG_{910} share even lower amino acid identities to the extracellular domain of SRK_{910}. For example, SLG_{A10} (from the non-functional S_{A10} allele), SLR2, and SLR1 are predicted to share 77%, 66% and 56% amino acid identity, respectivly, to the extracellular domain of SRK_{910}.

The results obtained following the transformation of SRK_{910} into the self-compatible Westar indicate that the signaling pathway to be activated by SRK in the pistil is functional and that the self-compatible nature of Westar is due to *S* locus mutations, as previously reported (Goring et al. 1993). Recently, the *SCR* gene has been shown to confer the pollen self-incompatibility trait and is predicted to encode a small polypeptide which is thought to be the ligand for SRK (Schopfer et al. 1999; Takayama et al. 2000). Therefore, one would predict that the transformation of the *SCR* gene and the *SRK* gene from one *S* allele into Westar should produce a fully self-incompatible plant.

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