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PCR detection of transcripts homologous to the self-incompatibility gene in anthers of *Brassica*

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Summary. The polymerase chain reaction (PCR) is particularly well suited for the detection of rare sequences. Taking advantage of the recent isolation of sequences associated with stigma self-incompatibility in *Brassica oleracea*, we used PCR amplifications with primers synthesized to the S6 cDNA sequence, to demonstrate the presence of mRNA homologous to stigma *S*-locus gene (SLG) in anthers during early microsporogenesis. In addition, other *S*-locus-related (SLR) sequences were shown to be transcribed in sexual as well as in vegetative tissues (roots, leaves), suggesting that the SLG family might be involved not only in pollen-stigma recognition, but more generally in various forms of plant cell signalling processes. This information corroborates the recent discovery of a cDNA-deduced protein kinase from maize roots, whose extracellular receptor displays high homology with *Brassica* S-locus-specific glycoproteins.

Key words: *Brassica oleracea* – Self-incompatibility – *S*-locus – Polymerase chain reaction – Anther

Introduction

Self-incompatibility in flowering plants is a mechanism of sexual reproduction control that prevents inbreeding and promotes outcrossing (de Nettancourt 1977). In the *Brassica* genus, the self-incompatibility reaction is characterized by pollen rejection at the stigma surface during the first steps of pollination. It is under the control of a single locus, the *S*-locus, with more than 50 alleles (Ockendon 1974), and occurs when the pollen and stigma bear identical *S*-alleles. In the sporophytic self-incompatibility system described in *Brassica*, pollen phenotype is determined by the alleles carried by the pollen-producing plant.

Glycoproteins associated with self-incompatibility, the *S*-locus-specific glycoproteins (SLSG), have been isolated from *Brassica oleracea* stigmas (Nasrallah et al. 1985), and cDNAs encoding these glycoproteins have been cloned from several genotypes (Nasrallah et al. 1985, 1987; Trick and Flavell 1989). Southern analysis of genomic DNA has shown that the *Brassica oleracea S*-gene system consists of a family of at least 11 related sequences with variable degrees of homology (Nasrallah et al. 1988). Some of these *S*-locus-related sequences have been described as pseudogenes, while two others were demonstrated to be active in mature stigmas. The SLG gene, characterized by extensive restriction polymorphism between *S*-homozygotes, is linked to the *S*-locus, as shown by the cosegregation of polymorphism with *S*-alleles in F_2 plant populations. The second gene, named SLR-1 for *S*-locus-related, is regulated as the SLG gene and has been demonstrated to be highly conserved between *S*-lines and unlinked to the *S*-locus (Lalonde et al. 1989; Trick and Flavell 1989). It is assumed that self-incompatibility is mediated at the biochemical level by the highly variable SLSG encoded by the SLG gene (Nasrallah et al. 1988; Trick and Flavell 1989). It has been proposed that the SLR-1 gene, which is also transcribed in the stigma of self-compatible lines of *Brassica campestris* and species like *Arabidopsis thaliana* (Lalonde et al. 1989) or *Brassica napus* (Trick and Flavell 1989), plays a role in interspecific incompatibility during the pollination process.

In spite of these fundamental advances made in the molecular study of self-incompatibility, little is known

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about the expression of the *S*-locus in the pollen at the biochemical and genetic levels (Gaude and Dumas 1987; Ebert et al. 1989). The sporophytic control of the self-incompatible pollen phenotype suggests that male *S*-products are synthesized in the tapetum, an anther diploid tissue, and are exported to the pollen wall when the tapetum degenerates at the end of the microspore stage (Heslop-Harrison 1968). The molecular basis of self-incompatibility in the male partner has already been investigated under the hypothesis that the *S*-allele identity of male and female, which governs the rejection of self-pollination, must be expressed at the molecular level. Until now, attempts to hybridize sequences expressed in anthers of self-incompatible lines with stigma SLG probes have only resulted in very limited support of this model of homology between male and female *S*-products: Nasrallah and Nasrallah (1986) announced in a review article that they had observed such hybridizations, but the original data are still unpublished. Recently, Scutt et al. (1990), after obtaining negative results from northern analyses, proposed that the determination of self-incompatibility in pollen and stigma might be under the control of different, though genetically linked, genes.

On the other hand, a monoclonal antibody directed against an antigenic site common to several SLSGs, when used as a probe on electroblots of pollen proteins separated by SDS-PAGE, allowed the detection of three weakly expressed pollen polypeptides (Gaude et al. 1988).

Taking therefore the model of "male and female *S*-allele homology" as a working hypothesis, our conviction was that the problem should also be stated in terms of level of expression of the responsible gene. In fact, previous biochemical work based on electrophoretic analyses of glycoproteins during pollen ontogeny demonstrated the complexity of anther and pollen proteins and the absence of detectable *S*-allele-specific bands among them (Detchepare et al. 1989). We attempted to specifically amplify the transcripts of *S*-related genes in male tissues using the polymerase chain reaction (PCR), known for its sensitivity and efficiency in detecting rare sequences (Gibbs and Chamberlain 1989).

Using primers synthesized to conserved domains of the S6-cDNA sequence published by Nasrallah et al. (1987), we have demonstrated that PCR, using mature stigma cDNA populations as templates, produced highly specific SLG fragments. SLG-specific PCR amplifications were also obtained with templates from cDNAs from early anthers, and showed that the corresponding transcripts have a size of ca. 4 kb, significantly different from that of the 2-kb stigma SLG transcripts. This observation suggests that male and female *S*-genes have homologous but nonidentical sequences. Other SLR sequences were shown to be transcribed in vegetative tissues (roots, leaves), suggesting that the SLG family might be involved not only in pollen-stigma recognition, but more generally in various forms of plant cell signalling processes.

Materials and methods

Plant materials

Brassica oleracea var. *acephala* plants homozygous for the S9 self-incompatibility allele were grown in the open field (seed collection of the Scottish Crop Research Institute of Invergrowrie, Dundee; courtesy of Dr. T. Hodgkin).

Anthers were collected at three stages of development, defined as microspore, bicellular pollen, and tricellular pollen stages by DAPI nuclear staining of developing anthers (Detchepare et al. 1989). Stigmas were dissected from flowers 1 or 2 days before anthesis.

RNA extractions

Anthers and stigmas were ground under liquid nitrogen, homogenized in 4 *M* guanidium thiocyanate, 0.1 *M* TRIS-HCl (pH 7.5), 0.5% lauryl sarcosinate, 1% betamercaptoethanol (Maniatis et al. 1989), and centrifuged to eliminate cell debris; the supernatants were layered on 5.7 *M* CsCl, 0.01 *M* EDTA (pH 7.5), then centrifuged at 20 °C for 24 h at 32,000 rpm in a TST41 (Kontron) rotor. The RNA pellets were rinsed with 70% ethanol, dried at room temperature, dissolved in 10 m*M* TRIS-HCl (pH 7.6), 1 m*M* EDTA, 0.1% SDS, and ethanol precipitated.

cDNA syntheses and PCR amplifications

First-strand cDNA was synthesized by Mu-MLV reverse transcriptase (BRL); 1–5 µg of total RNA was incubated for 1 h at 37 °C in 50 µl of Mu-MLV buffer (50 m*M* TRIS-HCl (pH 8.3), 75 m*M* KCl, 10 m*M* DTT, 3 m*M* $MgCl_2$), made 0.5 m*M* for each dNTP, 100 µg/ml BSA, 10 µg/ml oligo-dT. Twenty to thirty nanograms of ethanol-precipitated, first-strand cDNAs was used as a template for PCR amplification.

The PCRs were performed for 30 cycles in 100 µl of enzyme buffer [67 m*M* TRIS-HCl (pH 8), 6.7 m*M* $MgCl_2$, 16.6 m*M* $(NH_4)_2SO_4$], containing 1 m*M* of each dNTP, 1 m*M* of each primer, and 2.5 units of Taq DNA polymerase (Perkin Elmer Cetus). Primers were synthesized in a DNA synthesizer (381 A, Applied Biosystems). A1, A2, and A3 correspond to the 5′ part, and C1 and D1 to the 3′ part of the S6-cDNA sequence published by Nasrallah et al. (1987) (Fig. 1, Table 1). Annealing temperatures and extension times were determined for each pair of primers (Table 2). The amplification products were purified on microconcentrators, Centricon-30 (Amicon).

Southern, dot-blot, and northern analyses

Amplified DNA was electrophoresed on agarose gels and transferred onto nitrocellulose filters according to Southern (1975). For dot blots, 1 µg DNA and 100 ng of total RNA were denatured for 10 min at 65 °C in 3 × SSC, 7.2% formaldehyde, chilled on ice, and spotted on nitrocellulose filters (Maniatis et al. 1989). For northern blots, 10 µg of total RNA was denatured, run on a 1.5% agarose formaldehyde gel, and transferred to nitrocellulose (Maniatis et al. 1989). The size of the fragments was determined using an RNA reference ladder (BRL).

Radioactive probes were prepared from purified PCR products or from BS29-1 (SLR-1-like) and BS29-2 (SLG-like) cDNA clones (Trick and Flavell 1989), using random primer labeling (Boehringer) with ^{32}P-dCTP (Amersham). Hybridizations

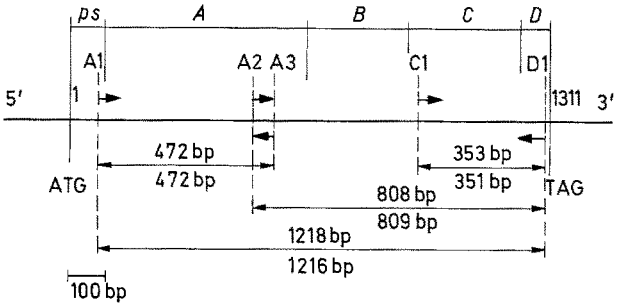

Fig. 1. *Localization of PCR primers along the S6-cDNA sequence* (Nasrallah et al. 1987). The SLG polypeptide sequence (*dashed line*) has been described as an alternation of conserved (*A*, *C*) and variable regions (*B*, *D*). *Solid line:* SLSG encoding region from ATG (+1) to TAG codons (+1,311). A1, A2, and C1 (*black arrowheads above* the line) are sense primers, while A3 and D1 (*black arrowheads below* the line) are antisense primers. The size of the SLG templates is indicated *above* or *below* the *arrows*, as deduced from the S6- or from the S29-cDNA sequences (Nasrallah et al. 1987; Trick and Flavell 1989)

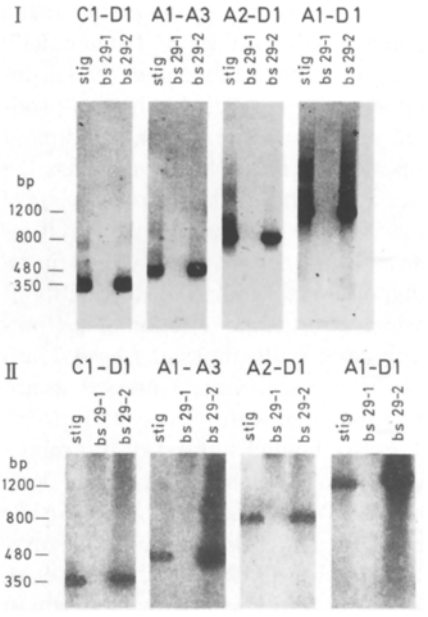

Fig. 2 I and II. *PCR amplifications from stigma cDNAs.* **I** Agarose gels of PCR fragments from C1–D1, A1–A3, A2–D1, and A1–D1 primer pairs using stigma cDNAs, BS29-1 (SLR-like) clone, and BS29-2 (SLG-like) as templates. The size of the bands was determined using a 123-bp DNA reference ladder (BRL). **II** Southern blots of these gels hybridized with the labelled BS29-2 (SLG-like) cDNA clone

were carried out for 18 h at 42 °C in 2 × SSC, 0.1% SDS, 1 × Denhardt's mix, 0.5 μg/ml denatured salmon sperm DNA, and 50% formamide. Filters were washed at 63 °C in 0.1% SDS – 2 × SSC, 1 × SSC, 0.5 × SSC, then 0.1 × SSC, dried, and exposed to Kodak XAR-5 film at −80 °C with intensifying screens.

Results

PCR amplifications from cloned SLG and SLR-1 cDNAs

The five synthetic oligonucleotides used as primers for PCR amplifications [A1, A2, C1 (sense), and A3, D1 (antisense)] were synthesized to regions of the S6-cDNA sequence (Nasrallah et al. 1987) conserved among several *S*-alleles (Fig. 1). The size of the template delimited by these primers was deduced for each pair from the S6- and S29-cDNA (Trick and Flavell 1989) sequences. The A1–A3 pair delimits a 470-bp fragment in the conserved domain A (nomenclature from Nasrallah et al. 1987) in the 5′ part of the two S-cDNAs; the C1–D1 pair corresponds to a 350-bp template in the conserved domain C in the 3′ part. The A2–D1 DNA fragment covers 810 bp from the end of the A region to the 3' boundary of the cDNAs. The longest DNA segment of 1,210 bp is delimited by the A1–D1 primer pair.

The two clones, BS29-1 (SLR-1-like) and BS29-2 (SLG-like), were used as control templates in PCR amplifications: PCR amplifications with BS29-2 regularly produced large amounts (Fig. 2-I) of single fragments with sizes of 350 bp (C1–D1), 480 bp (A1–A3), 800 bp (A2–D1), or 1,200 bp (A1–D1), consistent with those predicted from the sequences of S6- and S29-cDNAs (Fig. 1). When transferred to nitrocellulose filters and hybridized under stringent conditions (63 °C/0.1 × SSC washings), these PCR products gave strong single signals at 350 bp (C1–D1), 480 bp (A1–A3), 800 bp (A2–D1), or 1,200 bp (A1–D1) with the BS29-2 (SLG-like) probe, and no signal at all with the BS29-1 (SLR-1-like) probe (Fig. 2-II). In contrast, no synthesis of PCR products with these primers, even in minor quantities as checked by Southern hybridizations, was detected from a BS29-1 template.

Since the primers gave sequence homologies of 90–100% with BS29-2 (SLG) and only about 70–80% with BS29-1 (SLR-1) (Table 1), we infer that only DNA sequences showing more than 70–80% homology with these primer sets can be amplified by PCR under our experimental conditions.

PCR amplifications from mature stigma cDNAs populations

cDNAs from mature S9 stigmas were used as templates in PCR amplifications, after cDNA first-strand synthesis using Mu-MLV reverse transcriptase. The same results were obtained with reverse syntheses primed with oligo-dT or with anti sense (A3 or D1) PCR primers. Agarose gel electrophoresis (Fig. 2-I) and Southern hybridizations (Fig. 2-II) with the BS29-2 (SLG-like) clone demonstrated the production of large quantities of single PCR fragments with sizes of 350 bp (C1–D1), 480 bp (A1–A3), 800 bp (A2–D1), or 1,200 bp (A1–D1), con-

Table 1. *Nucleotide sequence of PCR primers.* PCR primers synthesized to the S6-cDNA sequence (Nasrallah et al. 1987) between positions indicated by numbers above the sequence. The percentage of homology of each primer sequence with eigher SLR-1 (Lalonde et al. 1989), BS29-2 (SLG-like), or BS29-1 (SLR-like) cDNA sequences (Trick and Flavell 1989) are indicated on the right

Code sequence		% of homology with		
		SLR-1	BS29-2	BS29-1
	92 114			
A1	5′ GAATTCGATCAACACACTTTGTCGTCT 3′	77	100	77
	502 529			
A2	5′ GAATTCCAGAGATGAAACTGGGTTACGACC 3′	70	96	70
	564 543			
A3	5′ GGATCCATGATGTAAGGAACCTGTT 3′	68	100	68
	957 986			
C1	5′ GTTTGTAACTGTATCCAAGGGTTCAATCCC 3′	77	90	77
	1308 1278			
D1	5′ AAGGTCAGCAACAGCCAATCTGACATAAA 3′	70	93	67

Table 2. *Reaction conditions for PCR amplification cycles.* For each cycle, DNA denaturation was performed at 92 °C for 1 min. The annealing temperatures of the primers and the extension times at 72 °C were the following:

Primers	Annealing T °C/min	Extension T °C/min
A1–A3	48/1	72/2
C1–D1	52/1	72/1
A2–D1	55/1	72/3
A1–D1	50/1	72/4

sistent with those predicted from the sequences of S6- and S29-cDNAs (Fig. 1). No fragment homologous with the BS29-1 (SLR-1-like) probe could be detected among these PCR products under the stringency of 63 °C/ 0.1 × SSC, although SLR-1 sequences are known to be expressed in mature stigmas (Lalonde et al. 1989; Trick and Flavell 1989; Scutt et al. 1990).

These stigma PCR products were labeled and used as probes against RNAs extracted from various S9 tissues at various stages. RNAs from mature stigmas hybridized strongly with these probes on dot blots as well as on northern blots (Figs. 4 and 5), where a unique band of ca. 2 kb corresponding to SLG transcripts was detected; no signal was detected with RNAs from vegetative tissues, in accord with the tissue specificity described in the literature for SLG transcripts. However, an important observation was the weak but reproducible hybridization of the stigma PCR probe primed with the A1–A3 oligonucleotides, with RNAs extracted from anthers at the microspore stage. This signal could only be detected on dot blots, probably as a result of the higher sensitivity of dot blotting versus northern blotting.

The BS29-1 (SLR-1-like) and BS29-2 (SLG-like) clones were used as control probes on dot blots (Fig. 4): while BS29-1 showed a strict stigma specificity consistent with that previously described, BS29-2 gave signals with stigma RNAs, but also weak hybridizations with leaf RNAs and early anther RNAs. These results are in contrast with published ones but support those described above. These hybridizations could not be obtained on northern blots, probably due to their lower sensitivity.

PCR amplifications from anther cDNA populations

PCR amplifications were performed from anther cDNAs in order to verify expression of the *S*-gene in anther tissues as suggested by the dot-blot hybridizations. With all primer pairs, PCR amplification produced single fragments (Fig. 3-I) with sizes of 350, 480, 800, or 1,200 bp, consistent with those predicted from the sequences of S6- and S29-cDNAs. The amounts of DNA produced were lower with RNAs from older anthers (tricellular pollen stage) than with RNAs from early ones (microspore stage), and were also lower than those produced from stigma RNAs, as estimated from ethidium bromide and UV observations. Transfer to nitrocellulose filters and probing with BS29-1 and BS29-2 clones showed exclusive hybridization with BS29-2 (SLG-like) under stringent conditions, and the absence of minor PCR fragments with sizes differing from 350, 480, 800, or 1,200 bp (Fig. 3-II).

Used as radioactive probes, the PCR products from microspore stage anther cDNAs gave different hybridization patterns with the RNAs of different origins, depending on the primer pair used for amplification. The A1–A3 and C1–D1 pairs gave particularly informative results: the PCR fragments corresponding to the 5′ do-

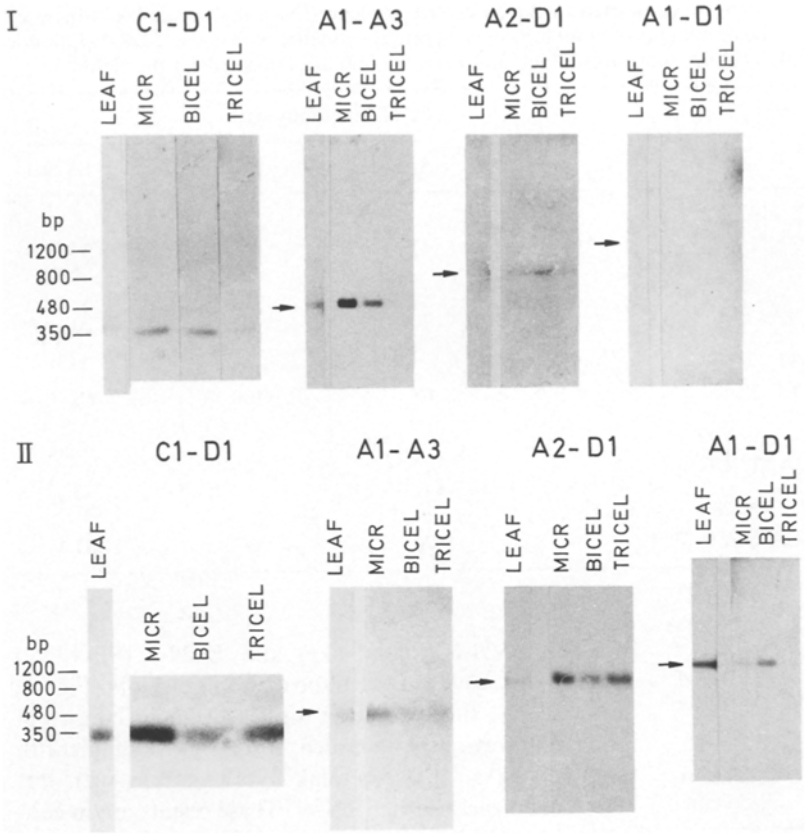

Fig. 3 I and II. *PCR amplifications from anther and leaf cDNAs.* **I** Agarose gels of PCR fragments performed with C1–D1, A1–A3, A2–D1, and A1–D1 primer pairs and templates of cDNA populations from leaves, anthers at microspore (micr), bicellular pollen (bicel), and tricellular pollen (tricel) stages. The size of the bands was determined using a 123-bp DNA reference ladder (BRL). **II** Southern blots of these gels hybridized with the labelled BS29-2 (SLG-like) cDNA clone

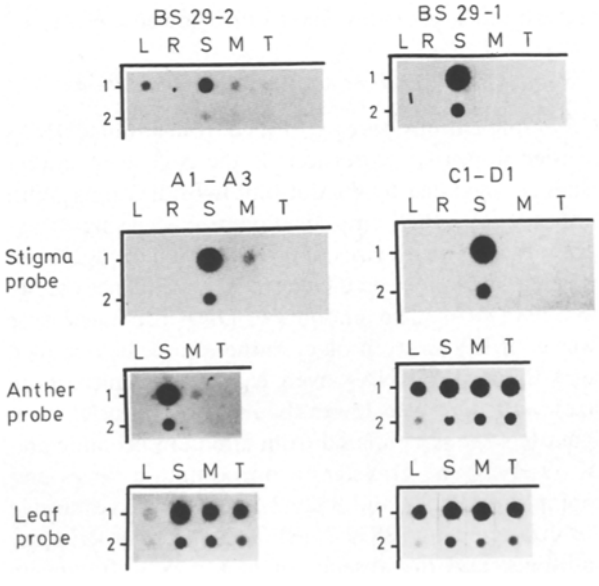

Fig. 4. *Dot-blot analysis of RNAs from various tissues with PCR fragments used as probes.* Onto nitrocellulose filters were spotted 1 μg (*1*) and 0.1 μg (*2*) of total RNA extracted from leaves (L), roots (R), stigmas (S), anthers at microspore (M) or tricellular pollen (T) stage. They were hybridized with the PCR products resulting from amplifications from templates of cDNAs from stigmas, anthers at microspore stage, or leaves with A1–A3 or C1–D1 primer pairs. Two other RNA dot-blot analyses (at top of the figure) were carried out as controls using BS29-2 (SLG-like) and BS29-1 (SLR-like) cDNA clones as probes

main A of the SLG sequence (primers A1–A3) hybridized exclusively with RNAs from mature stigmas and from early stage anthers; northern blots allowed the identification of the corresponding transcripts as a single abundant band of ca. 2 kb in the stigmas, and a single tenuous band of ca. 4 kb in the anthers (Fig. 5). Those fragments corresponding to the 3′ domain C of the SLG sequence (C1–D1 primers) gave complex and puzzling hybridization patterns in dot blots (Fig. 4) as well as in northern blots (Fig. 5). The RNAs from all tissues, whether vegetative (leaves) or sexual (stigmas, anthers), and regardless of their developmental stage (early or mature), gave dot hybridizations. Northern blots identified the corresponding transcripts as at least three different bands – SLR-2, 3, and 4 – apparently common to all these tissues (Fig. 5). In addition to these three bands, the mature stigmas showed large amounts of the SLG transcript (2 kb), strongly hybridizing these probes under stringent conditions (63 °C/0.1 × SSC).

PCR amplifications from leaf cDNA population

The expression of the SLG gene has been described hitherto as specific to the mature stigma (Nasrallah et al. 1985; Trick and Flavell 1989; Scutt et al. 1990); we thus performed PCR amplifications from leaf cDNAs, expecting negative or aberrant results. To our great surprise,

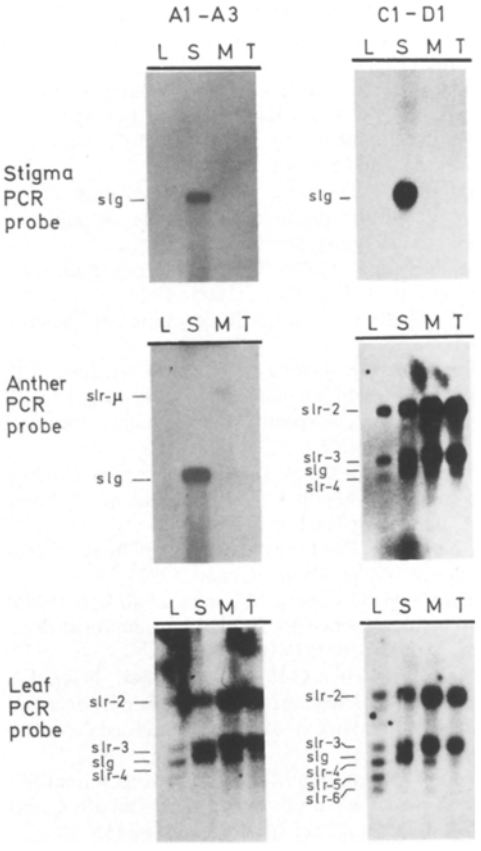

Fig. 5. *Characterization of S-related transcripts by northern analysis.* Total RNA extracted from leaves (L), stigmas (S), anthers at microspore (M) and tricellular pollen stages (T) were electrophoresed on formaldehyde gels and transferred onto nitrocellulose filters. They were hybridized with probes of PCR products amplified with A1–A3 or C1–D1 primer pairs from cDNAs from mature stigmas, anthers at microspore stage, or leaves

however, very specific SLG-type syntheses of PCR fragments were systematically obtained from leaf cDNA templates: with all primer pairs, PCR amplifications produced single fragments with sizes (Fig. 3-I) consistent with those predicted from the sequences of S6- and S29-cDNAs. These single fragments hybridized specifically BS29-2 (SLG-like) but not BS29-1 (SLR-1-like) clones under stringent conditions; they were produced in low amounts, often barely detectable by ethidium bromide and UV observations, but always clearly shown by Southern hybridization with the BS29-2 clone (Fig. 3-II).

Dot-blot hybridizations using these PCR probes gave intense signals with RNAs extracted from stigmas and anthers (both early and mature), and somewhat fainter signals with RNAs extracted from leaves (Fig. 4). Northern blot hybrizations with leaf PCR probes showed five RNA bands, some common to all tissues analyzed (Fig. 5), three of them having also been detected with PCR probes made from anther cDNAs. In addition to these bands, the mature stigmas showed large amounts of the SLG transcript of ca. 2 kb.

Discussion

The specificity of the PCR reactions primed in this work with oligonucleotides synthesized to the S6-cDNA sequence can be estimated from the comparison of amplifications using the two cDNA clones, BS29-1 (SLR-1-like) and BS29-2 (SLG-like), as well as stigma cDNA populations.

Only *bona fide*, single, SLG-type PCR fragments with size rigorously consistent with those predicted from the sequences of S6- and S29-cDNAs, and hybridizing exclusively with the BS29-2 probe, have been obtained with BS29-2 and stigma cDNA templates.

No SLR-1-like amplification products were observed using BS29-1 or stigma cDNAs known to contain SLR-1 transcripts (Nasrallah et al. 1985; Trick and Flavell 1989; Scutt et al. 1990).

Our experimental conditions did not allow the annealing of the SLG-type primers used here on SLR-1-type templates, due to a sequence divergence of 20–30% (Table 1). We can thus assume that the sequences amplified in our PCRs must be more closely related to the SLG gene than to the SLR-1 gene. This means that they belong to a class of sequences diverging by less than about 20–30% from the SLG gene, at least at the sites of the primers A1–A3 and C1–D1.

It is noteworthy that sequences more homologous to SLG were preferentially amplified when a mixture of different SLG-related cDNAs were used as templates, probably as the result of variation in primer/template homologies. This situation occurs clearly in the case of mature stigmas known to contain SLG and SLR-1 transcripts, and shown in Fig. 5 to contain also SLR-2, 3, and 4 transcripts: PCR amplifications from stigma cDNAs populations yielded PCR fragments hybridizing exclusively to the 2-kb transcript of SLG among stigma RNAs, and to no other RNA species at all among RNAs from other tissues (Fig. 5). The corollary of this is that no systematic reciprocal hybridizations of PCR probes from one tissue onto the others were observed.

The same situation of preferential amplification of SLG-type sequences among a mixture of related but non-identical templates is encountered in the case of microspore stage RNAs with the A1–A3 primer pair; our conditions allowed the synthesis of tissue-specific PCR fragments that hybridized the SLR-μ (4 kb) as well as the SLG (2-kb) transcripts on northern filters (Fig. 5). Since the SLG probes made from stigma cDNAs did not allow the reciprocal detection of the SLR-μ transcript, it can be concluded that SLR-μ and SLG are not perfectly homologous at the nucleotide sequence level. The two genes

thus differ by their structure (4-kb versus 2-kb transcripts) and by their nucleotide sequences in the domains bordered by the A1–A3 primers. The molecular description of the self-incompatibility gene system in *Brassica* should therefore incorporate some elements of the "male and female homology" model, others from the hypothesis proposed by Scutt et al. (1990), together with a very low level of expression of the SLR-μ in the anthers. The expression of the SLR-μ gene in early stage anthers is quite consistent with the characteristics of a sporophytic control of self-incompatibility (Heslop-Harrison 1968).

In vegetative tissues (leaves) or in early anthers with C1–D1 primers from the 3′ region of the SLG sequence, various sequences related to SLG (since they hybridize with SLG probes in stringent conditions) have been amplified and also detected in northern hybridizations. The finding of so many abundant transcripts closely related to SLG in vegetative tissues, and thus apparently not directly involved in the control of self-incompatibility, seemed at first very puzzling to us. However, the report of homologies detected between the 3′ cystein-rich region of *Brassica* SLSGs and a group of animal glycoproteins involved in protein-protein binding and in the formation of extracellular matrices – the precursor to the Von Willebrand factor and collagen VI (J.M. Crabbe and H.G. Dickinson, personal communication) – shows that these SLG-related sequences are not exceptional. Perhaps still more significant, the recent discovery of a cDNA-deduced protein kinase from maize roots whose extracellular receptor displays high homology with *Brassica* SLSGs, in the cystein-rich domain (Walker and Zhang 1990), further supports the fact that these sequences are not exceptional. The SLG gene therefore appears to be a member of a large family of related genes potentially involved in various plant cell recognition and signalling systems, among which recognition of self and nonself pollen would be only one particular case.

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Note added in proof

Since the communication of the present manuscript, an additional family of the SLR sequences, named SLR2, has been demonstrated to be expressed in the stigma of *Brassica oleracea* (Boyes, D.C., Chen, C.H., Tantikanjana, T., Esch, J.J., and Nasrallah, J.B. 1991. Isolation of a second SLR cDNA from *B. oleracea*: genetic relationships between the S locus and two related loci. Genetics 127:221–228). We have checked that the homologies between the primers used in this work, and the SLR2 published sequences, are too low in most cases, to have allowed potential priming with this family of sequences. This point will be verified experimentally.