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(54) Title: SELF-INCOMPATIBILITY ALLELES OF BRASSICA (57) Abstract The present invention is directed to isolated cDNA sequences corresponding to alleles of the self-incompatibility locus (S-locus) in <i>Brassica campestris</i> and <i>Brassica rapus</i> ssp. <i>rapifera</i> . The cDNA code for glycoproteins which play a role in the self-incompatibility mechanism of <i>Brassica</i> species. The present invention also relates to a method for the isolation of other cDNAs and genes having homology to genes of the S-locus of <i>Brassica</i> species. The present invention also relates to the transformation of self-compatible plants into self-incompatible plants by the introduction of the cDNAs of the present invention into self-compatible (SC) plants, plant cells and/or protoplasts taken from self-compatible plants. The present invention also relates to a method for the rapid screening of seedlings for the presence of S-locus alleles.		

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SELF-INCOMPATIBILITY ALLELES OF BRASSICA
FIELD OF THE INVENTION

The present invention is directed to cDNA sequences which code for glycoproteins involved in the self-incompatibility reaction in *Brassica* species. The present invention is also directed to a method for the identification and isolation of other cDNAs and genes having homology to genes of the self-incompatibility locus of *Brassica* species and other plants. The present invention is further directed to a method for conferring the self-incompatible phenotype on compatible plants.

BACKGROUND OF THE INVENTION

Self-incompatibility (SI) is a genetically controlled self-recognition system which prevents inbreeding in plants. This self-recognition system results in the rejection of self-pollen by the female somatic tissues of the plant. There are two major homomorphic types of self-incompatibility which differ with respect to the determination of specificity expressed by pollen. (Uyenoyama, M.K., Genetics, 128:453-469, 1991). In gametophytic self-incompatibility, specificity is determined in the gametophyte stage by the genotype of the pollen tube itself. If the pollen carries the same allele as one of the two alleles in the pistil, fertilization is not achieved. In sporophytic self-incompatibility, the incompatibility reaction occurs between factors carried by the pollen but are specified by the diploid tissues of the pollen parent and a product of the female pistil. This is thought to be due to the expression of SI factors in the tapetum.

Some naturally occurring SI lines have been found in rutabagas for example in *B. napus* L. ssp. *rapifera* Metzg. Sinsk., (Gowers, S., Euphytica 23:205-208, 1974.) and oilseed rape, *B. napus* L. ssp. *oleifera* Metzg. . . (Olsson, G., Hereditas

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46:241-252, 1960). Self-incompatible rutabagas have also been produced using SI rapeseed plants found in Swedish winter rapeseed cultivars that trace at least part of their ancestry to artificial (resynthesized) *B. napus* (Olsson, G., Kungl. Lantbruksakademiens Tidskrift 92:394-402, 1953; Olsson, G., Hereditas 46:241-252, 1960; Olsson, G., Hereditas 46:351-386, 1960; Gowers, S., Euphytica 23:205-208, 1974; Gowers, S., Euphytica 24:537-541, 1975; Gowers, S., Proceedings of the Eucarpia 'Cruciferae 1979' Conference, Wageningen, The Netherlands. October 1-3, 1979). There are no apparent fertility barriers between these subspecies of *B. napus*.

Self-incompatibility in *Brassica* is sporophytic and is controlled by a single genetic locus, the S-locus. The diploid species, *B. campestris* L. and *B. oleracea* L., are generally found to have active SI systems composed of multiple alleles at the S-locus. *B. oleracea* has been shown to have nearly 50 different naturally occurring S-alleles (Ockendon, D.J., Heredity 33:159-171, 1974; Ockendon, D.J., Euphytica 31:325-331, 1982). However, the allotetraploid species, *B. napus* L., which is derived from genomes of *B. campestris* and *B. oleracea*, generally occurs in a self-compatible (SC) form (Downey, R.K. and Rakow, G.F.W., Principles of Cultivar Development, MacMillan Publishing Co., New York, 1987). *B. napus* can be made SI by the introgression of S-alleles from one of the progenitor species, as described by Mackay and Gowers, who introgressed S-alleles from *B. campestris* into *B. napus* and who have described the fertility and cytology of progeny from this cross, and considered the most efficient breeding strategies for introgressing alleles from *B. campestris* into *B. napus* (Mackay, G.R., Euphytica 26:511-519, 1977; and Gowers, S., Euphytica 31:971-

976, 1982).

SI has been associated with the production of high levels of S-locus glycoproteins (SLG) in the papillae cells of the stigma (Nasrallah, M.E., et al., Heredity 25:23-27, 1970; Hinata, K. & Nishio, T., Heredity 41:93-100, 1978). It has been shown that there is a highly abundant glycoprotein in stigma extracts which segregates with individual SI alleles (Nasrallah, M.E., et al., Heredity 25:23-27, 1970; Hinata, K. & Nishio, T., Heredity 41:93-100, 1978). Genes for the SLGs have been isolated from *B. campestris* and *B. oleracea* (Nasrallah, J.B., et al., Nature 326:627-619, 1987; Trick, M. & Flavell, R.B., Mol. Gen. Genet. 218:112-117, 1989; Chen, C.H. & Nasrallah, J.B., Mol. Gen. Genet. 222:241-248, 1990; Dwyer et al., Plant Mol. Biol. 16:481-486, 1991). The *B. oleracea* S-alleles have been found to fall into 2 classes based on SI characteristics determined from a survey of 30 different *B. oleracea* lines (Thompson, K.F. & Taylor, J.P., Heredity 21:345-362, 1966). Class I, which is represented by five of the cloned SLG genes, phenotypically have strong SI reactions and are generally dominant or co-dominant to other S-alleles (Nasrallah, J.B., et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:393-422, 1991). Class II, which is represented by one cloned SLG-allele, shows a weak SI phenotype and is recessive to other S-alleles in the pollen. At the DNA sequence level, Class I genes generally show greater than 80% homology to each other, but are only about 70% homologous to the pollen recessive SLG-allele. The single *B. campestris* SLG allele which has been isolated, contains strong sequence similarities to the Class I alleles (Dwyer et al., Plant Mol. Biol. 16:481-486, 1991).

Other gene families, which show sequence similarities to the SLG alleles, have also been isolated. The SLR-1 locus (S-locus related) is

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present in all *Brassica* species, but segregates independently of the S-locus (Lalonde, B.A., et al., Plant Cell 1:249-258, 1989). The SLR-2 locus is also found in all *Brassica* species (Boyes, D.C., et al., Genetics 127:221-228, 1991). However, this locus does show linkage to the S-locus and shares strong sequence homology to the pollen recessive SLG. Neither the SLR-1 nor the SLR-2 loci appear to be involved in the SI reaction. Very recently another gene at the S-locus in *B. oleracea* has been characterized (Stein et al., Proc. Natl. Acad. Sci. 88:8816-8820, 1991). This gene codes for a putative receptor kinase and is expressed at low levels in both the anthers and stigmas.

The cDNA sequences of three gametophytic self-incompatibility genes (SI-genes) and the genomic sequence of one of these cDNAs from *Nicotiana glauca* and *Lycopersicon peruvianum* are described in U.S. Patent No. 5,053,331 ('331) by Clark et al. The SI genes described in the '331 patent are associated with self-incompatibility in plants expressing the proteins coded for by the SI genes. The '331 patent also discloses regulatory sequences which direct the tissue specific expression of the SI-genes in reproductive tissues and signal sequences which allow export of the SI-proteins from the golgi. Interestingly, the genomic sequence of an upstream regulatory region of one of these genes (S_2) exhibits considerable homology with a mitochondrial DNA gene. In addition, the '331 patent discloses a method for isolating gametophytic SI-genes by differential hybridization using gametophytic S-allele specific cDNA probes. The '331 patent also discloses a method for the purification of gametophytic SI-gene encoded glycoproteins.

Unlike the gametophytic incompatibility taught in the '331 patent, the present invention is

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directed to genes involved in the expression of sporophytic self-incompatibility. Only a few of the alleles that code for sporophytic self-incompatibility in certain *Brassica* species have been isolated and characterized. It is an object of the present invention to isolate and characterize two previously unisolated and uncharacterized alleles of the SI locus in *Brassica* species that play a role in self-incompatibility.

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SUMMARY OF THE INVENTION

The present invention relates to isolated cDNAs corresponding to alleles of the self-incompatibility locus (SI-locus) of *Brassica napus* spp. *rapifera* (A14) (SEQ ID NO: 1) and *Brassica campestris* (910) (SEQ ID NO: 2) and having of the sequences set forth in Figures 3 and 9 respectively. The alleles of the present invention code for glycoproteins which play a role in the self-incompatibility (SI) mechanism of *Brassica* species.

The present invention also relates to a vector comprising a cDNA of the sequence set forth in Figure 3, (SEQ ID NO: 1) as well as a vector comprising a cDNA of the sequence set forth in Figure 9 (SEQ ID NO: 2). The invention also relates to the vectors comprising the sequences set forth in Figures 3 (SEQ ID NO: 1) or 9 (SEQ ID NO: 2), further comprising the plasmid pTZ18 or pBSc.

More particularly, the present invention relates to the A14 cDNA (SEQ ID NO: 1) from the SI-locus of *Brassica napus* spp. *rapifera* which was isolated after introgression into *Brassica napus* spp. *oleifera* and which comprises a cDNA of approximately 1471 nucleotides having close homology to Class I *Brassica* S-linked glycoprotein alleles (SLG). The glycoprotein coded for by the A14 cDNA is from about 76% to about 87% homologous to other Class I SI-locus glycoproteins. The A14 cDNA is also from about 84% to about 90% homologous to Class I S-locus genes.

The present invention also relates to the 910 (SEQ ID NO: 2) cDNA from the S-locus of self-incompatible *Brassica campestris* which was isolated after introgression into self-compatible *Brassica napus* spp. *oleifera* and which consists essentially of a cDNA of approximately 1424 nucleotides. The glycoprotein coded for by the 910 cDNA is from about 74% to about 80% homologous to Class I

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glycoproteins. The 910 cDNA is from about 84% to about 86% homologous to Class I S-locus genes.

5 The present invention is also directed to two oligonucleotides having homology to SI-locus alleles and which are useful in the identification and isolation of cDNAs and genes having homology to S-locus. More particularly, the oligonucleotides of the present invention consist essentially of the sequences CTTGTGGCAAAGTTTCGATT (SEQ ID NO: 3), and
10 CTGACATAAAGATCTTG (SEQ ID NO: 4).

The present invention is also directed to a method of identifying and amplifying DNA sequences from plants which have homology to S-locus genes comprising the polymerase chain reaction utilizing
15 two oligonucleotides (SEQ ID NO:3 and SEQ ID NO:4, or SEQ ID NO:6 and SEQ ID NO:10) of the present invention as primers in the polymerase chain reaction.

The present invention is further directed to a
20 method of identifying and isolating DNAs homologous to genes of the S-locus by screening cDNA libraries or genomic DNA libraries from plants with either of the oligonucleotide probes of the present invention.

The present invention is also directed to a
25 transfer vector comprising the cDNAs of Figure 3 (SEQ ID NO: 1) or 9 (SEQ ID NO: 2). Particularly, the present invention relates to a transfer vector comprising the cDNA of Figure 3 (SEQ ID NO: 1) or 9 (SEQ ID NO: 2) and a Ti plasmid. More particularly,
30 the present invention relates to a transfer vector comprising the plasmid pBI101.2. The present invention also relates to a transfer vector comprising the plasmid pBsc.

The present invention also relates to an
35 *Agrobacterium tumifaciens* useful in the transformation of a plant, plant cell and/or plant protoplast comprising the transfer vector consisting essentially of the sequence set forth in Figure 3

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(SEQ ID NO: 1) or 9 (SEQ ID NO: 2). The present invention further relates to a method for conferring the self-incompatible phenotype on a self-compatible plant. The method comprises the transfer of a transfer vector as described above into a self-compatible plant capable of assimilating said vector and expressing self-incompatibility.

The present invention further relates to a method for the rapid screening of *Brassica* seedlings for the presence of S-locus alleles utilizing (+) strand and (-) strand oligonucleotides taken from unique regions of S-locus alleles. A rapid screening method for the 910 allele in *Brassica* seedlings comprising the steps of:

1) obtaining genomic DNA from a *Brassica* seedling suspected of having the 910 allele;

2) combining the genomic DNA with a (+) strand oligonucleotide having the sequence CTCGTCATTCGATACTCCAA (SEQ ID NO: 5) or TCTTCACCAGTGGATACCAG (SEQ ID NO: 6) with a (-) strand oligonucleotide having the sequence ACTGGACCCTTCTCTCAGAT (SEQ ID NO: 7);

3) amplifying the allele using the polymerase chain reaction to render said allele detectable; and

4) determining the presence of 910 specific amplification products in the amplification mixture.

Similarly, the present invention is also directed to a method for screening seedlings for the A14 gene using the polymerase chain reaction and in combination a (+) strand probe having the sequence

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ACAACCGCTCAAGTCGATT (SEQ ID NO: 8) with a (-) strand probe having the sequence TGTGAGTCGAATGGAAGAG (SEQ ID NO: 9).

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents the crossing and selection scheme for generating the T2 self-incompatible line.

Figure 2 represents a genomic blot analysis of the candidate SLG cDNAs. Genomic DNA was digested to completion with HindIII. Sources of genomic DNA are lane 1: Topas; lane 2: T2; lane 3: *B. napus* ssp. *rapifera* Z-line; lane 4: *B. oleracea* S2; lane 5: *B. oleracea* S13; and lane 6: an SI canola line in the Westar background. The genomic DNA was probed with each of the six cDNAs: a)A35, b)A29, c)A26, d)A34, e)A10, and f)A14. The data show that the A14 cDNA hybridized to a single band in plants SI with the Z-allele.

Figure 3 illustrates the cDNA sequence and the putative amino acid sequence of the A14 cDNA and its protein respectively (SEQ ID NO: 1). The putative amino acids encoded by the cDNA are shown above the sequence. The underlined region at the start represents a signal sequence. The *** symbol represents conserved cysteine residues, the ... symbol represents potential N-glycosylation sites, and the ▲▲▲ symbol marks amino acids conserved in *B. oleracea* SLG proteins.

Figure 4 is a blot of genomic DNA taken from F2 plants derived from a cross between an SI plant homozygous for T2 to a SC plant homozygous for t to generate a heterozygous F1 T2/t which was then self-pollinated to produce an F2 population of T2/T2 (lane 1), T2/t (lane 2), and t/t plants (lane 3), F2 generation from T2 X t2 (lanes 4-19). DNA was digested with HindIII and probed with the A14 cDNA. Plants from lane 1, 2, and lanes 4-12 are SI.

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Plants from lanes 3, and 13-19 are SC. The data shows that the A14 gene segregates with the T2 self-incompatibility.

5 Figure 5 represents an RNA blot analysis of A14 gene expression in leaves, petals, anthers, and stigma. Lanes 1 to 10 contain 30 μ g of total RNA, and lanes 11 to 18 contain 10 μ g of total RNA. The blot was probed with the A14 cDNA. On the upper
10 scale, the numbers 1 to 7 represent bud sizes increasing from about 1mm to 6mm, and A represents anthesis.

15 Figure 6 illustrates the crossing and selection procedure for generating the W1 self-incompatible line.

20 Figure 7 illustrates a genomic blot analysis of related SLG sequences. Genomic DNA samples were digested with Hind III, hybridized with the A14 cDNA and washed at reduced stringency to detect cross hybridizing genes. The genomic DNA samples are the SC westar (lane 1), SI W1 (lanes 2 & 3), and progeny
25 from two different 3-way crosses involving W1 and various SC canola lines. Lanes 3-6 represent one cross, and lanes 7-19 represent the second cross. The plants were tested for self-incompatibility by seed set. Lanes 4, 6, 8-14 are SI, and lanes 5, 7, 15-19 are SC. The arrows mark two cross-hybridizing
30 bands which are only present in the genomic DNA samples from SI plants.

35 Figure 8 illustrates the PCR primers used in the isolation of the 910 cDNA. SI-1 (SEQ ID NO: 4) and SI-2 (SEQ ID NO: 3) represent conserved regions shown in published SLG sequences. Primers were made from these sequences and used in the PCR reaction to amplify the W1 associated bands from genomic DNA.

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The adaptor and dT₁₇-adaptor primers were designed according to Frohman et al. (Proc. Natl. Acad. Sci. 85:8998-9002, 1988), with different restriction enzyme sites incorporated into the adaptor primer.

5 The 910-2 (SEQ ID NO:7) and 910-3 (SEQ ID NO: 10) specific primers were chosen by comparing the partial 910 genomic sequence to published SLG sequences and looking for variable regions. The most closely related SLG sequences in these two

10 areas are shown.

Figure 9 illustrates cDNA sequence and the putative amino acid sequence of the 910 SLG and its protein respectively (SEQ ID NO: 2). The putative

15 amino acid encoded by the cDNA are shown above the DNA sequence. The underlined region at the 5' end represents a putative signal sequence. The *** symbol represents conserved cysteine residues, the ... symbol represents potential N-glycosylation

20 sites, and the ▲▲▲ symbol marks amino acids conserved in *B. oleracea* SLG proteins.

Figure 10 is a genomic DNA blot illustrating the segregation of the 910 SLG gene with W1 Self-

25 Incompatibility. Sources of genomic DNA are W1/W1 (lane 1), W1/w (lane 2), w/w (lane 3), F2 generation from a W1 X Westar cross (lanes 4-19). Plants from lanes 1,2,4-11 are SI, while plants from lanes 3 and 12-19 are SC. Genomic DNA was digested with HindIII

30 and the blots were hybridized with the 910 genomic probe.

Figure 11 illustrates an RNA blot analysis of 910 gene expression in leaves, petals, stigmas and

35 anthers of SI plants. Each lane contains 10μg of total RNA. The blot was hybridized with the 910 genomic probe. On the upper scale, the numbers 1 to

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7 represent bud sizes increasing from about 1mm to 6mm, and A represents anthesis.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolating and characterizing cDNAs from the self-incompatibility locus (S-locus) of *Brassica* species which correspond to genes encoding S-linked glycoproteins (SLGs) which are associated with the expression of sporophytic self-incompatibility. More particularly, the present invention includes the isolation and characterization of S-locus cDNAs from the self-incompatible plants *Brassica campestris* and *Brassica napus* spp. *rapifera* after introgression into self-compatible *Brassica napus* ssp. *oleifera*. The present invention is further directed to identifying and characterizing conserved DNA sequences found in the cDNAs of the present invention which are useful in the isolation and characterization of other S-locus cDNAs and genes.

The present invention is also directed to a transfer vector consisting essentially of the cDNAs of Figures 3 (SEQ ID NO: 1) and 9 (SEQ ID NO: 2) which are useful in the transformation of plants, plant cells and plant protoplasts. More particularly the present invention relates to transfer vectors comprising the cDNAs of Figures 3 (SEQ ID NO: 1) or 9 (SEQ ID NO: 2) and the plasmid pBI101.2. Even more particularly the present invention relates to an *Agrobacterium tumefaciens* further comprising a transfer vector consisting essentially of the cDNA of Figures 3 (SEQ ID NO: 1) or 9 (SEQ ID NO: 2) and the plasmid pBI101.2. The present invention also relates to a method for conferring the self-incompatible phenotype on self-compatible plants as the method comprising the step of infecting the plant, plant cells, or protoplasts with the *Agrobacterium tumefaciens* of the present invention.

The present invention further relates to a self-compatible plant made self-incompatible by the

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method of the present invention.

Finally, the present invention is also directed to a method for the rapid screening of seedlings for the presence of an S-locus allele.

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MATERIALS AND METHODS

Standard chemical materials and standard molecular biological methods were used in this invention. Modifications to the protocols were made as described herein.

Genomic DNA Extraction

Genomic DNA was extracted from leaves using a modified version of Fedoroff et al. (Genet. 2:11-29, 1983.) Approximately 1g of tissue was homogenized in a mortar and pestle in the presence of liquid nitrogen. Six milliliters (mls) of extraction buffer (8M urea, 350mM NaCl, 50mM Tris-Cl, pH 7.5, 20mM EDTA, 2% Sarcosine) were added to the tissue and grinding was continued until the materials were thawed. The mixture was then transferred to an 15ml polypropylene tube, and 0.6ml 10% SDS and 6ml phenol/chloroform/isoamyl-alcohol (75:24:1) were added. The mixture was gently shaken for 10 min. and separated by centrifugation. The supernatant was then extracted with 1 volume of phenol/chloroform/isoamyl-alcohol (25:24:1) followed by an extraction with chloroform/isoamyl-alcohol (24:1). The nucleic acids were precipitated with a 1/10th volume of 3M sodium acetate and 2 volumes ice-cold ethanol. Nucleic acid was then resuspended in 2ml 10mM Tris-Cl, pH 8.0, 45mM EDTA and treated with 60µg RNase A at 37°C for 30 min. The DNA was ethanol-precipitated and resuspended in 100-200µl TE (10mM Tris, 1mM EDTA, pH 7.5). A scaled down version which involved grinding one leaf in an eppendorf tube was utilized for the F2 plants. For the purpose of rapidly screening seedlings for the presence of S-alleles, DNA was prepared by the method of Edwards and Thompson, (Nucl. Acids. Res. 19:1349, 1991).

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Genomic DNA Blots

Approximately 5 to 10 μ g of genomic DNA was digested to completion with the restriction endonuclease HindIII (Bethesda Research Laboratories, Bethesda, MD). Digested DNA was then fractionated through a 0.7% agarose gel, and transferred to a ZetabindTM membrane (Cuno Labs Inc., Meriden, CT) by blotting in 20X SSC (20X SSC = 3M sodium acetate, 0.3M Na₃ citrate $\cdot$ 2H₂O. After drying, the membrane was prewashed in 0.1X SSC, 0.5% sodium dodecylsulfate solution (SDS) for 30 min. at 60°C. The membranes were prehybridized at 42°C in 5X SSPE, 10X Denhardt's (10X Denhardt's=1g Ficoll 400, 1g polyvinylpyrrolidone, 1g bovine serum albumin [Pentax fraction V], in 500ml of distilled water), 0.5% SDS for approximately one hour, hybridized overnight at 42°C in 50% formamide, 10% dextran sulfate, 5X SSPE, 0.5% SDS, and 50 μ g/ μ l sheared salmon sperm DNA. Filters were then washed at 68°C to 70°C in 0.1X SSC, 0.1% SDS. Hybridization probes consisting of full length cDNAs were digested with the appropriate restriction endonucleases to excise the cDNA from the vector. The excised cDNA was separated from the vector by electrophoresis on an agarose gel. Probes were labelled by random-priming using the method of Feinberg & Vogelstein, (Anal. Biochem. 132:6-13, 1983.)

DNA Sequencing

The 5' and 3' cDNA end clones were partially sequenced using dideoxy sequencing method of Sanger and the Sequenase enzyme (United States Biochemicals, Cleveland Ohio) (Sanger, F., et al., Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467). To sequence the full length cDNA clones, deletions were made using exonuclease III and Mung Bean nuclease

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according to the procedure in the Stratagene kit (Stratagene, LaJolla CA). Overlapping deletions were sequenced for both strands. All DNA and protein sequence analysis was performed on the DNASIS and PROSIS software. (Pharmacia, Piscataway, NJ).

RNA extraction

Total RNA was extracted from about 100-200mg of tissue using the method of Jones et al. (EMBO J. 4:2411-2418, 1985.) 10 to 30µg of RNA was fractionated through a 1.2% formaldehyde-agarose gel (Sambrook et al., A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, 1989) and transferred to Zetabind™ membrane (Cuno Labs. Inc.) in 20X SSC. Hybridization and washing conditions were the same as used for the genomic blots.

The objects of the present invention are illustrated by way of the following non-limiting examples.

EXAMPLE 1

Isolation and Characterization of the A14 cDNA from *B. napus* spp. rapifera.

Plant Crosses and Growth Conditions

The SI Z-rutabaga (*B. napus* ssp. rapifera), which was used as a donor for the self-incompatible (SI) allele, was obtained from the Scottish Crop Research Institute (Invergowrie, Dundee, Scotland). Plants were grown either in a green house or in a growth room under 16 hour daylight and 8 hour dark conditions. Plants were tested for SI by measuring seed set, or staining pistils for pollen tube growth with aniline blue which stains the carbohydrate, callose, which is associated with the growing pollen tube, and examining them under a fluorescence

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microscope (Kho., Y.O., & Baer, J., Euphytica 17:298-302, 1968.)

5 Generation of the T2 and R2 Self-Incompatible Canola Lines

 The generation of the T2 self-incompatible line is illustrated in Figure 1. Introgression of the SI allele from the SI Z-rutabaga line was conducted by a straight forward backcrossing routine utilizing a field vernalized stock of the SI donor material crossed with each of the spring canola recipient varieties, Regent (SeCan, Ottawa, Canada) and Topas (Bonis and Co., Manitoba, Canada) producing the R2 and T2 lines, respectively (Paul Banks, Thesis, Univ. of Guelph). F1 plants in these backcross schemes were briefly vernalized by maintaining the plants at 4°C for 6-8 weeks followed by removal to 20°C and checked for SI by placing pollination bags over inflorescence to avoid cross pollination. F1 plants, which were SI, were further backcrossed using pollen of the corresponding recipient (recurrent parent). Self-compatible (SC) F1 plants were self-pollinated to produce an F2 generation which was subjected to selection for SI plants and subsequent backcrossing. Topas-2 was derived from a S3 derivative of Topas/z//4*Topas. Regent 2 was derived from a S3 derivative of Regent/z//4*Regent. Crosses of these lines to SC canola lines showed that SI was inherited as a single dominant (or partially dominant) locus.

Construction and Screening of the cDNA Library

 Stigmas were collected from R2 buds 1 to 2 days before anthesis. Poly A⁺ RNA was extracted and used to make a plasmid cDNA library in a modified PTZ18 vector according to the procedure of Bellemare et al. (Gene 52:11-19, 1987) with the improvements

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outlined by Bellemare et al (Gene 98:231-235, 1991.)
In this method, a vector (pPBS27) was constructed by
introducing a poly(dT) tail adjacent to the XbaI
site of pTZ18R. pTZ18R can exist as a plasmid in *E.*
5 *coli* or as a single stranded (ss) phage DNA. The
vector was then linearized with XbaI using a
restriction-site-directed fragment (RSDF, 5'-
GGATCCTCTAGAAAA-3') and used to anneal a mixture of
poly-A+ RNA for cDNA synthesis by Moloney murine
10 leukemia virus (M-MLV) reverse transcriptase.
Second strand synthesis and RNA replacement was then
performed according to Gubler (Nucl. Acids Res.
16:2726, 1988), followed by closure and ligation of
the blunt-ended double stranded cDNA. The cDNA
15 mixture was then used to transform high efficiency
E. coli DH5alphaF' competent cells (Bethesda
Research Lab. Bethesda Maryland) which were then
plated on LB agar plates containing 50 mg/l of
ampicillin. All of the transformation reactions
20 were plated out and colonies were scraped and
collected to generate the final cDNA library. The
library was then screened twice. In the first
screening, 12 plates with approximately 3000
colonies per plate were prepared, and colony lifts
25 were performed according to Sambrook et al. (A
Laboratory Manual. 2nd ed. Cold Spring Harbor
Laboratory Press, 1989.) Filters were prehybridized
at 42°C in 5X SSPE, 10X Denhardts, 0.5% SDS for
approximately one hour; hybridized overnight at 42°C
30 in 50% formamide, 5X SSPE, 0.5% SDS, and 50µg/µl
salmon sperm DNA and washed for one hour at 50°C in
1X SSC, 0.1% SDS. Six plates were hybridized with
the BS29-1 probe and six plates were hybridized with
the BS29-2 probe both of which represent S-locus
35 glycoprotein alleles, isolated from *Brassica*
oleracea S29 (Trick, M., and Flavell, R.B.; Mol.
Gen. Genet. 218:112-117, 1989). Hybridizations and
washing conditions were chosen to allow cross-

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hybridization between two cDNAs which share approximately 70% homology. Eleven (11) colonies hybridizing to the BS29-1 and five (5) colonies hybridizing to 29-2 were further purified and characterized.

In the second screening, 5 plates of 6000 colonies were plated for each probe. Hybridizations were the same as above except the formamide was reduced to 35%. Filters were washed at 50°C in 1X SSC and 0.1% SDS, exposed to X-ray film, then washed at 60°C in 0.1X SSC and 0.1% SDS, and exposed to X-ray film again. The two exposures were lined up to determine which signals were washed off at the higher temperature.

Isolation of Candidate SLG cDNAs

Approximately 30 cDNAs were isolated and partially sequenced by the dideoxy method of Sanger to determine their identity. The cDNAs were found to fall into 6 groups, and a representative cDNA from each group was then fully sequenced as described above (A10, A14, A26, A29, A34, A35) with the exception of A35.

The A35 cDNA was identical to 29-1 which belongs to a class of related genes termed SLR-1 locus and is not linked to the S-locus (Lalonde, B.A., et al., Plant Cell 1:249-258, 1989). A second cDNA, A29, was very similar to SLR-1 showing homology at around 94% at the DNA level. As shown in Fig. 2 (a & b), both of these genes hybridized to bands in all SI and SC plants. Due to the high level of homology between two genes, gene specific bands could not be detected on the DNA blots. However, the different intensities of the two bands in some lanes suggests that A29 represents one copy while A35 represents the second copy. A third group (A26) was found to have 62% homology to the SLR-L and approximately 70% homology to most of the SLG

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sequences with the exception of the pollen recessive SLG allele, S2 (Chen, C.H. and Nasrallah, J.B., Mol. Gen. Genet. 222:241-248, 1990) which showed 92% sequence identity. The highest homology (96%) was subsequently found to a second class of related genes termed SLR-2, and as has been shown for this locus (Boyes, D.C., et al., Genetics 127:221-228, 1991), A26 is present in all plants (Fig. 2c). The SLR-2 locus is distinct from the S-locus, however, linkage has been detected between these two loci (Boyes, D.C., et al., Genetics 127:221-228, 1991). The A34 cDNA was also very similar to SLR-2 (89%), the pollen-recessive SLG-2 (92%), and A26 (85%). However, weak hybridization of the A34 cDNA to all of the plants screened for its presence suggests that it was cross-hybridizing to related sequences and not present in any of the plants (Fig. 2d). This cDNA was detected at a very low level in the library and may have been present in only a fraction of the plants used to make the cDNA library.

The remaining two cDNAs, A10 and A14, were found to contain high levels of homology to the Class I high activity SLG alleles (greater than 80%). Hybridization signals for the A10 gene could be detected in some of the lanes (Fig. 2e, lane 6), however, it was not found in the T2 (Fig. 2e, lane 2) or Z-tester (Fig. 2e, lane 3) plants. A more extensive analysis of R2, T2, Regent and Topas lines has shown this gene to be present at a low frequency in the Topas background and at a higher frequency in the Regent background. The A14 cDNA was found to hybridized to a single band in plants SI for the Z-allele such as the original *B. napus* ssp. *rapifera* line carrying the Z-allele (Fig. 2f, lane 3), the T2 line (Fig. 2f, lane 2), and the R2 line.

The ends of the 30 clones obtained from the two screenings were sequenced using the standard Sanger dideoxy sequencing method and the sequenase enzyme

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(United States Biochemical, Cleveland, OH). The six full length clones (A10, A14, A26, A29, A34, A35) which were obtained from the second library screening were further characterized. To sequence the full length cDNA clones, deletions were made using exonuclease III and Mung Bean nuclease according to the procedure outline in the Stratagene kit (Stratagene, LaJolla, CA). Overlapping deletions were sequenced for both strands. All DNA and protein sequence analysis was performed on the DNASIS and PROSIS software (Pharmacia, Piscataway, NJ.)

Sequence Analysis of the A14 cDNA (SEQ ID NO: 1)

Complete DNA sequence analysis revealed that the A14 cDNA is 1477 bp long with 3 base pairs upstream of the ATG start codon and 163 bp untranslated region at the 3'-end after cDNA. There is an open reading frame for a protein of 437 amino acids. (Fig. 3) (SEQ ID NO: 1). The cDNA has several features which are characteristic of other SLG sequences. The first 31 amino acids (Fig. 3, underlined) encode a signal sequence which is absent in the mature protein form of other SLGs (Nasrallah, J.B. et al., Nature 326:617-619, 1987; Takayama, S., et al., Nature 326:102-105, 1987; Isogai, A., et al., Plant Cell Physiol. 28:1279-1291, 1987). There are 12 potential N-glycosylation sites at Asn-X-Ser/Thr (Fig. 3, dotted lines) in keeping with fact that N-glycosidic saccharide chains are present on the native protein (Takayama, S. et al., Nature 326:102-105, 1987; Takayama, S., et al., Agric. Biol. Chem. 53:713-722, 1989). However, not all of the sites are thought to be glycosylated in the SLG protein (Takayama, S., et al., Nature 326:102-105, 1987; Isogai, A., et al., Plant Cell Physiol. 28:1279-1291, 1987). At the carboxy terminus of the protein, there are 12 exactly spaced cysteine

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residues (Fig.3, stars) which are highly conserved in all SLG proteins (Isogai, A., et al., Plant Cell Physiol. 28:1279-1291, 1987; Dwyer, K.G., et al., Plant Mol. Biol. 16:481-486, 1991) and thought to be important for the structure of the protein. Lastly, the A14 amino acid sequence has 15 of the 16 amino acids (Fig. 3., triangles) that have been found to be conserved in *B. oleracea* alleles (Dwyer, K.G. et al., Plant Mol. Biol. 16:481-486, 1991). The only amino acid residue that is not conserved is a threonine at position 32, which is an isoleucine in the *B. oleracea* genes.

A DNA homology comparison of the A14 DNA sequence (SEQ ID NO: 1) to other SLG sequences shows that it is very similar to the Class I SLG alleles with homologies ranging from 81% to 90% (Table I), while only 73% similar to the Class II, pollen recessive allele, SLG-2. At the amino acid level, the percentage of homology drops to the range of 71% to 87%. The A14 coding region is most closely related to the SLG-6 allele isolated from *B. oleracea* (Nasrallah, J.B., et al., Nature 326:617-619, 1987).

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TABLE 1

5 HOMOLOGY OF THE A14 cDNA TO OTHER SLG ALLELES.

	GENE HOMOLOGY	DNA HOMOLOGY	AMINO ACID
10	SLG-2	73%	65%
	SLG-6	90%	82%
	SLG-8	87%	78%
	SLG-13	88%	80%
15	SLG-14	NA*	87%
	SLG-22	NA*	77%
	SLG-29	87%	76%
	910	84%	76%

20 The coding region of the A14 cDNA was aligned to published DNA and amino acid sequences (with the exception of 910). S2 (Chen and Nasrallah, 1990), S6 (Nasrallah, et. al., 1987), S13 (Dwyer, et. al., 1991), S14 (Nasrallah, et. al., 1987), S22 (Nasarallah, et. al., 1987), and S29 (Trick and
 25 Flavell, 1989), were isolated from *B. oleracea*. S8 (Dwyer, et. al., 1991), was isolated from *B. campestris*. Gene 910 was isolated from a W1 SI canola line. NA* means not available.

30

Segregation of the A14 gene in T2 plants

35 To confirm that the A14 cDNA is associated with SI in T2 plants, the inheritance of this allele in a segregating F2 population was studied. A homozygous T2 plant (T2/T2) was crossed to a SC Topas (t/t) plant by standard breeding methods to generate a heterozygous F1 population (T2/t) which was then
 40 self-pollinated to produce a F2 population of T2/T2, T2/t and t/t plants. These plants were determined

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to be SI or SC by self-pollination and reciprocal crosses to the parental T2 and Topas lines followed by testing for the SI phenotype as described above. In addition, the genomic DNA was subjected to DNA blot analysis as described above to determine which plants carried the A14 gene in their genome. Of the 28 plants tested, 21 gave a positive signal for the A14 gene on genomic blots.

In Fig. 4, lanes 1-3 represent genomic DNA from a homozygous SI T2 plant (T2/T2), a SC Topas plant (t/t), and the F1 T2/t plant which was self-pollinated to produce the F2 generation. Both the T2 and T2/t lanes show hybridization to the A14 probe while the SC Topas plant does not. Lanes 4-12 contain genomic DNA from F2 plants determined to be SI; based on the ability of the stigma to inhibit germination of self-pollen and T2 pollen (T2/T2 and T2/t) as determined by the methods described above. All of these plants contain the A14 gene. The plants whose DNA showed no hybridization to the A14 probe (lanes 13-19) were also found to be compatible to T2 and self-pollen (t/t) with the exception of one plant. This plant revealed a SI phenotype when self-pollinated. However, seed set was observed in reciprocal crosses to T2 plants suggesting that in this plant, SI was resulting from another S-locus that was either absent in the T2 plants or recessive to the A14 S-locus. Thus, the A14 gene does segregate with T2 SI in the F2 population.

Expression of the A14 gene During Flower Development

To determine the expression patterns of the A14 gene in the T2 line, total RNA samples from anthers and stigmas of different bud sizes, and from leaves and open flower petals were subjected to RNA blot analysis using the A14 cDNA as a probe. As seen in Fig. 5, A14 mRNA could not be detected in leaves (lane 1), petals (lane 2), or anthers (lanes 3-10).

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However, high levels of message could be detected in the stigmas (lanes 11-18). In the smallest buds (lane 11), the level of A14 transcripts is relatively small. With increasing buds sizes, a stronger signal was detected. As the buds approached anthesis, maximum levels of A14 message were detected (lanes 13-16). By anthesis, levels of A14 mRNA decline (lane 18). At this stage, the stigmas have acquired the SI phenotype. This pattern of expression is similar to that seen for other SLG genes (Nasrallah, J.B., et al., Nature 318:263-267, 1985).

EXAMPLE 2

Isolation and Characterization of the 910 cDNA (SEQ ID NO: 2) from *B. campestris*.

Plant Crosses and Growth Conditions

The self-incompatible (SI) *B. campestris* line is triazine tolerant version of the candle cultivar developed at the University of Guelph. Plants were grown either in a green house or in a growth room under 16 hour daylight and 8 hour dark conditions. Plants were tested for SI by measuring seed set, or staining pistils for pollen tube growth with aniline blue examining them under a fluorescence microscope according to the method of Kho & Baer as described above.

Generation of the W1 SI Canola Line.

Figure 6 illustrates the generation of the WI self-incompatible line. The SI *B. campestris* cultivar Atr-Candle (SeCan, Ottawa) was employed as a pollen parent in a cross with the self-compatible (SC) *B. napus* ssp. *oleifera* spring canola cultivar Westar (Agriculture Canada, Saskatoon). Resulting triploid F1s ($2n=3x=29AAC$) were self-pollinated and subjected to fluorescence microscopy according to

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the method of Kho and Baer (Euphytica 17:298-302, 1968) to detect pollen growth. The F1 plants that expressed SI were chain crossed to identify full sibs carrying the same S-allele. Subsequent backcrossing involved the SI triploid F1s as females and the corresponding recurrent parent as recurring pollen parent, for convenience. Each cycle of backcrossing was coupled with fluorescence microscopy evaluation of pollen tube growth as described above to identify SI plants for subsequent backcrossing. This scheme ensured that the SI allele from *B. campestris* was dominant to SC associated with the *B. napus* recurrent (pollen) parent. The variety Westar-1 evolved from this backcross series as an F3 derivation of (W1) Westar/Atr-C-1//4Westar; or the progeny tested homozygotes of the backcross for derivative of Westar by Atr-candle using Westar as the recurrent pollen parent. Crosses of W1 to SC *B. napus* canola lines showed that SI was inherited as a single dominant (or partially dominant) locus.

Identification of W1 SLG-like Genes and the Cloning of the 910 SLG cDNA.

The initial characterization of the W1 line involved hybridization of the A14 cDNA described in Example 1 to a genomic DNA blot washed with reduced stringency at 50°C in 1xSSC, 0.1% SDS, which allows hybridization to sequences having about 65% homology and greater. Under these conditions, multiple bands could be detected in both SI and SC plants as illustrated (Fig. 7). However, two hybridizing bands were found to be present in W1 genomic DNA (Fig. 7, lanes 2 & 3) and in SI plants (Fig. 7, lanes 4, 6, 8-14) derived from two different crosses involving W1. The SC Westar line (Fig. 7, lane 1) and SC progeny (Fig. 7, lanes 5, 7, 15-19) from the

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crosses did not contain these fragments.

To isolate the W1 associated fragments, oligomers for PCR amplification were designed to highly conserved regions in published SLG sequences as illustrated in Figure 8. The SI-2 (+)-strand primer (SEQ ID NO: 3) corresponds to nucleotides 461-481 of the conserved region of the A14 cDNA and SI-1 (-)-strand primer (SEQ ID NO: 4) corresponds to a sequence complimentary to nucleotides 1290-1270 of the conserved region of the A14 cDNA. PCR amplification was performed according to a modification of the method described by Saiki et al. (Science 230:1350, 1985). Two different sources of DNA were used; the W1 homozygote (Fig. 7, lane 2) and the 1581 plant (Fig. 7, lane 4). W1 and 1581 genomic DNA were digested with HindIII and fractionated on a 0.7 % agarose gel. The regions in the gel spanning 3.6 to 3.9kb and 6.5 to 6.9kb were excised and the DNA was isolated by electroelution. Approximately 50ng of the fractionated genomic DNA was used in a 100 μ l PCR reaction with 1 μ M of each primer (SI-1 and SI-2), 200 μ M each dNTP, and 2.5 units of Taq polymerase. The PCR conditions were 94°C for 1.5 min., 45°C for 1 min., and 72°C for 1.5 min. for a total of 30 cycles. The PCR products were cloned into pBluescript (Stratagene, LaJolla, CA) by standard methods. The expected product size was roughly 800 bp starting approximately 400 bp from the 5' end. The cloned PCR products were partially sequenced as described above to determine their identity, and then used as probes on genomic blots. From the 6.5kb region, two different clones were obtained, one was specific for the 1581 plant and the second clone, 910, hybridized to the upper W1 specific band (Fig. 7). From the 3.6kb region, only one clone, 1631, was obtained and it was found to hybridize to the lower W1 specific band. RNA blot analysis performed (not shown) as described

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above revealed that only the 910 gene was highly expressed in the stigma as expected for a putative SLG gene, and consequently, the 910 gene was further characterized.

5 To isolate the cDNA for the 910 SLG allele, a modified rapid amplification of cDNA ends (RACE) technique was used (Frohman *et al.*, Proc. Natl. Acad. Sci. 85:8998-9002, 1988). The 3' end was isolated using the SI-1 primer (SEQ ID NO: 4) and
10 the Adaptor primer to amplify first strand cDNA synthesized from total RNA with the T₁₇-adaptor primer (Fig. 8). The PCR products were cloned by standard methods and screened with the 910 genomic probe to identify positive clones. To isolate the
15 5' region of the cDNA, first strand cDNA, tailed at the 5' end with dA residues using terminal transferase, was amplified with the 910-1 specific-primer at the 3' end and the dT₁₇-adaptor and Adaptor primers at the 5' end, followed by a
20 second round of amplification with the 910-2 specific-primer (SEQ ID NO: 7) and the adaptor primer (Fig. 8). Partial sequence analysis of the cloned PCR products confirmed that they were derived from the 910 gene.

25

PCR Amplification of 910 cDNA

The 3' end of the cDNA was amplified using the RACE procedure (Frohman, M.A., *et al.*, Proc. Natl. Acad. Sci. 85:8998-9002, 1988). The 1st strand cDNA
30 was synthesized from 10µg of total stigma RNA using the dT₁₇-adaptor primer shown in Figure 8 using the method of Krug & Berger (Meth. Enzymol. 152:316-325, 1987). The PCR reaction contained 1/5th of the cDNA in 100µl with 400nM of the SI-2 primer (SEQ ID NO:
35 3) and the Adaptor primer for a total of 30 cycles. The PCR products from two separate PCR reactions were cloned and screened by colony hybridization

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with the 910 genomic probe to identify the desired clone.

5 The 5' end was amplified using a modified procedure of the RACE method (Harvey, R.J. and Darlison, M.G., Nucl. Acid Res. 19:4002, 1991) as follows. The 1st strand cDNA was synthesized from approximately 1 μ g of poly A⁺ stigma RNA, and subsequently tailed. Serial dilutions of cDNA were amplified using 300nM of the 910-2 (SEQ ID NO: 7) primer, 200nM of the Adaptor primer and 100nM of the dT₁₇-adaptor primer for 30 cycles of 94°C for 1.5 min., 51°C for 1 min., and 72°C for 2.5 min. The resulting products (very faint smears) were fractionated on a 1% low melting-point agarose gel and agarose plugs were removed with pasteur pipettes (Zintz, C.B. and Beebe, D.C., BioTechniques 11:158-162, 1991) around the expected size of 1kb. The DNA containing agarose plugs were melted at 70°C for 10 min. and used for a second round of PCR amplification using 200nM each of the adaptor and 910-3 (SEQ ID NO:10) primer for 30 cycles shown in Figure 8. A distinct band of the expected size was obtained when 1/500 and 1/2500 of the stigma cDNA was used in the PCR reaction. PCR products from two separate PCR reactions were cloned and characterized.

DNA Sequencing

30 The 5' and 3' ends of cDNA clones were partially sequenced using dideoxy sequencing method of Sanger and the sequenase enzyme (United States Biochemicals, Cleveland Ohio) to confirm that they were derived from the 910 gene. To avoid errors which may have been introduced during the PCR reaction, two 5' end and three 3' end cDNAs derived from separate PCR reactions were sequenced. To

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sequence the full length cDNA clones, deletions were made using exonuclease III and Mung Bean nuclease according to the procedure in the Stratagene kit. Overlapping deletions were sequenced for both
5 strands. All DNA and protein sequence analysis was performed on the DNASIS and PROSIS software. (Pharmacia, Piscataway, NJ).

Sequence Analysis of the 910 cDNA.

10 The complete sequence of the 910 cDNA was determined as described above. The sequence of the 910 cDNA obtained from the overlapping 5' and 3' PCR products is shown in Fig. 9 (SEQ ID NO: 2). The
15 cDNA is 1424 bp in length and encodes a protein of 409 amino acids. This protein is shorter than the 434 to 438 amino acids encoded by other SLG alleles, and is caused by a deletion of one nucleotide at position 1216 resulting in a frameshift and
20 premature termination. The frameshift does not appear to be due to a cloning artifact as it was present in 3 different cDNA clones in addition to the genomic clone.

The putative 910 protein has several key features in common with other SLG alleles isolated
25 from *B. oleracea* and *B. campestris*. The first 31 amino acids (Fig. 9., underlined) encode a signal sequence (Nasrallah, J.B., et al., Nature 326:617-619, 1987; Takayama, S., et al., Nature 326:102-105, 1987) which is absent in the mature protein form of
30 other SLGs (Takayama, S., et al., Nature 326:102-105, 1987; Isogai, A., et al., Plant Cell Physiol. 28:1279-1291, 1987). There are 5 potential N-glycosylation sites at Asn-X-Ser/Thr (Fig. 9., dotted lines) in keeping with fact that N-glycosidic
35 saccharide chains are present on the native protein (Takayama, S., et al., Nature 326:102-105, 1987; Agric. Biol. Chem. 53:713-722, 1989. Generally, it has been found that SLG sequences contain both

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shared and unique N-glycosylation sites. However, the 910 sequence does not appear to have any unique sites. Alignment of the Class I SLG sequences (SLG-6, SLG-8, SLG-13, SLG14, SLG-22, SLG-29; Dwyer, K.G., et al., Plant Mol. Biol. 16:481-486, 1991) as well as the A14 SLG described above has shown that the five N-glycosylation sites are present in all SLG amino acid sequences, with the exception of SLG-29 which lacks the first site. At the carboxy terminus, there are 11 of the 12 exactly spaced cysteine residues (Fig 9., stars) which are highly conserved in all SLG proteins (Isogai, A., et al., Plant Cell Physiol. 28:1279-1291, 1987; Dwyer, K.G., et al., Plant Mol. Biol. 16:481-486, 1991) and thought to be important for the structure of the protein. The last cysteine residue has been lost as a result of the shifted reading frame.

DNA homology comparison of the 910 DNA sequence to other SLG sequences shows that it is very similar to the Class I SLG alleles with homologies of about 84% (Table 2), while only 75% similar to the Class II, pollen recessive allele, SLG-2. At the amino acid level, the percentage homology drops to the range of 74% to 80% for the Class I proteins and 68% for Class II proteins. While the 910 gene originated from a *B. campestris* genome, the coding region appears to be equally similar to both *B. oleracea* and *B. campestris* SLG-alleles. Dwyer, K.G., et al., (Plant Mol. Biol. 16:481-486, 1991) has suggested that there are 16 amino acids which are specific to *B. oleracea* SLG alleles. However, the predicted protein sequence of the 910 gene contains 13 of the 16 amino acids indicating that they are not species-specific.

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TABLE 2
HOMOLOGY OF THE 910 cDNA TO OTHER SLG ALLELES.

5	GENE	DNA HOMOLOGY	AMINO ACID
	HOMOLOGY		
	SLG-2	75%	68%
	SLG-6	86%	80%
	SLG-8	85%	79%
10	SLG-13	86%	79%
	SLG-14	NA*	74%
	SLG-22	NA*	76%
	SLG-29	86%	78%
	A14	84%	76%

15 The coding region of the 910 cDNA was aligned to published DNA and amino acid sequences (with the exception of A14). S2 (Chen and Nasrallah, 1990), S6 (Nasrallah, et. al., 1987), S13 (Dwyer, et. al., 1991), S14 (Nasrallah, et. al., 1987), S22 (Nasrallah, et. al., 1987), and S29 (Trick and Flavell, 1989), were isolated from *B. oleracea*. S8 (Dwyer, et. al., 1991), was isolated from *B. campestris*. A14 was isolated from a T2 SI canola line. NA* means not available.

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Inheritance of the 910 gene in a F2 Population

In the initial plant crosses, the SI plants were confirmed to carry the same SI allele as the W1 line. Consequently, a more extensive study was performed to confirm that the 910 gene represented the active SLG allele in the W1 plants. A homozygous W1 plant (W1/W1) was crossed to the SC Westar line (w/w), described above. The resulting F1 (W1/w) was self-pollinated to produce a F2 population of W1/W1, W1/w, and w/w plants. Each of these plants were tested for self-pollination, and cross-compatibility with the parental W1 and Westar

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plants. The genomic DNA taken from plants resulting from the crosses was digested with HindIII run on an agarose gel, blotted and analyzed for the presence of the 910 gene using the 910 probe. In Fig. 10, lanes 1-3 represent a homozygous SI W1 plant, the W1/w F1 plant and a homozygous SC Westar plant, respectively. Lanes 4-19 represent the F2 generation. All of the plants which were demonstrated to be SI and did not show any fertilization in reciprocal crosses to W1 were positive for the 910 gene (W1/W1, W1/w; lanes 4-11). The plants which were SC and compatible in crosses to W1 and Westar did not show any hybridization to the 910 probe (w/w; lanes 12-19). Thus, the 910 gene was established to segregate with the W1 SI phenotype.

910 mRNA Levels

To determine which tissues contained 910 transcripts, total RNA derived from leaf, petal, anther, and stigma tissues was subjected to RNA blot analysis as described above using the 910 probe (Fig. 11). The anther and stigma transcripts were extracted from different bud sizes ranging from approximately 1mm (1) to 4mm (7), and at anthesis (A). 910 transcripts could not be detected in the leaf (Fig. 10, lane 1), petal (Fig. 10, lanes 2 & 3), or anther samples (Fig. 10, lanes 12-19). However, strong signals were detected in the stigma RNA samples with the highest levels around the mid-bud size (Fig. 10, lanes 4-11).

EXAMPLE 3

Introduction of A14 cDNA or 910 cDNA into Plants, Plants Cells and/or Plant Protoplasts

Both the A14 gene and the 910 gene have been shown to segregate with the SI-phenotype in

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Brassica. Additionally, neither gene appears to be present in self-compatible plants. Both genes show a tissue specific expression pattern in SI plants which corresponds to the tissues responsible for self-incompatibility in *Brassica*. The specific association between these genes and their expression with the SI phenotype in plants, clearly establishes the importance of these genes in the self-incompatibility mechanism of *Brassica*.

On this basis, the present invention also relates to a transfer vector consisting essentially of the cDNA of Figure 3 (SEQ ID NO: 1) or Figure 9 (SEQ ID NO: 2) which is useful in the transformation of SC plants, plant cells from SC plants and/or protoplasts from SC plants which are capable of expressing the SI phenotype. The vectors of the present invention may be introduced into SC plants, plant cells and/or protoplasts by standard methodologies including but not limited to calcium-phosphate co-precipitation techniques, protoplast fusion, electroporation, microprojectile mediated transfer, by infection with bacteria (e.g., *Agrobacterium tumefaciens*), viruses or other infectious agents capable of delivering nucleic acids to recipient plants, plant cells and/or protoplasts capable of expressing SI genes and the SI phenotype.

By way of example, the bacteria *Agrobacterium tumefaciens* may be used to introduce the vectors of the present invention into SC plants, plant cells and/or plant protoplasts. More specifically, the A14 (SEQ ID NO: 1) or 910 cDNA (SEQ ID NO: 2) maybe cloned into the Ti plasmid pBI101.2 by standard cloning procedures. The chimeric plasmid comprising pBI101.2 and either of the cDNAs of the present invention may be introduced into *Agrobacterium tumefaciens* LBA4404 (Oomstal, Gene 14; 33-50, 1981) by standard transformation techniques well known in

the art. (Horsh et al., Science 277:1229-1231, 1985; Arnoldo, M., et al., Genome [in press]). The resulting *Agrobacterium tumefaciens* may then be used to introduce the SI cDNA into SC plants such as *Brassica napus* ssp. *oleifera* or other SC plants by standard infection procedures.

It is contemplated, that the introduction of the vectors of the present invention into SC plants, plant cells and/or plant protoplasts such as those described above, will result in the expression of the SI phenotype in plants which were previously self-compatible.

Example 4

Rapid Screening of Seedlings for the Presence of S-locus Alleles

Typically, in order to screen plants for the presence of a particular SI allele the plants being tested are grown to flowering and then crossed to tester plant lines carrying known alleles as described above. This is a time-consuming and expensive process. In order to overcome these problems, the present invention also relates to a method for the rapid screening of seedlings for the presence of S-locus alleles. The method comprises the polymerase chain reaction using genomic DNA obtained from *Brassica* seedlings and oligonucleotide probes selected from unique regions of the S-locus alleles. In particular, the rapid screening method for the 910 allele comprises the steps of:

- 1) obtaining genomic DNA from a *Brassica* seedling suspected of having the 910 allele;
- 2) combining the genomic DNA with a (+) strand oligonucleotide having the sequence CTTGTCATTCGATACTCCAA (SEQ ID NO: 5) or TCTTCACCAGTGGATACCAG (SEQ ID NO: 6) with a (-) strand oligonucleotide having the sequence

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ACTGGACCCTTCTCTCAGAT (SEQ ID NO: 7);

3) amplifying the allele using the polymerase chain reaction to render the allele detectable; and

4) determining the presence of the 910 allele via its specific amplification products.

10 By way of non-limiting example, the 910 allele was detected in 2 week-old seedlings of *Brassica* species carrying the 910 allele by the method of the present invention. In these examples, genomic DNA was prepared from 2 week-old *Brassica* seedlings by
15 the method of Edwards and Thompson, (Nucl. Acids. Res. 19:1349, 1991.) Genomic DNA was then used in a polymerase chain reaction using a 910 specific (+) strand probe representing nucleotides 436-454 of the 910 cDNA having the sequence CTTGTCATTCGATACTCCAA
20 (SEQ ID NO: 5) and a 910 specific (-) strand probe representing nucleotides corresponding to a sequence complimentary to nucleotides 1042- 1023 of the 910 cDNA and having the sequence ACTGGACCCTTCTCTCAGAT (SEQ ID NO: 7). (Figure 8, 910-2). This method
25 resulted in the detection of a 910 specific amplification product in DNA from 2 week-old seedling. Similarly 910 specific amplification products were detected using a (+) strand oligonucleotide representing nucleotides 896-915 of
30 the 910 cDNA (SEQ ID NO: 6) and having the sequence TCTTCACCAGTGGATACCAG in combination with the 910-2 (-) strand (SEQ ID NO: 7) oligonucleotide described above.

35 The A14 gene was also detected in 2 week-old *Brassica* seedlings using the polymerase chain reaction and A14 gene specific oligonucleotides. In these examples, an A14 specific (+) strand oligonucleotide corresponding to nucleotides 446-464

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and having the sequence ACAACCGCTCAAGTCGATT (SEQ ID NO: 8) was used in conjunction with an A14 specific (-) strand oligonucleotide corresponding to a sequence complimentary to nucleotides 899-881 of the A14 cDNA and having the sequence TCTGAGTCGAATGGAAGAG (SEQ ID NO: 9).

It is also contemplated that other oligonucleotides may be selected from other regions of S-locus alleles in order to screen for the presence of that allele in young seedlings by the method of the present invention.

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We Claim:

1. An isolated cDNA of the self-incompatibility locus of *Brassica napus* ssp. *rapifera* comprising the sequence set forth in Figure 3 (SEQ ID NO: 1).
2. An isolated cDNA of the self-incompatibility locus of *Brassica campestris* comprising the sequence set forth in Figure 9 (SEQ ID NO: 2).
3. An isolated cDNA of the self-incompatibility locus of *Brassica napus* ssp. *rapifera* consisting essentially of the sequence set forth in Figure 3 (SEQ ID NO: 1).
4. An isolated cDNA of the self-incompatibility locus of *Brassica campestris* consisting essentially of the sequence set forth in Figure 9 (SEQ ID NO: 2).
5. A vector comprising the cDNA of Claim 1.
6. A vector comprising the cDNA of Claim 2.
7. The vector of Claim 3 or 4 further comprising the plasmid pTZ18.
8. The vector of Claim 3 or 4 further comprising the plasmid pBSc.
9. The cDNA of Claim 1 or 2 wherein said cDNA codes for an S-locus glycoprotein.
10. The cDNA of Claim 1 wherein said cDNA codes for an SI-locus glycoprotein having from about

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76% to about 87% homology to Class I SI-locus glycoproteins.

11. The cDNA of Claim 2 wherein said cDNA codes for a S-locus glycoprotein having from about 74% to about 80% homology to the Class I S-locus glycoproteins.

12. The cDNA of Claim 1 wherein said cDNA is from about 84% to about 90% homologous to Class I S-locus genes.

13. The cDNA of Claim 2 wherein said cDNA is from about 84% to about 86% homologous to Class I S-locus genes.

14. The cDNA of Claim 1 or 2 wherein the cDNA is expressed in the stigma of a self-incompatible plant.

15. An oligonucleotide having homology to S-locus genes of *Brassica* consisting essentially of the sequence CTTGTGGCAAAGTTTCGATT (SEQ ID NO: 3).

16. An oligonucleotide having homology to S-locus genes of *Brassica* consisting essentially of the sequence CTGACATAAAGATCTTG (SEQ ID NO: 4).

17. A method of identifying and amplifying DNA sequences having homology to S-locus genes comprising, performing a polymerase chain reaction utilizing two primers, the two primers consisting essentially of the sequences CTTGTGGCAAAGTTTCGATT (SEQ ID NO: 3), and CTGACATAAAGATCTTG (SEQ ID NO: 4), respectively.

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18. A method of identifying and isolating DNAs homologous to genes of the S-locus of *Brassica* species comprising the step of screening a plant cDNA library with an oligonucleotide consisting essentially of the sequence CTTGTGGCAAAGTTTCGATT (SEQ ID NO: 3) or CTGACATAAAGATCTTG (SEQ ID NO: 4).

19. A method of identifying and isolating DNAs homologous to genes of the S-locus of *Brassica* species comprising the step of screening a plant genomic DNA library with an oligonucleotide consisting essentially of the sequence CTTGTGGCAAAGTTTCGATT (SEQ ID NO: 3) or CTGACATAAAGATCTTG (SEQ ID NO: 4).

20. A transfer vector comprising the cDNA of Claim 1.

21. A transfer vector comprising the cDNA of Claim 2.

22. A transfer vector of Claim 20 or 21 further comprising a Ti plasmid.

23. A transfer vector comprising the plasmid pBI101.2.

24. An *Agrobacterium tumefaciens* useful in the transformation of a plant, plant cell and/or plant protoplast, said *Agrobacterium tumefaciens* comprising the transfer vector of Claim 20 or 21.

25. A method for conferring the self-incompatible phenotype on a self-compatible plant, the method comprising transferring the transfer vector of Claim 20 into a self-compatible plant capable of assimilating said vector and expressing self-incompatibility.

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26. A method for conferring the self-incompatible phenotype on a self-compatible plant, the method comprising transferring the transfer vector of Claim 21 into a self-compatible plant capable of assimilating said vector and expressing self-incompatibility.

27. A method for conferring the self-incompatible phenotype on a self-compatible plant, the method comprising transferring the transfer vector of Claim 22 into a self-compatible plant capable of assimilating said vector and expressing self-incompatibility.

28. The method of Claim 25, 26 or 27 wherein the self-compatible plant is of the genus *Brassica*.

29. A method for conferring the self-incompatible phenotype on a self-compatible plant, the method comprising infecting a receptive plant, plant cell, or plant protoplast with *Agrobacterium tumifaciens* that has been transformed by inclusion of the vector of Claim 5 or 6, said plant, plant cell or plant protoplast capable of assimilating said vector and expressing self-incompatibility.

30. A method for the rapid detection of an S-locus allele in a *Brassica* seedling comprising:

(a) obtaining genomic DNA from a *Brassica* seedling suspected of containing the S-locus allele;

(b) combining said genomic DNA with a (+) strand oligonucleotide taken from a unique region of the S-locus

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allele and a (-) strand oligonucleotide corresponding to a region complementary to a region of the S-locus allele;

5

(c) amplifying the genomic DNA suspected of containing said S-locus allele via a polymerase chain reaction utilizing said oligonucleotides as primers to render said S-locus allele detectable; and

10

(d) determining the presence of said amplified S-locus allele its specific amplification products.

15

31. A method for the rapid detection of the 910 allele in a *Brassica* seedling comprising:

(a) obtaining genomic DNA from a *Brassica* seedling suspected of containing said 910 allele;

5

(b) combining said genomic DNA with the oligonucleotides CTTCGTCATTGACTCCAA (SEQ ID NO: 5) and ACTGGACCCTTCTCTCAGAT (SEQ ID NO: 7);

10

(c) amplifying the genomic DNA suspected of containing said 910 allele via a polymerase chain reaction utilizing the oligonucleotides of Step (b) as primers to render said 910 allele detectable; and

15

20

(d) determining the presence of said

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910 allele via its specific amplification products.

32. A method for the rapid detection of 910 allele in a *Brassica* seedling comprising:

5 (a) obtaining genomic DNA from a *Brassica* seedling suspected of containing said 910 allele;

10 (b) combining said genomic DNA with the oligonucleotides TCTTCACCAGTGGATACCAG (SEQ ID NO: 6) and ACTGGACCCTTCTCTCAGAT (SEQ ID NO: 7);

15 (c) amplifying the genomic DNA suspected of containing said 910 allele via a polymerase chain reaction utilizing the oligonucleotides of Step (b) as primers to render said 910 allele detectable; and
20

(d) determining the presence of said 910 allele via its specific amplification products.

33. A method for the rapid detection of the A14 allele in a *Brassica* seedling comprising:

5 (a) obtaining genomic DNA from a *Brassica* seedling suspected of containing said A14 allele;

10 (b) combining said genomic DNA with the oligonucleotides ACAACCGCTCAAGTCGATT (SEQ ID NO: 8)

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and TGTGAGTCGAATGGAAGAG (SEQ ID NO:
9);

5 (c) amplifying the genomic DNA
suspected of containing said A14
allele via a polymerase chain
reaction utilizing the
oligonucleotides of Step (b) as
primers to render said A14 allele
10 detectable; and

(d) determining the presence of said
A14 allele via its specific
amplification products.

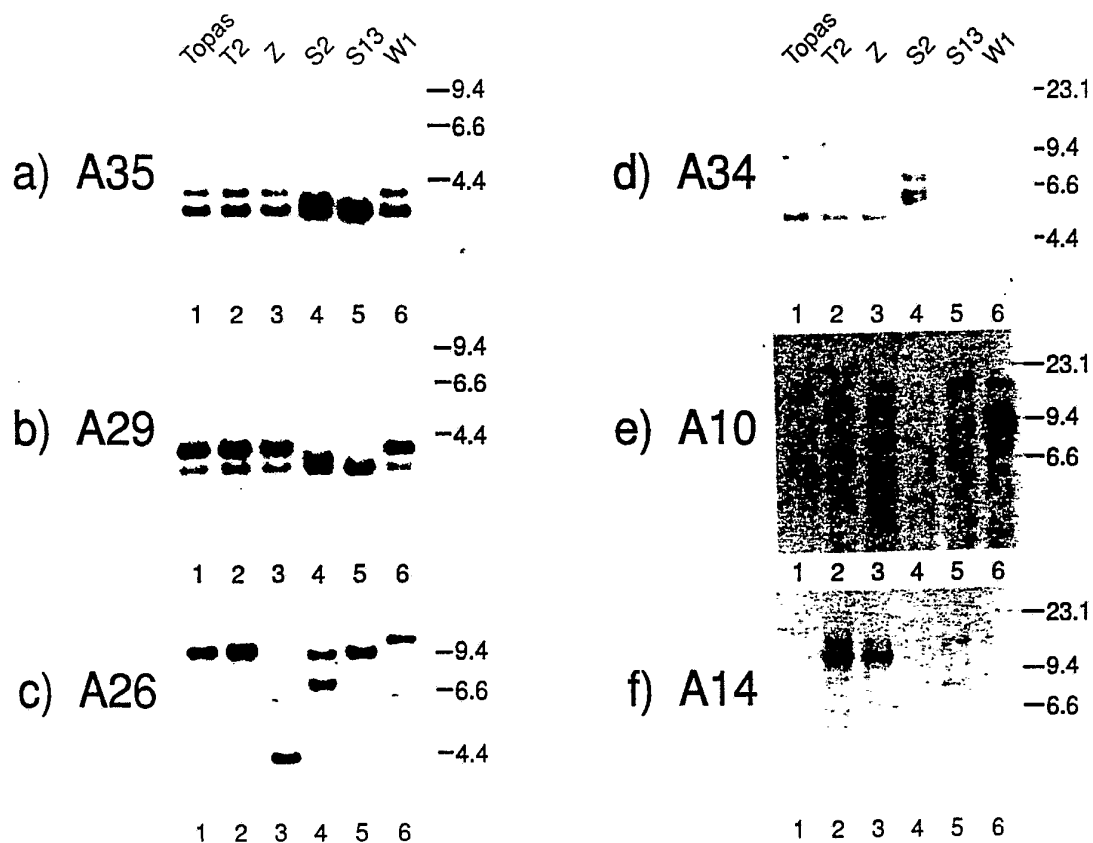
34. The method of Claim 30, 31, 32 or 33
wherein the seedling is about 2 weeks old.

FIGURE 1
Crossing and selection procedure
employed to produce T2.

POLLINATIONS			SELECTION/PROCESS
♀		♂	
TOPAS	X	LINE Z (rutabaga)	vernalize "z"; hybridize
	↓		
F ₁	X	TOPAS	select SI hybrid and backcross
	↓		
BC ₁ F ₁	X	TOPAS	select SI hybrid and backcross
	↓		
BC ₂ F ₁	X	TOPAS	select SI hybrid and backcross
	↓		
BC ₃ F ₁	X	TOPAS	select SI hybrid and backcross
	↓		
BC ₄ F ₁			select SI hybrid and self pollinate
	⊗		
	↓		
BC ₄ F ₂			select SI hybrid and self pollinate
	⊗		
	↓		
BC ₄ F ₃			select low glucosinolate/ low erucic acid SI line (T2)

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FIGURE 2



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FIGURE 3

(SEQ ID NO:1)

```

1      Met Lys Gly Val Arg Lys Thr Tyr Asp Asn Ser Tyr Thr Leu Ser Ser Leu Phe Val
1      GAG ATG AAA GGC GTA AGA AAA ACC TAC GAT AAC TCT TAC ACC TTA TCC TCT TTG TTT GTC
      ▲▲▲
20     Phe Phe Val Leu Ile Ile Leu Arg Pro Ile Phe Ser Thr Thr Thr Leu Ser Ser Thr Glu
61     TTT TTC GTC TTG ATT ATA CTT CGT CCT ATC TTT TCG ACC ACA ACT TTG TCG TCA ACA GAA
      ▲▲▲
40     Ser Leu Thr Ile Ser Ser Asn Arg Thr Leu Val Ser Pro Gly Asn Ile Phe Glu Leu Gly
121    TCT CTT ACA ATC TCA AGC AAC AGA ACA CTT GTA TCT CCC GGT AAT ATC TTC GAG CTC GGC
      ▲▲▲
60     Phe Phe Asn Thr Thr Ser Ser Ser Arg Trp Tyr Leu Gly Ile Trp Tyr Lys Lys Ile Ser
181    TTC TTC AAT ACC ACC TCA AGT TCT CGT TGG TAT CTC GGG ATA TGG TAC AAG AAA ATC TCC
      ▲▲▲
80     Glu Arg Ser Tyr Val Trp Val Ala Asn Arg Asp Asn Pro Leu Ser Asn Ala Val Gly Thr
241    GAA AGG TCC TAC GTA TGG GTT GCC AAC AGA GAT AAC CCT CTC TCC AAT GCC GTT GGA ACC
      ▲▲▲
100    Leu Lys Ile Ser Gly Asn Asn Leu Val Leu Leu Gly His Ser Asn Arg Ser Val Trp Ser
301    CTC AAA ATC TCA GGC AAT AAT CTT GTC CTC CTT GGT CAC TCC AAT AGA TCA GTT TGG TCG
      ▲▲▲
120    Thr Asn Leu Thr Arg Glu Asn Glu Arg Ser Pro Val Val Ala Glu Leu Leu Ala Asn Gly
361    ACG AAT CTT ACT AGA GAA AAT GAG AGA TCT CCG GTG GTG GCA GAG CTT CTC GCT AAT GGA
      ▲▲▲
140    Asn Phe Val Met Arg Asp Thr Asn Asn Asn Arg Ser Ser Arg Phe Leu Trp Gln Ser Phe
421    AAC TTC GTG ATG CGA GAC ACC AAT AAC AAC CGC TCA AGT CGA TTC TTG TGG CAA AGT TTC
      ▲▲▲
160    Asp Tyr Pro Thr Asp Thr Leu Leu Pro Glu Met Lys Leu Gly Tyr Glu Pro Lys Thr Gly
481    GAT TAC CCT ACA GAT ACT TTG CTT CCA GAG ATG AAG CTG GGT TAC GAA CCC AAA ACA GGG
      ▲▲▲
180    Leu Asn Arg Phe Leu Thr Ser Trp Arg Ser Ser Asp Asn Pro Ser Ser Gly Asp Phe Ser
541    CTG AAC AGG TTC CTT ACA TCA TGG AGA AGT TCT GAT AAT CCA TCA AGC GGG GAT TTC TCA
      ▲▲▲
200    Tyr Lys Leu Glu Ala Arg Arg Leu Pro Glu Leu Tyr Leu Ser Ser Gly Ile Phe Arg Val
601    TAC AAG CTC GAA GCC CGA AGG CTT CCT GAG CTT TAT CTA TCG AGT GGG ATC TTT CGA GTG
      ▲▲▲
220    His Arg Ser Gly Pro Trp Asn Gly Ile Arg Phe Ser Gly Ile Pro Asp Asp Lys Lys Leu
661    CAT CGG AGC GGC CCA TGG AAC GGA ATC CGA TTT AGT GGC ATA CCA GAT GAC AAA AAG CTG
      ▲▲▲
240    Ser Tyr Leu Val Tyr Asn Phe Thr Glu Asn Ser Glu Glu Val Ala Tyr Thr Phe Leu Met
721    AGT TAC CTG GTG TAC AAT TTC ACA GAG AAT AGT GAA GAA GTC GCT TAT ACA TTC CTA ATG
      ▲▲▲
260    Thr Asn Lys Ser Ile Tyr Ser Arg Leu Thr Val Ser Phe Ser Gly Tyr Leu Glu Arg Gln
781    ACC AAC AAA AGC ATC TAC TCG AGA TTG ACA GTA AGT TTC TCA GGG TAT TTA GAG CGA CAG
      ▲▲▲
280    Thr Trp Asn Pro Thr Leu Gly Met Trp Asn Val Ser Trp Ser Leu Pro Phe Asp Ser Gln
841    ACG TGG AAT CCG ACA TTA GGG ATG TGG AAC GTG TCA TGG TCT CTT CCA TTC GAC TCA CAG
      ***
300    Cys Asp Ser Tyr Arg Met Cys Gly Pro Tyr Ser Tyr Cys Asp Val Asn Thr Ser Pro Ile
901    TGC GAT TCG TAC AGG ATG TGT GGG CCT TAC TCT TAC TGT GAC GTT AAC ACA TCA CCG ATT
      ***
320    Cys Asn Cys Ile Gln Gly Phe Ile Pro Ser Asn Val Glu Gln Trp Asp Gln Arg Val Trp
961    TGT AAC TGT ATC CAA GGG TTC ATT CCC TCG AAT GTG GAG CAG TGG GAT CAG AGA GTC TGG
      ***
340    Ala Asn Gly Cys Ile Arg Arg Thr Arg Leu Ser Cys Ser Gly Asp Gly Phe Ile Arg Met
1021   GCA AAT GGG TGT ATA AGG AGG ACG CGG CTT AGC TGC AGT GGA GAT GGT TTT ATA AGG ATG
      ***
360    Lys Asn Met Lys Leu Pro Glu Thr Thr Met Ala Thr Val Asp Arg Ser Ile Gly Val Lys
1081   AAG AAT ATG AAG TTG CCA GAA ACT ACG ATG GCT ACT GTC GAC CGC AGT ATT GGT GTG AAA
      ***
380    Glu Cys Glu Lys Lys Cys Leu Ser Asp Cys Asn Cys Thr Ala Phe Ala Asn Ala Asp Ile
1141   GAA TGT GAG AAG AAA TGC CTT AGC GAC TGT AAT TGT ACC GCG TTT GCA AAT GCG GAT ATC
      ***
400    Arg Asn Gly Trp Thr Gly Cys Val Phe Trp Thr Gly Arg Leu Asp Asp Met Gln Asn Tyr
1201   CGG AAT GGT TGG ACA GGT TGT GTG TTT TGG ACC GGC CGG CTT GAC GAT ATG CAG AAT TAC

```

FIGURE 3 (cont'd)

420 Val Thr Asp His Gly Gln Asp Leu Tyr Val Arg Leu Ala Ala Ala Asp Leu Val □□□
1261 GTT ACT GAT CAC GGT CAA GAT CTT TAT GTC AGA TTG GCT GCT GCT GAC CTT GTT TAG CTG
1321 TTT CTC TTA AAA TAA ACC ACG GAT CCG ACT ATG TTA TCG AGA AAT CCG AAT ATG TGA CGC
1381 AAC TAT CTT AAT ATG TCG ATC CTT AAT ATA TTT CTG AAT CAA TAA AGA TAT ACA GAT TGT
1441 ATC CAA ATC TAT AAT ATA GAA AAC ATG CCA TAT AAA T

FIGURE 4

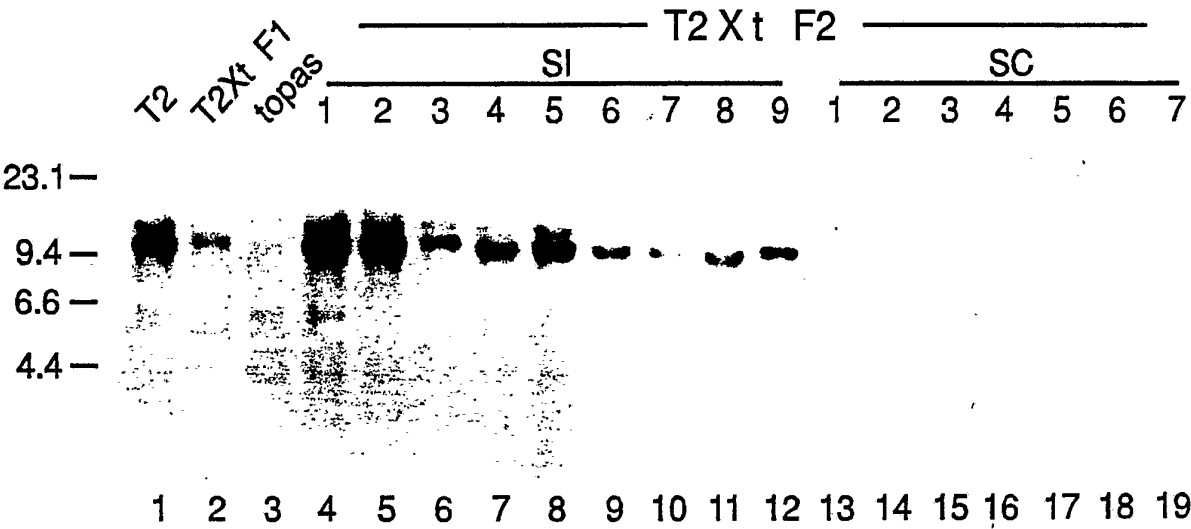


FIGURE 5

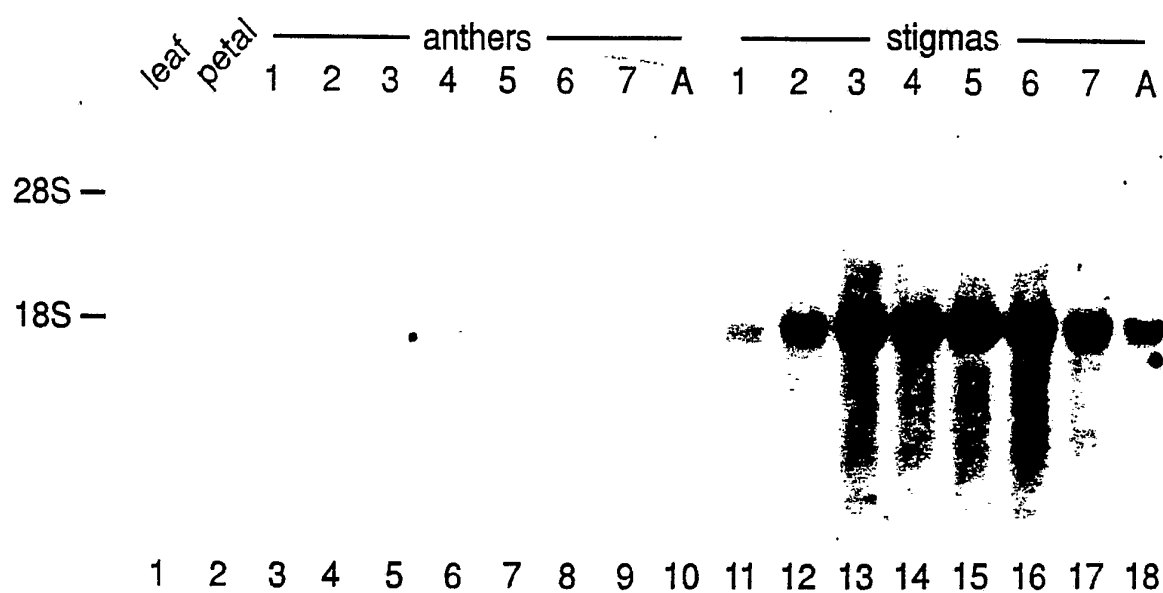


FIGURE 6

Crossing and selection procedure
employed to produce W1.

POLLINATIONS		SELECTION/PROCESS
♀	♂	
WESTAR (2N=38; AACCC)	X GP 1 (2N=20; AA)	produce interspecific hybrid
F ₁	X WESTAR	select SI hybrid and backcross
BC ₁ F ₁	X WESTAR	select SI hybrid and backcross
BC ₂ F ₁	X WESTAR	select SI hybrid and backcross
BC ₃ F ₁	X WESTAR	select SI hybrid and backcross
BC ₄ F ₁		select SI hybrid and self pollinate
BC ₄ F ₂		select SI hybrid and self pollinate
BC ₄ F ₃		select low glucosinolate/ low erucic acid SI line (W1)

FIGURE 7

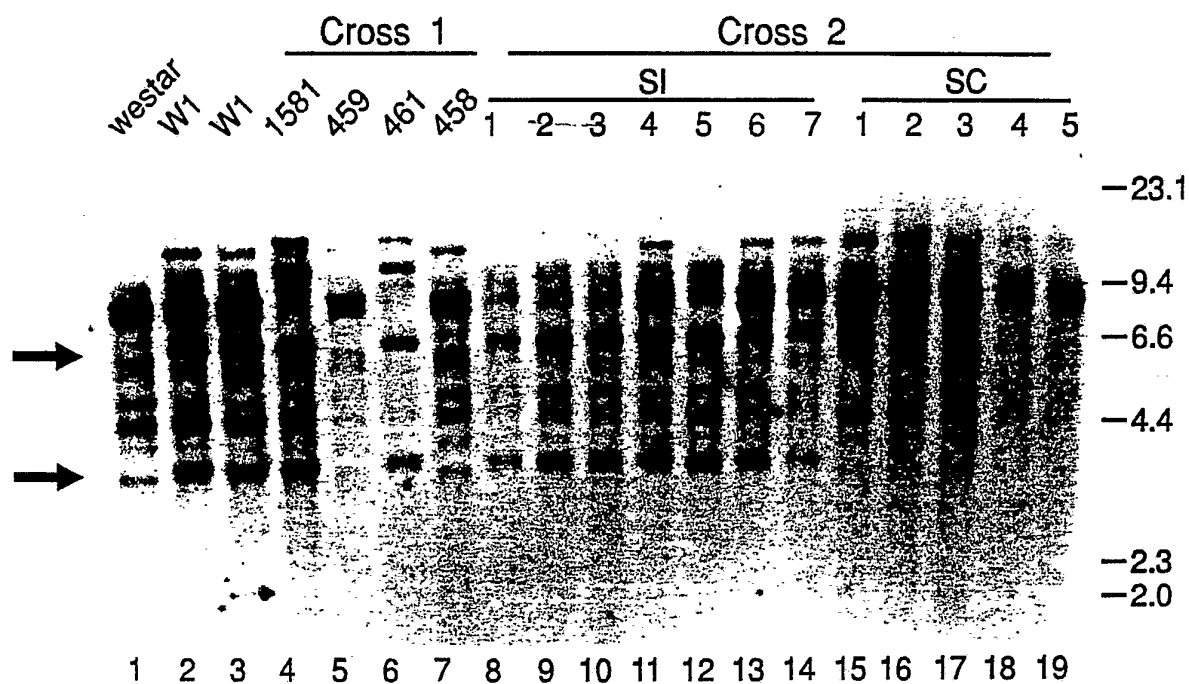


FIGURE 8

SI-2
(+strand)

5' GTCAAGCTT GTGGCAAAGTTTCGATT 3'
 ↑
 SI gene nt. 461-481

SI-1
(-strand)

5' Hind III GTCAAGCTTCTGACATAAAGATCTTGACC 3'
 ↑ SI gene nt. 1290-1270

dT17-Adaptor

5' GATCCAGATCTCGAGAAGCTTTTTTTTTTTTTTTTTT 3'

Adaptor

5' BamH1 Xho I 3'
GCGGATCCAGATCTCGAGAAGCTT
 Bgl II Hind III

910-2
(-strand)

5' ACTGGACCCTTCTCTCAGAT 3'

ACCTGACCAGGCTCTCAGAC (SLG-29)

910-3
(-strand)

5' ⁹¹⁵CTGGTATCCACTGGTGAAGA⁸⁹⁶ 3'
 TGGGGGTCCACTGGAGAAGA (SLG-6)

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FIGURE 9

(SEQ ID NO:2)

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1      Met Lys Gly Val Arg Lys Thr Tyr Asp Asn Ser Cys Thr Leu Ser Phe
1      G GGG AGA GAG ATG AAA GGC GTA AGA AAA ACC TAC GAC AAT TCT TGC ACC TTA TCC TTT
17      Leu Leu Val Phe Phe Val Leu Ile Leu Phe His Pro Ala Leu Ser Ile Tyr Ile Asn Thr
59      CTG CTT GTC TTT TTC GTC TTA ATT CTA TTT CAT CCT GCC CTT TCG ATC TAT ATC AAC ACT
37      Leu Ser Ser Thr Glu Ser Leu Thr Ile Ser Asn Asn Arg Thr Leu Val Ser Pro Gly Asn
119     TTG TCG TCT ACA GAA TCT CTT ACA ATC TCA AAC AAC AGA ACA CTT GTA TCC CCA GGT AAT
57      Val Phe Glu Leu Gly Phe Phe Arg Thr Thr Ser Ser Ser Arg Trp Tyr Leu Gly Ile Trp
179     GTC TTC GAG CTC GGC TTC TTT AGA ACC ACC TCA AGT TCT CGT TGG TAT CTC GGG ATA TGG
77      Tyr Lys Lys Val Ser Glu Arg Pro Tyr Val Trp Val Ala Asn Arg Asp Asn Pro Leu Ser
239     TAC AAG AAA GTG TCC GAG AGA CCC TAT GTA TGG GTT GCC AAC AGA GAT AAC CCT CTC TCC
97      Asn Ser Ile Gly Thr Leu Lys Ile Ser Cys Asn Asn Leu Val Leu Leu Gly His Ser Asn
299     AAT TCC ATT GGA ACC CTC AAA ATC TCA TGC AAT AAT CTT GTC CTC CTT GGT CAC TCC AAT
117     Lys Ser Val Trp Ser Thr Asn Leu Thr Arg Arg Asn Glu Arg Ser Pro Val Val Ala Glu
359     AAA TCT GTT TGG TCG ACG AAT CTG ACT AGA AGA AAT GAG AGA TCT CCG GTG GTG GCA GAG
137     Leu Leu Ala Asn Gly Asn Phe Val Ile Arg Tyr Ser Asn Asn Asn Asp Ala Ser Gly Phe
419     CTT CTG GCT AAC GGA AAC TTC GTC ATT CGA TAC TCC AAT AAC AAC GAC GCA AGT GGA TTC
157     Leu Trp Gln Ser Phe Asp Phe Pro Thr Asp Thr Leu Leu Pro Glu Met Lys Leu Gly Tyr
479     TTG TGG CAA AGT TTC GAT TTC CCT ACA GAT ACT TTG CTT CCA GAG ATG AAA CTA GGC TAC
177     Asp Arg Lys Lys Gly Leu Asn Arg Phe Leu Thr Ala Trp Arg Asn Ser Asp Asp Pro Ser
539     GAC CGC AAA AAA GGG CTG AAC AGA TTC CTT ACA GCA TGG AGA AAT TCA GAT GAT CCC TCA
197     Ser Gly Glu Ile Ser Tyr Gln Leu Asn Thr Gln Arg Gly Met Pro Glu Phe Tyr Leu Leu
599     AGC GGG GAA ATC TCG TAC CAA CTA AAC ACT CAA AGG GGA ATG CCT GAG TTT TAT CTA TTG
217     Lys Asn Gly Leu Arg Val His Arg Ser Gly Pro Trp Asn Gly Val Arg Phe Asn Gly Ile
659     AAA AAC GGC TTA CGA GTC CAC CGG AGC GGT CCA TGG AAT GGA GTC CGA TTT AAT GGA ATA
237     Pro Glu Asp Gln Lys Leu Ser Tyr Met Val Tyr Asn Phe Ile Glu Asn Ser Glu Glu Val
719     CCA GAG GAC CAA AAG TTG AGT TAC ATG GTG TAC AAT TTC ATA GAG AAT AGT GAG GAG GTT
257     Ala Tyr Thr Phe Arg Val Thr Asn Asn Ser Ile Tyr Ser Ile Leu Lys Ile Ser Ser Glu
779     GCT TAT ACA TTT CGA GTG ACC AAC AAC AGC ATT TAC TCG ATA TTG AAA ATT AGT TCC GAA
277     Gly Phe Leu Glu Arg Leu Thr Trp Thr Pro Thr Ser Thr Ala Trp Asn Leu Phe Trp Ser
839     GGG TTT TTG GAG CGA CTG ACG TGG ACC CCG ACA TCA ACT GCA TGG AAC TTG TTC TGG TCT
297     Ser Pro Val Asp Thr Arg Cys Asp Val Tyr Met Thr Cys Gly Pro Asn Ala Tyr Cys Asp
899     TCA CCA GTG GAT ACC AGG TGC GAT GTT TAC ATG ACT TGT GGG CCT AAC GCT TAC TGT GAC
317     Val Ser Thr Ser Arg Val Cys Asn Cys Ile Gln Gly Phe Met Pro Ser Asn Ala Gln Gln
959     GTG AGC ACA TCA CGG GTG TGT AAC TGT ATC CAA GGG TTC ATG CCC TCG AAT GCG CAG CAG
337     Trp Asp Leu Arg Glu Gly Ser Ser Gly Cys Ile Arg Arg Thr Arg Leu Thr Cys Ser Gly
1019    TGG GAT CTG AGA GAA GGG TCC AGT GGG TGT ATA AGG AGG ACG CGG CTT ACC TGC AGT GGT
357     Asp Gly Phe Thr Arg Met Arg Arg Met Lys Leu Pro Glu Thr Thr Lys Ala Ile Val Asp
1079    GAT GGC TTT ACA AGG ATG AGG ATG AAG TTG CCA GAG ACT ACG AAG GCG ATT GTC GAC
377     Arg Ser Ile Gly Val Lys Glu Cys Glu Lys Arg Cys Leu Ser Asp Cys Asn Cys Thr Ala
1139    AGG AGT ATC GGT GTG AAA GAA TGT GAG AAG AGG TGC CTT AGC GAT TGC AAC TGT ACC GCG
397     Phe Ala Asn Ala Asp Ile Val Met Val Gly Arg Ile Val ***
1199    TTT GCA AAT GCG GAT ATC GTA ATG GTG GGG CGG ATT GTG TGA TTT GGA CCG GAG AGC TCG

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FIGURE 9 (con'td)

1259 AGG ATA TCC GGA CTT ACT ATG ATG ATG GTC AAG ATC TTT ATG TCA GAT TGG CTC CTG CTA

1319 ACC TTG TTT AGC TCT TTC TCT TAA AAT AAA AAA CGG ATC CGA CTA TGA TAT TGA GAA ATC

1379 CGG ATA TGT AAC GCA ACT ATC TTA ATA TAA TAT ATA TAT CTG AAT C

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FIGURE 10

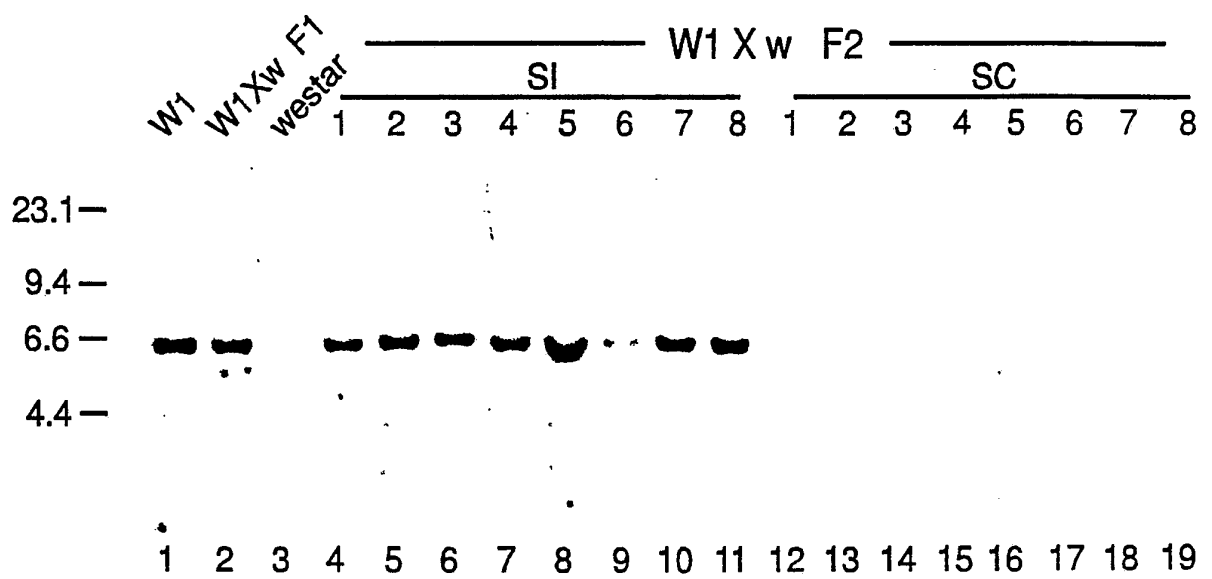
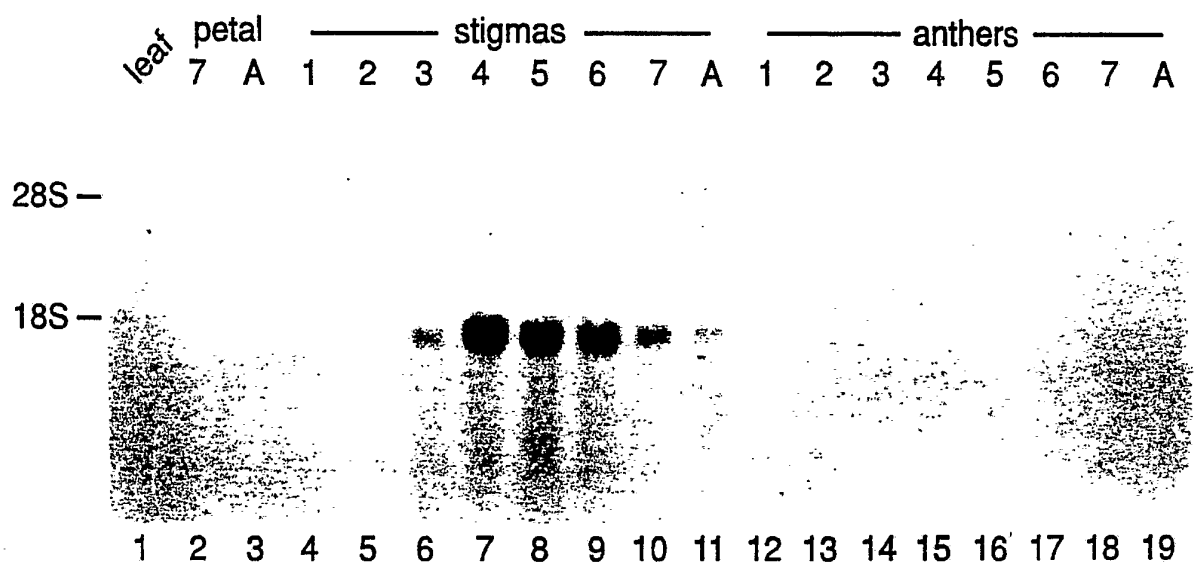


FIGURE 11



INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/04530

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12N15/29; C12N15/82; C12Q1/68; C12N1/21		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12N ; C12Q	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EMBL NUCLEIC ACID SEQUENCE DATABASE ACCESSION NO. Z11725 24-FEB-1992 " B.napus mRNA for S-locus glycoprotein"	1,3,5,9, 10,12,14
Y		20,22, 24,25, 27,28,30
X	EMBL NUCLEIC ACID SEQUENCE DATABASE ACCESSION NO. Z11724 24-FEB-1992 " B.napus mRNA for S-locus glycoprotein"	2,4,6,9, 11,13
Y		21,22, 24,26-30
X	WO,A,9 117 252 (NOVO NORDISK) 14 November 1991 see page 24, line 25	16
¹⁰ Special categories of cited documents : ¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
11 NOVEMBER 1992		24. 11. 92
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		MADDOX A.D. 

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category ^o	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 88, October 1991, WASHINGTON US pages 8816 - 8820 STEIN, J.C., ET AL. 'Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of Brassica oleracea' see page 8816, right column ----	15
X	GB,A,2 197 653 (JEFFERSON) 25 May 1988 see page 5, line 25 - page 6, line 4; figure 9 ----	23
Y	EP,A,0 436 467 (CIBA-GEIGY) 10 July 1991 see the whole document ----	20-22, 24-29
Y	THEOR. APPL. GENET. vol. 82, no. 4, September 1991, pages 466 - 472 GUILLUY, C.-M., ET AL. 'PCR detection of transcripts homologous to the self-incompatibility gene in anthers of Brassica' see the whole document ----	30
Y	WO,A,9 012 097 (GENELABS) 18 October 1990 see page 30 - page 34; claim 12 ----	30
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