Transformation of Arabidopsis with a Brassica SLG/SRK region and ARC1 gene is not sufficient to transfer the self-incompatibility phenotype

Abstract
Self-incompatibility (SI) promotes outbreeding in flowering plants, and in Brassica SI is genetically controlled by the S locus. Self-incompatible Brassica and self-fertile Arabidopsis belong to the same crucifer family. In addition, a comparative analysis reveals a high degree of microsynteny between the B. campestris S locus and its homologous region in Arabidopsis – with the notable exception that the Brassica SI genes, SLG and SRK, are missing. Brassica ARC1 encodes a component of the SRK signal transduction pathway leading to self-pollen rejection, and no closely related ARC1 homolog has been identified in Arabidopsis. The purpose of the research reported here was to introduce Brassica SI components into Arabidopsis in an attempt to compensate for the missing genes and to investigate whether the SI phenotype can be transferred. Inserts of approximately 40 kb from the fosmid clones F20 and F22, which span the B. napus W1 SLG-SRK region, were cloned into the plant transformation vector pBIBAC2. Transgenic plants were generated that expressed the Brassica SI genes in the flower buds. In addition, the endogenous, SLG-like, gene AtS1 was not co-suppressed by the Brassica SLG transgene. No SI phenotype was observed among the T1 BIBAC2-F20 and BIBAC2-F22 transgenic plants. When the ARC1 gene was transformed into BIBAC2-F20 or BIBAC2-F22 plants, the resulting BIBAC2-F20-ARC1 and BIBAC2-F22-ARC1 plants still set seeds normally, and no rejection response was observed when self-incompatible B. napus W1 pollen was placed on BIBAC2-F20-ARC1 or BIBAC2-F22-ARC1 Arabidopsis stigmas. Taken together, our results suggest that complementing Arabidopsis genome with Brassica SLG, SRK and ARC1 genes is unlikely to be sufficient to transfer the SI phenotype.

Key words
Self-incompatibility · SLG (S locus glycoprotein) · SRK (S locus receptor kinase) · ARC1 (Arm repeat containing) · Transgenic Arabidopsis

Introduction
Self-incompatibility (SI) is one of the mechanisms present in flowering plants that prevents self-fertilization and promotes out-crossing. In Brassica, SI is controlled genetically by multiple alleles of the S locus, and is sporophytic in nature, in that the pollen phenotype is determined by the parental genotype (Dodds et al. 1996). Several S locus genes have been discovered, and there is evidence that two of these are required for the SI phenotype. They encode, respectively, an S locus glycoprotein (SLG) and a plasma membrane-spanning receptor protein kinase (SRK). Both genes are expressed specifically in the stigma papillae cells and they are thought to be responsible for the female side of the SI phenotype when the communication between pistil and pollen begins (Nasrallah and Nasrallah 1993; Nasrallah et al. 1994a). The expression of SLG and SRK is necessary for the SI function, as plants carrying mutated or down-regulated SLG or SRK genes are self-fertile (Nasrallah et al. 1992; Goring et al. 1993; Nasrallah et al. 1994b; Conner et al. 1997; Stahl et al. 1998). The current model for SI in Brassica proposes that the pollen-borne ligand interacts with SLG and SRK, activates the SRK protein kinase and thus triggers a signal cascade that eventually leads to the rejection of self-pollen (Nasrallah et al. 1994a). According to the model, at least one gene required for the SI phenotype on the male side...
should be encoded in the S locus complex. Although S locus genes that are specifically expressed in the anther have been isolated, such as SLL1 and SLA (Boyes and Nasrallah 1995; Yu et al. 1996), they do not fulfill all the requirements that one would expect for the male determinant (Yu et al. 1996; Pastuglia et al. 1997). Very recently, a new gene named SCR (S locus cysteine-rich) was isolated in Brassica (Schoepfer et al. 1999). It is encoded at the S locus and is a highly polymorphic, anther-expressed gene. Functional studies proved that it is necessary and sufficient for determining pollen SI specificity, suggesting that it is a ligand for the stigmatic S receptor complex. Meanwhile, in an attempt to dissect the SRK-mediated signal transduction pathway, an ARCI (Arm Repeat Containing) gene was isolated by employing the yeast two-hybrid system. It encodes a protein that interacts specifically with the SRK kinase domain, and its expression is restricted to the stigma, the site of the SI response (Gu et al. 1998). In addition, down-regulation of ARCI mRNA levels in transgenic plants that express ARCI antisense RNA is correlated with a partial breakdown of SI, providing evidence that ARCI is a positive effector of this response (Stone et al. 1999).

Recently, a fosmid contig of ~65 kb from a functional S-haplotypic (B. napus W1 line) was isolated and analyzed (Cui et al. 1999). In our preliminary studies on transgenic B. napus plants, the 40-kb fragments (from fosmid F20 or F22) each appeared to be capable of conferring the SI female phenotype (Cui et al. 2000). In this work, we attempted to transform these SLG/SRK-spanning regions into Arabidopsis, based on the following considerations. First, the out-crossing Brassica and the self-fertilizing Arabidopsis belong to the same crucifer family. A high-resolution comparative analysis of the organization of the S locus region in one genotype of B. campestris (Sₜ haplotype) and a region of the Arabidopsis genome has already been conducted by Conner et al. (1998). The Arabidopsis region was identified as homologous to the Brassica S locus and their results revealed a high degree of microsynteny between the Brassica S locus region and its homolog in Arabidopsis, with the notable exception that sequences related to Brassica SI genes were missing in the Arabidopsis genome (Conner et al. 1998). Thus, by addressing whether these Brassica SI components could transfer the phenotype into Arabidopsis, we were hoping to shed some light on the mechanisms underlying transitions in mating systems in this crucifer family. Second, the life cycle of Arabidopsis is significantly shorter than that of Brassica, so that further investigations could be done considerably more quickly if an interesting change in phenotype was observed in the transgenic plants. Therefore, we transformed Arabidopsis with the genomic fragment containing the SLG and SRK genes from this 910 S-haplotypic (B. napus W1 line) and with the Brassica ARCI gene, and analyzed the resulting transgenic plants for the expression of the Brassica genes as well as examining them for any changes in phenotype.

Materials and methods

Vector constructs and Agrobacterium strains used for plant transformation

F20 and F22 were identified from a fosmid library (Cui et al. 1999). The F20, F22 and BIBAC2 (Hamilton 1997) DNAs were prepared by using a Qiagen kit. NorI digestion released the F20 or F22 insert. After pulsed field gel electrophoresis (PFGE), the 40-kb insert band was excised from the agarose gel and subjected to digestion with Gelase (Epigenic). The BIBAC2 vector was dugested with NorI and dephosphorylated with shrimp alkaline phosphatase (Epigenic). Aliquots (100 ng) of F20 or F22 insert were co-precipitated with 40 ng of NorI-digested, dephosphorylated BIBAC2 vector. The precipitates were dissolved in 20 μl of distilled water. Ligation was carried out under standard conditions and the ligated DNA was electroporated into E. coli strain DH10B (GIBCO-BRL Life Technologies). The resulting construct (pBIBAC2-F20 or pBIBAC2-F22) was introduced into the Agrobacterium strain COR338 (Hamilton 1997) by triparental mating (Draper et al. 1988). COR338 is the Agrobacterium strain GV3101 (Koncz and Schell 1986) containing the plasmid pCH32, which carries the extra virulent genes required for the transfer of large fragments (Hamilton et al. 1986; Hamilton 1997). After several generations, Southern hybridization was performed to confirm that the plasmid was stably maintained in COR338 (data not shown). The resulting Agrobacterium strains, pBIBAC2-F20/COR338 and pBIBAC2-F22/COR338, were used for Arabidopsis transformation. The transformation vector carrying the ARCI sense cDNA under the direction of the SLR1 promoter was introduced into Agrobacterium strain GV2200 (Deblaere et al. 1985) by triparental mating (Draper et al. 1988).

Arabidopsis transformation and screening for transgenic plants

Transgenic Arabidopsis plants of ecotype Columbia were generated by using the A. tumefaciens-mediated whole-plant infiltration protocol (Bechtold et al. 1993), except that flowers were dipped for 6 min in the infiltration medium supplied with Silwet L-77 (Lehle Seeds) instead of using vacuum infiltration. Seeds were harvested and then germinated on germination medium (1/2 MS, 10 g/l sucrose, pH 5.8; Clarke et al. 1992) containing 50 mg/l kanamycin for selection. One week later, kanamycin-resistant seedlings were transferred into soil.

Southern analysis

Genomic DNA was extracted from leaf tissues of transgenic plants and non-transformed control plants using the DNA extraction kit Phytopure (Amersham). Aliquots (1 μg) of DNA were digested with either EcoRI or NorI. EcoRI-digested DNA was fractionated on a 0.7% agarose gel and NorI-digested DNA was fractionated by PFGE. The DNA was transferred to a nylon membrane (Boehringer Mannheim) and probed with the DIG-labeled SLG cDNA (for EcoRI digestion) or with the radioactively labeled SLG and SRK cDNAs (for NorI digestion). Hybridizations were carried out under standard conditions and filters were washed twice at 65 °C with 0.1 x SSC, 0.1% SDS for 20 min (Sambrook et al. 1989). Detection of the signal from the DIG probe was done according to the manufacturer’s (Boehringer Mannheim) protocol.

Northern analysis

Total RNA was extracted according to Verwoerd et al. (1989) from transformed and untransformed Arabidopsis flower buds or from W1 stigmas. Aliquots of total RNA from Arabidopsis (15 μg) and from W1 stigmas were loaded and fractionated on formaldehyde gels as described in Brugière et al. (1999), and transferred to nylon.