Unilateral incompatibility gene *ui1.1* encodes an S-locus F-box protein expressed in pollen of *Solanum* species

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Unilateral interspecific incompatibility (UI) is a postpollination, prezygotic reproductive barrier that prevents hybridization between related species when the female parent is self-incompatible (SI) and the male parent is self-compatible (SC). In tomato and related *Solanum* species, two genes, *ui1.1* and *ui6.1*, are required for pollen compatibility on pistils of SI species or hybrids. We previously showed that *ui6.1* encodes a Cullin1 (CUL1) protein. Here we report that *ui1.1* encodes an S-locus F-box (SLF) protein. The *ui1.1* gene was mapped to a 0.43-cM, 43.2-Mbp interval at the S-locus on chromosome 1, but positional cloning was hampered by low recombination frequency. We hypothesized that *ui1.1* encodes an SLF protein(s) that interacts with CUL1 and Skp1 proteins to form an SCF-type (Skp1, Cullin1, F-box) ubiquitin E3 ligase complex. We identified 23 *SLF* genes in the *S. pennellii* genome, of which 19 were also represented in cultivated tomato (*S. lycopersicum*). Data from recombination events, expression analysis, and sequence annotation highlighted 11 *S. pennellii* genes as candidates. Genetic transformations demonstrated that one of these, *SpSLF-23*, is sufficient for *ui1.1* function. A survey of cultivated and wild tomato species identified *SLF-23* orthologs in each of the SI species, but not in the SC species *S. lycopersicum, S. cheesmaniae,* and *S. galapagense*, pollen of which lacks *ui1.1* function. These results demonstrate that pollen compatibility in UI is mediated by protein degradation through the ubiquitin–proteasome pathway, a mechanism related to that which controls pollen recognition in SI.

interspecific incompatibility | self-incompatibility | *Solanum lycopersicum* | *Solanum pennellii* | S-locus F-box protein

Self-incompatibility (SI) in *Solanum* and other Solanaceae is the S-RNase–based, gametophytic type, in which S-specificity is determined by S-RNases in the pistil (1) and S-locus F-box proteins (SLFs) in pollen (2). F-box proteins, together with Skp1 and Cullin1 proteins, are components of SCF-type (Skp1, Cullin1, F-box) ubiquitin E3 ligases that mark target proteins for degradation by the 26S proteasome (3, 4). The ubiquitin–proteasome pathway controls the pollen compatibility phenotype in SI (5). In the "collaborative non–self-recognition" model (6), the S-locus encodes multiple SLF proteins that together recognize different sets of S-RNases. In a compatible pollination, the SLF/S-RNase interaction leads to protection of pollen tubes against cytotoxic S-RNase, whereas in incompatible pollinations, a failure to recognize self–S-RNase results in pollen tube arrest. In addition, modifier genes, such as those encoding HT-B, NaStEP, and 120-kDa proteins in the pistil, and CUL1 and SSK1 proteins in pollen, are required for SI function but do not confer S-specificity (7–11).

Unilateral incompatibility (UI) is a reproductive barrier related to SI in which pollen from one species or population is rejected on pistils of a related species or population, whereas in the reciprocal crosses, no pollen rejection occurs. Pollen of SC species or populations is almost always rejected on pistils of related SI species or populations, whereas in the reciprocal crosses (SC pollinated by SI), pollen rejection rarely occurs. This unidirectional pattern of pollen rejection is referred to as the "SI × SC rule" (12). Although the mechanisms of pollen recognition and rejection by UI are complex (13), several SI factors, including S-RNase, CUL1, and HT, also function in UI (8, 14, 15).

Cultivated and wild tomatoes provide a powerful model system to study the mechanisms of reproductive barriers in the Solanaceae (16). They display wide variation in mating systems, both between and within species (17). Cultivated tomato, *S. lycopersicum,* is SC and accepts pollen of its SI or SC wild relatives; conversely, pollen of *S. lycopersicum* is rejected by pistils of the SI species. Three other red- or orange-fruited species, *S. cheesmaniae, S. galapagense*, and *S. pimpinellifolium*, are bilaterally cross-compatible with each other and with *S. lycopersicum*. There are notable exceptions to SI × SC rule in the tomato clade (18). One is that pollen of all of the red/orange-fruited species (SC) are rejected on pistils of the SC green-fruited species. Another exception is that pollen of some SC biotypes of SI species are compatible on pistils of conspecific SI populations. Therefore, pollen rejection is complex and likely involves more than one mechanism (13). The ability to reject tomato pollen is dominant in interspecific F_1 hybrids between cultivated tomato and related wild SI species (i.e., SC × SI hybrids), although pollen tube arrest occurs lower in the style of hybrids, suggesting that expression of the pistil side barrier is weakened (19). Allotriploid hybrids comprised of two genomes from *S. lycopersicum* (SC) and one genome from *S. lycopersicoides* (SI) reject tomato pollen tubes lower in the style than corresponding diploid hybrids (19).

We previously reported that two pollen factors from *S. pennellii*, *ui1.1* and *ui6.1*, are required and sufficient to overcome the UI barrier on allotriploid *S. lycopersicum* × *S. lycopersicoides* hybrids (19, 20). These two factors are not sufficient for compatibility on

Significance

Self-incompatibility (SI) in plants prevents inbreeding by rejection of pollen from closely related individuals of the same species. Unilateral interspecific incompatibility (UI) blocks cross-hybridization between related species, typically when the pollen donor is self-compatible and the pistil parent is self-incompatible. In this study, we show that *ui1.1*, a pollen UI factor in tomato, encodes an S-locus F-box protein that is homologous to an SI gene that in *Petunia* determines pollen specificity. We previously showed that another pollen factor, *ui6.1*, encodes a Cullin1 protein that functions in both UI and SI. Cullin1 and F-box proteins are components of SCF-type (Skp1, Cullin1, F-box) ubiquitin ligase complexes. The results provide further evidence that pollen rejection in UI involves biochemical mechanisms related to SI.

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diploid *S. lycopersicum* × *S. lycopersicoides* hybrids (19). The *ui6.1* locus encodes a pollen specific Cullin1 (CUL1) protein (21) that functions in pollen recognition by UI and SI (8). Pollen lacking *ui6.1* are incompatible on pistils expressing active S-RNases, but compatible on pistils expressing a mutant S-RNase lacking RNase activity (8). This observation suggested that *ui6.1*—and by extension, *ui1.1*—is required for pollen resistance to S-RNase–based rejection in the pistil. Interestingly, neither *ui6.1* nor *ui1.1* is required for resistance to S-RNase itself, because tomato pollen is fully compatible on pistils expressing active S-RNase in the absence of a functional HT protein (15, 22). Thus, both SI and UI require multiple pollen and pistil factors. The *ui1.1* locus is located at or near the *S* locus region on the short arm of chromosome 1, suggesting it might encode one or more SLF proteins. The goal of the present research was to isolate *ui1.1* from *S. pennellii* to elucidate the nature of pollen rejection by UI and its relationship to SI.

Results

***ui1.1* Maps to the S-Locus.** We previously mapped *ui1.1* to a relatively broad (16 cM) genetic interval that spans the S-locus region on chromosome 1 of tomato (20). In the present study, we used recombination data to refine the genetic position of *ui1.1*, assess the intensity of linkage to the S-locus, and evaluate the prospects for map-based cloning. A backcross-type mapping population was developed from a cross between two plants that were homozygous for the *S. pennellii* allele at *ui6.1*, one of which was heterozygous at *ui1.1* (i.e., $ui1.1^{+/p}$-$ui6.1^{p/p}$ × $ui1.1^{+/+}$-$ui6.1^{p/p}$, where + represents the *S. lycopersicum* allele and *p* the *S. pennellii* allele). This cross ensured that all pollen express functional *ui6.1*; because both pollen factors are required for a compatible reaction on the allotriploid tester stock, inferring the genotype of *ui1.1* is only possible if pollen express *ui6.1*. The heterozygous *ui1.1*/+ plant was used as female parent in the cross because recombination frequency is normally higher in female than in male gametes of tomato (19, 23, 24).

A total of 1,632 individuals were genotyped with two CAPS (cleaved amplified polymorphic sequence) markers, TG184 and C2_At5g18590, spanning the S-locus region. A total of 176 recombinants were recovered, representing a recombination frequency of 10.8%. These flanking markers are separated by ~17 cM on the reference map of tomato, EXPEN-2000 (solgenomics.net), indicating that recombination was suppressed in our mapping population. Eight additional markers [seven CAPS and one SSR (simple sequence repeat)] situated between TG184 and C2_At5g18590 were

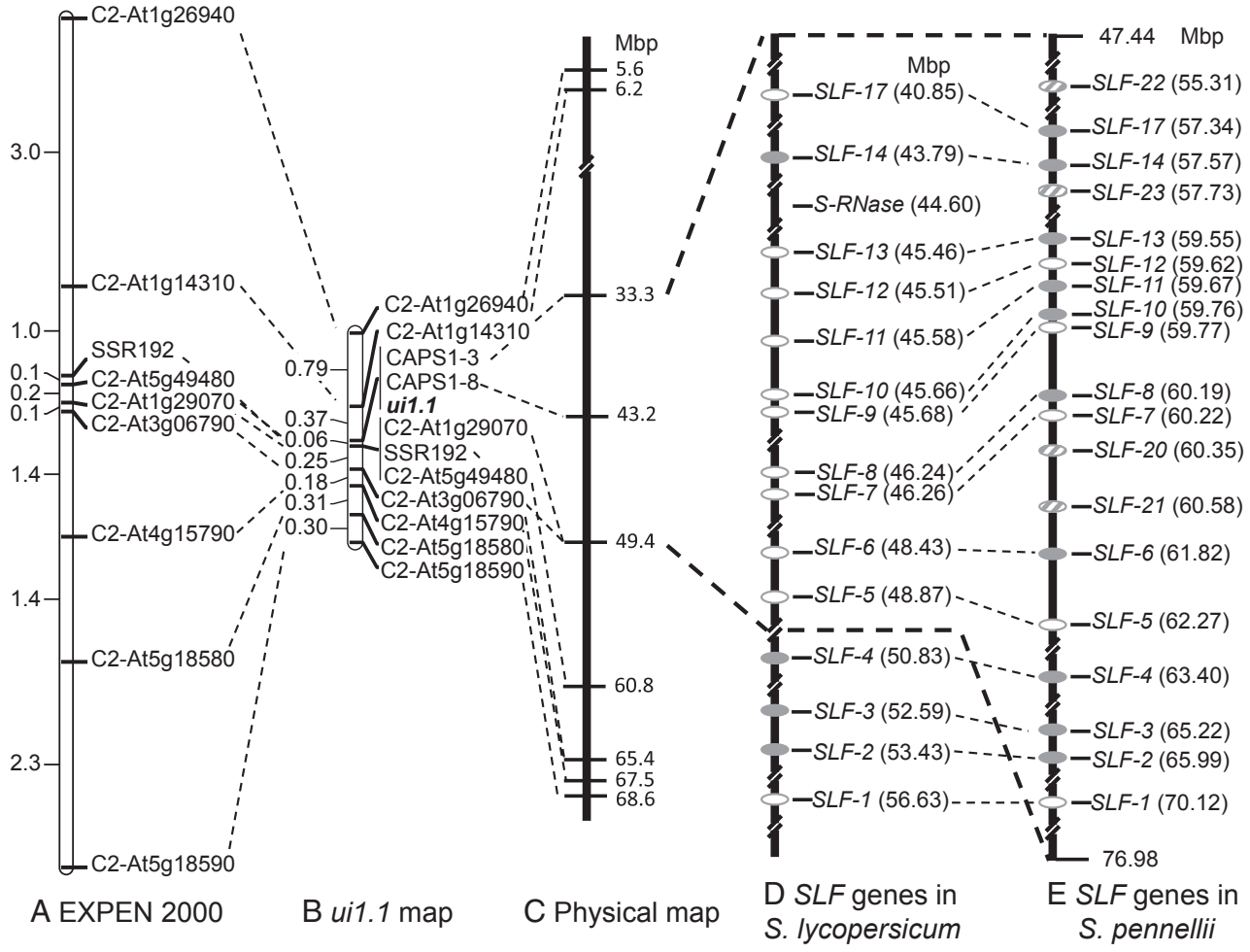

Fig. 1. Genetic and physical maps of the *ui1.1* region, showing positions of *SLF* genes in *S. lycopersicum* and *S. pennellii*. (*A*) Genetic map of the S-locus region on chromosome 1, from the EXPEN 2000 interspecific mapping population. (*B*) Genetic map of the *ui1.1* region from a BC mapping population of 1,632 individuals. Recombination suppression reduced the genetic distance of this region to less than 25% of the reference map. (*C*) Physical map of markers based on the Tomato WGS Chromosomes (ver. SL2.31). (*D*) Physical map of *SLF* genes identified in the Tomato WGS Chromosomes. Note: 19 *SLF* genes were found this region, of which 4 were ruled out as *ui1.1* candidates based on the positions of flanking markers. Open ovals, genes with likely loss-of-function mutations; shaded ovals, putative functional genes. (*E*) *SLF* genes identified in the *S. pennellii* genome. Hatched ovals, four *S. pennellii SLF* genes not present in *S. lycopersicum*.

used to further genotype the recombinants (Fig. 1*B* and *SI Appendix*, Table S1). The markers were also anchored to the tomato reference genome sequence: Tomato WGS Chromosomes version SL2.31 (Fig. 1*C*). The genetic distance between C2_At1g26940 and C2_At5g18590 was 2.26 cM, which is less than one quarter of the distance on the tomato reference map (Fig. 1*A*).

Thirty-two recombinants with cross-overs between markers C2_At1g26940 and C2_At5g18580 were tested for pollen compatibility on pistils of the allotriploid tester stock, GH266 (*SI Appendix*, Table S4). The results indicated that *ui1.1* is located between markers C2_At1g14310 and C2_At1g29070, a genetic distance of 0.43 cM on the genetic map and 43.2 Mbp on the physical map (Fig. 1 *B* and *C*). The ratio of physical to genetic distance in the *ui1.1* region was thus ~100 Mb/cM, which is >100× higher than the genome-wide average of 750 kb/cM in tomato (25).

In an attempt to refine the map position of *ui1.1,* we developed additional CAPS markers using publically available genome sequences from the region. Two CAPS markers, CAPS1-3 and CAPS1-8, separated by 10 Mb, cosegregated perfectly with *ui1.1* (Fig. 1 *B* and *C*). An *S-RNase* pseudogene was found at position 44.6 Mb in the *S. lycopersicum* genome, confirming that *ui1.1* cosegregates with the S-locus. Because the prospects for map-based cloning of *ui1.1* were poor, given the low recombination frequency in this region, we instead pursued a candidate gene approach.

The *ui1.1* Region in *S. pennellii* Includes 23 *SLF* Genes. The release of the tomato reference genome sequence (26) provided the opportunity to identify candidate genes underlying *ui1.1*. We hypothesized that one or more pollen-expressed *SLF* genes are responsible for *ui1.1* function, for the following reasons. First, we showed that *ui6.1* encodes CUL1, and both CUL1 and SLF proteins are components of SCF-type ubiquitin E3 ligase complexes. Second, our genetic data established that both *ui1.1* and *ui6.1* are required to overcome the pistil UI barrier in SC × SI hybrids, which is consistent with the biochemical model in which each gene encodes components of the SCF complex. Third, we showed that CUL1 is required for pollen-side SI function only if pistils express active S-RNase (8). Finally, the pollen determinants of SI specificity in the Solanaceae and related families with the S-RNase–based gametophytic self-incompatibility system are SLF proteins (2, 6).

To test this prediction, we chose a *Petunia* F-box protein associated with the S_1-haplotype (GenBank no. AAS79484) as a query to search the partially released tomato scaffold sequence database and then the fully released Tomato WGS Chromosomes (version SL2.31) (solgenomics.net) using the sequence alignment program tBLASTn. We identified 19 *SLF* genes (*SlSLF-1* to *-19*) in the *S. lycopersicum* genome that mapped to the S-locus region on chromosome 1 (*SI Appendix*). We also searched the *S. pennellii* accession LA0716 genome sequence (27) using the same *Petunia* SLF protein to find related proteins. Four additional SLF paralogs (*SpSLF-20* to *-23*) were identified in the *S. pennellii* genome that were absent from tomato (*SI Appendix*). All of the genes found in *S. lycopersicum* were represented by orthologs in *S. pennellii*. The *SLF* genes were anchored to the corresponding positions on the Tomato WGS Chromosomes (ver. SL2.31) and the *S. pennellii* genome sequence (Fig. 1 *C* and *D* and *SI Appendix*, Table S5). Eight loci (*SLF-1, -2, -3, -4, -15, 16, -18,* and *-19*) were ruled out for further analysis based on their physical positions on the tomato WGS of chromosome 1 relative to markers C2_At1g14310 and C2_At1g29070 flanking *ui1.1*. We later discovered a difference in the position of marker C2_At1g29070 in the *S. lycopersicum* (49.4 Mb) vs. the *S. pennellii* (76.98 Mb) genome sequences (Fig. 1 *D* and *E*); if the *S. pennellii* sequence is correct, then *SpSLF-1, -2, -3,* and *-4* are located within the marker-delimited *ui1.1* region and should not have been ruled out. We identified an *S-RNase* pseudogene in the *S. lycopersicum* genome (Fig. 1*D* and *SI Appendix*) at position 44.6 Mb. No sequences showing homology to S-RNases were found in the *S. pennellii* genome, suggesting the SI to SC mutation in accession LA0716 was caused by a deletion of the *S-RNase* gene.

The Expression of Candidate *SLF* Genes Is Pollen Specific. The expression patterns of the 23 *ui1.1*-linked *SLF* genes were evaluated in leaves and pollen of tomato cultivar VF36 and *S. pennellii* LA0716 by RT-PCR (Fig. 2). With the exception of *SLF-15, -16, -18,* and *-19*, which are located outside the flanking markers, all genes were abundantly expressed in pollen but not in leaves. Nine *SLF* paralogs (*SLF-1, -2, -3, -4, -5, -8, -10, -13,* and *-14*) were expressed at roughly equal levels in pollen of *S. lycopersicum* and *S. pennellii*. Eight genes (*SpSLF-6, -7, -11, -17, -20, -21, -22,* and *-23*) were expressed only in pollen of *S. pennellii*. Four of these, *SpSLF-20* to *-23*, are not represented by orthologs in the *S. lycopersicum* genome sequence, so no expression in this species was expected. Two paralogs, *SLF-9* and *-12*, were more highly expressed in pollen of *S. lycopersicum* than in pollen of *S. pennellii*.

***F-Box* Gene Sequences Are Highly Conserved Across Species.** We annotated the pollen-expressed *SLF* genes (15 from the *S. lycopersicum* genome and 19 from *S. pennellii*) using Genescan gene structure prediction software or manually by querying the NCBI (National Center for Biotechnology Information) protein database. The sequences of 11 *SlSLF* genes showed evidence of loss of function mutations, whereas the remaining four appeared functional (*SI Appendix*, Table S5). Five *SpSLF* genes contained mutations, whereas the other 14 appeared functional. The five *SLF* loci with mutations in *S. pennellii* also exhibited mutations in the corresponding *S. lycopersicum* orthologs.

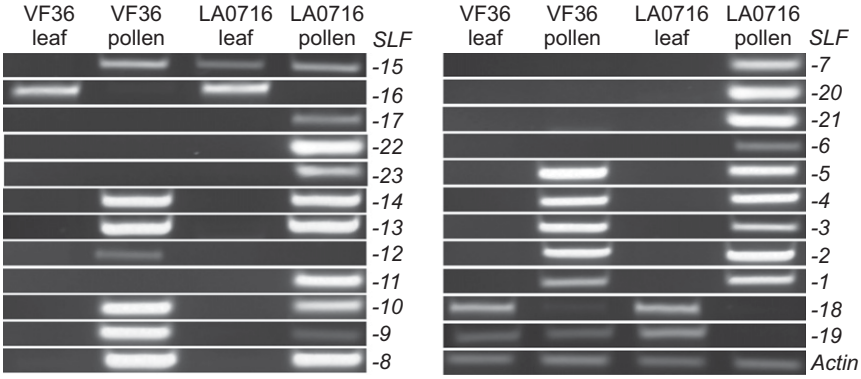

Fig. 2. Analysis of *SLF* mRNA levels by RT-PCR. Leaves and pollen of *S. lycopersicum* cv. VF36 and *S. pennellii* LA0716 were compared for expression levels of *SLF* genes. Pollen-specific expression was observed for all genes except four located outside the *ui1.1* region. The constitutively expressed *Actin* gene is included as a control.

We studied the sequence divergence between each *SLF* ortholog in *S. pennellii* and *S. lycopersicum* and among the different *SLF* paralogs within each species. We performed allele sequence alignment between the 10 putative *SpSLF* genes and the corresponding *SlSLF* genes, regardless of whether they were functional or mutated in the tomato genome. The four *SpSLF* genes absent from *S. lycopersicum* were excluded from this analysis. The results showed that each *SLF* ortholog is highly conserved between *S. lycopersicum* and *S. pennellii* (*SI Appendix*). Sequence identity ranged from 95% to 99%, despite mutations in six of the *SlSLF* genes. We also conducted multiple sequence alignment among the 14 putative *SpSLF* paralogs using Clustalw2. The results indicated that the similarity between paralogs is low, with the percentage of sequence identity ranging from 50.1% to 71.1% at the nucleotide level and from 25.5% to 51.4% at the amino acid level (*SI Appendix*, Tables S6 and S7). Except for a relatively conserved F-box domain, no other conserved region was found among the SLF proteins (*SI Appendix*).

SpSLF-23 Is Sufficient for *ui1.1* Function. Of the 23 *SpSLF* genes identified in *S. pennellii*, 8 of them were ruled out based on their map location (see above), and 4 (*SpSLF-5, -7, -9,* and *-12*) were excluded based on the sequence annotation that showed mutations. The remaining 11 *SpSLF* genes were used for genetic transformations to test for *ui1.1* function. Each gene was introduced by Agrobacterium-mediated transformation into *S. lycopersicum* IL 6–1, a line homozygous for an *S. pennellii* introgression which provides functional *ui6.1*. We obtained two to eight independent transformants for each *SpSLF* paralog (*SI Appendix*, Table S8).

Expression of the transgenes was evaluated by RT-PCR, either in the T_0 or T_1 generation. Two T_0 plants or two T_1 progeny arrays representing each *SpSLF* paralog (one for *SpSLF-6*) were tested. All but one of the *SpSLF* transgenes were expressed in anthers of T_0 and/or T_1 plants; one plant transformed with *SpSLF-10* did not show detectable expression (*SI Appendix*, Fig. S1). The pollen compatibility phenotypes of T_0 plants were evaluated on pistils of two independent allotriploid tester lines, GH266 and 10L2411, derived from different accessions of *S. lycopersicoides* (LA1964 and LA2951, respectively) and containing different *S-RNase* genes (*SI Appendix*). Of 11 transformations tested (52 plants total), only transgenic plants expressing *SpSLF-23* showed a compatible pollen phenotype (Fig. 3 and *SI Appendix*, Table S8). All four tested *SpSLF*-23 transformants were compatible on both allotriploid testers. Transformants for all of the other *SpSLF* genes elicited only incompatible pollen reactions on the allotriploid tester lines. We further tested for cosegregation between the *SpSLF-23* transgene and pollen compatibility phenotypes in the T_1 generation. A T_1 family derived by self-pollination of a randomly chosen T_0 plant segregated 18 transgenic:5 nontransgenic individuals, consistent with the 3:1 ratio expected for a single transgene insertion in the T_0 parent [χ^2 (3:1) = 0.13^{ns}]. We then tested phenotypes of these T_1 plants on pistils of allotriploid tester GH266: all of the transgenic progeny were compatible, and all of the nontransgenic plants were incompatible (Fig. 3). We did not distinguish T_1 plants hemizygous or homozygous for the T-DNA; however, both were expected to give an overall compatible reaction on the tester lines because at least 50% of the pollen would be transgenic in either case. These results show that expression of *SpSLF-23* in pollen is sufficient to confer *ui1.1* function.

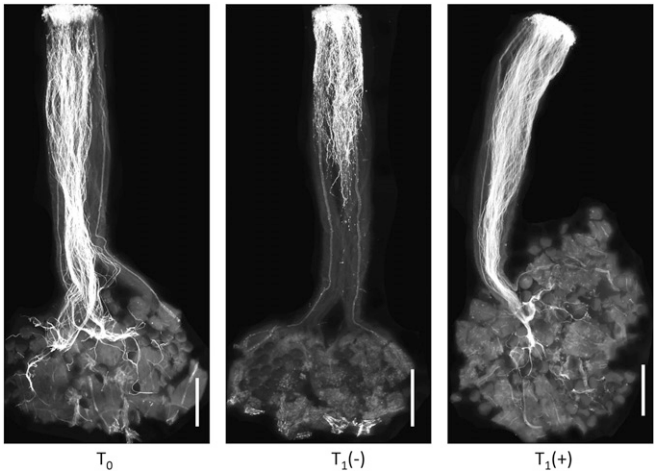

Fig. 3. Compatibility phenotypes of *SpSLF-23* transgenic plants on pistils of an allotriploid tester line. T_0 transgenic plants showed a compatible phenotype on pistils of allotriploid tester lines, demonstrating that *SpSLF-23* is sufficient to confer *ui1.1* function in pollen. The compatible phenotype cosegregated with the transgene in the T_1 generation. T_0, a representative T_0 transgenic plant; T_1(–), a T_1 plant lacking the transgene; T_1(+), a T_1 plant with the transgene. (Scale bar: 1 mm.) (Full data are in *SI Appendix*, Table S8.)

SLF-23 Orthologs in *Petunia* and Other Tomato Species. We searched the NCBI database using the DNA sequence of *SpSLF-23* to find the most closely related *SLF* genes from petunia. The type-2 *SLF* genes of petunia showed the highest sequence similarity with the *SpSLF-23* gene. We downloaded the DNA and protein sequences of six type-2 *SLF* genes of petunia and conducted sequence alignment among them. Nucleotide sequence identity between *SpSLF-23* and petunia type-2 *SLF* genes ranged from 77.28% to 89.92%, and the amino acid sequence identity was 64.4–87.5%, suggesting that *SpSLF-23* is a type-2 *SLF* gene (*SI Appendix*, Tables S9 and S10).

No ortholog of *SpSLF-23* was found in the Tomato WGS Chromosomes (ver. SL2.50). PCR tests with other *S. lycopersicum* genotypes, including IL 6–1 and cv. VF36, also failed to detect this gene. We examined whether *SLF-23* orthologs are represented in the genomes of other wild tomato relatives. Five randomly chosen accessions from 15 related *Solanum* species were genotyped for the *SLF-23* gene by genomic PCR with ortholog-specific primers (Fig. 4). Our results show that in addition to *S. lycopersicum,* this gene is also absent from the genomes of the two yellow- or orange-fruited SC species, *S. galapagense* and *S. cheesmaniae*. Several primer pairs were tested, and no amplification was ever observed in these three species. Conversely, an *SLF-23* ortholog could be amplified from the closely related red-fruited SC species *S. pimpinellifolium,* as well as from each of the green-fruited species, which include SC and SI taxa (*SI Appendix*, Table S11).

Discussion

An S-Locus F-Box Protein Is Sufficient for *ui1.1* Function in Pollen. The major finding of this research is that a pollen-expressed SLF protein underlies *ui1.1* function in *Solanum*. Our results demonstrate that a single SLF transgene, *SpSLF-23*, is sufficient, in the presence of functional *CUL1,* to convert the pollen phenotype on allotriploid tester lines from incompatible to compatible. The absence of *SLF-23* from the genome of *S. lycopersicum* explains why pollen of this species are incompatible in assays for *ui1.1* function. SLF proteins interact with Skp1 and CUL1 proteins to form SCF ubiquitin E3 ligase complexes and regulate SI by recognition of specific S-RNase forms (5, 10). Thus, the biochemical and genetic data support a model in which pollen rejection by UI, like SI, is controlled by the ubiquitin/proteasome pathway.

Two wild relatives of cultivated tomato, *S. cheesmaniae* and *S. galapagense*, both SC, also lack an *SLF-23* ortholog, and their pollen is also rejected on pistils of SI species (16, 18). These taxa also lack functional *ui6.1* (21). The genome of *S. pimpinellifolium* does contain an *SLF-23* ortholog, yet pollen of this species are

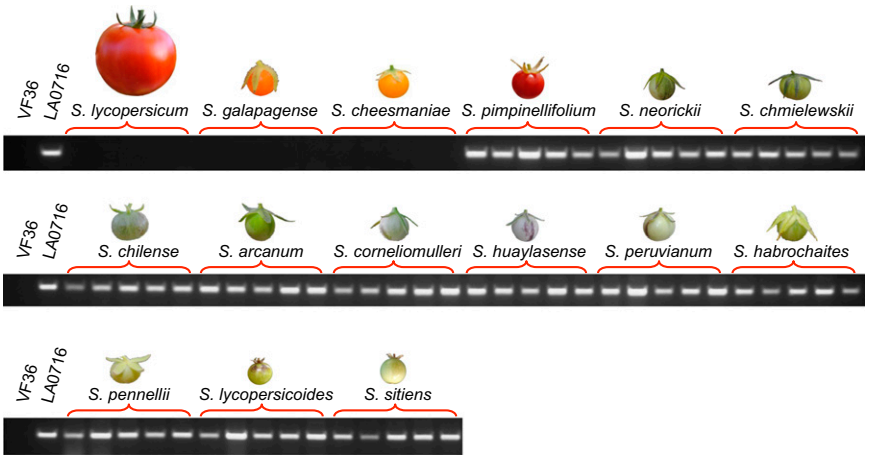

Fig. 4. Survey of cultivated and wild tomato species for the presence or absence of *SLF-23* orthologs. Five accessions from each species were tested for the presence of *SLF-23* using gene-specific primers (*SI Appendix*, Table S2). The SC red- or orange-fruited species *S. lycopersicum, S. galapagense,* and *S. cheesmaniae* lack *SLF-23* orthologs.

nonetheless rejected on pistils of the SI species and the allotriploid tester line. However, because *S. pimpinellifolium* also lacks functional *ui6.1* (21), we cannot draw conclusions about the functionality of *ui1.1* or SLF-23 in this species. All of the green-fruited species, which are mostly SI, contain an ortholog of *SLF-23*. Thus, the presence/absence of this gene correlates relatively well with the SI × SC rule in tomato.

Role of SLF-23 in UI and SI. Our results show that SLF-23 functions in pollen recognition and rejection during UI in *Solanum*. SpSLF-23 shows a high degree of amino acid sequence similarity to the *Petunia* type-2 SLF protein, which has been shown to function in SI by recognizing S_9-, S_{11}-, and S_{19}-RNases (6). We have no evidence that SLF-23 is specialized for interspecific pollen rejection: its sequence similarity to type-2 SLF in *Petunia* suggests it could also function in SI (i.e., in an SI ancestor of SC *S. pennellii* LA0716), as we previously demonstrated for UI factor CUL1. We show that SpSLF-23 confers pollen resistance to S-RNases in two independent allotriploid tester lines containing different *S-RNase* genes. The role of SLF-23 in SI is being tested by silencing this gene in *S. pennellii* to determine whether this affects pollen transmission on SI accessions of this species, as was the case for *CUL1* (8).

Role of Other SLF Proteins in *S. lycopersicum*. We found at least 23 *SLF* genes in the genome of *S. pennellii* and 19 in *S. lycopersicum,* most of which are pollen specific in their expression. In *Petunia inflata*, the same set of 17 SLF genes is expressed in pollen of two different S-haplotypes, suggesting that pollen specificity is determined by this combination of genes in each haplotype (28). In the collaborative non–self-recognition model (6), each *SLF* gene encodes a unique protein capable of recognizing different S-RNase forms. Of the 23 genes identified in *S. pennellii* by sequence homology, all but 4 are expressed in pollen (the others are located outside the S-locus region). Thus, the number of expressed genes in *P. inflata* and *S. pennellii* is similar, consistent with the conservation of S-RNase alleles (shared ancestral polymorhpisms) in divergent Solanaceae species (29).

Another question raised by this study is whether proteins encoded by one of the four nonmutated pollen-expressed *SLF* genes in *S. lycopersicum* (*SlSLF-2,- 3, -4,* or *-14*) are capable of protecting pollen from specific S-RNases. Diploid F_1 hybrids of *S. lycopersicum* with SI species such as *S. peruvianum, S. chilense, S. pennellii*, and *S. lycopersicoides* are self-sterile (30–33). In each of these studies, two to several independent F_1 hybrids, presumably with different S-haplotypes, were examined, and none were SC. This trend suggests that the S-haplotype of *S. lycopersicum* (herein S_c) does not encode active SLF proteins that interact with functional S-RNases from the SI parents. On the pistil side, *S. lycopersicum* is deficient in S-RNase expression (13) and thus cannot reject pollen with the S_c haplotype. Therefore, if any of the *SLF* genes expressed in *S. lycopersicum* pollen (*SlSLF-2, -3, -4,* and *-14*) were functional, then at least some F_1 interspecific hybrids with SI species are predicted to be partially SC, but this has not been reported. The collaborative non–self-recognition model (6, 34) predicts that each functional SLF protein recognizes one to two different S-RNase alleles; therefore, if the four genes in *S. lycopersicum* are functional, the expectation is that they should be compatible on four to eight S-haplotypes. However, only 25% of the pollen from the interspecific F_1 hybrids would contain both a functional *CUL1* gene (from the SI parent) and the S_c allele, including *SlSLF* genes, from *S. lycopersicum.* Any other essential pollen SI factors that are nonfunctional in *S. lycopersicum* would further reduce the expected proportion of self-compatible pollen from these interspecific hybrids. Segregation for multiple pollen loci can be ruled out in allotetraploid SC × SI hybrids, because 100% of diploid pollen should express all necessary pollen factors from the SI parent. Allotetraploid *S. lycopersicum* × *S. chilense* and *S. lycopersicum* × *S. lycopersicoides* hybrids are also self-sterile (35, 36). These observations imply that the S-haplotype of *S. lycopersicum* is incapable of overcoming SI through the competitive interaction mechanism in heteroallelic pollen. Thus, the apparent dominance of SI in interspecific SC × SI hybrids suggests the *SLF* genes expressed in *S. lycopersicum* pollen are not sufficient to overcome pollen rejection by SI.

SI to SC Transition in *S. pennellii* LA0716. We did not detect an *S-RNase* gene in the genome sequence of *S. pennellii* LA0716, suggesting this gene was lost, perhaps through a deletion or unequal crossing over event. Previous studies have shown that this SC accession lacks *S-RNase* mRNA, protein, and activity in the pistil (13, 37). Nonetheless, LA0716 is capable of rejecting pollen of red-fruited species such as *S. lycopersicum*. Also, a QTL contributing to the strength of this pollen rejection response maps to the same genetic region as the HT-A and HT-B genes, suggesting these genes in LA0716 are probably functional (13). Pollen of LA0716 is fully compatible on pistils of SI accessions of *S. pennellii*, indicating full pollen function has been retained. These observations suggest that the SI to SC transition in LA0716 was caused by a loss of the *S-RNase* gene. This loss is likely a recent mutational event, because no other pollen or pistil SC mutations have been detected in this accession.

GENETICS

Kondo et al. (22) failed to amplify *S-RNase* genes from any of the red-fruited species, which suggested they might be absent from these species. However, as we and others (34) report, the *S. lycopersicum* genome does contain an *S-RNase* pseudogene. This gene is predicted to be nonfunctional based on sequence analysis, consistent with the lack of S-RNase activity in styles of *S. lycopersicum* and the other red-fruited species (13, 22). Thus, SI to SC transitions in both *S. pennellii* LA0716 and in *S. lycopersicum* were accompanied or caused by loss of *S-RNase* expression, whereas loss of the pollen factors *ui1.1* and *ui6.1* occurred in *S. lycopersicum* (and other red/orange-fruited species) but not *S. pennellii*. These observations suggest that mutations in pollen SI factors are relatively late events, preceded in these cases by one or more pistil mutations, and reinforce, rather than initiate, the breakdown of SI.

Materials and Methods

Details about materials and methods are provided in *SI Appendix, SI Materials and Methods*.

Plant Materials. To map the *ui1.1* locus, we used a backcross-type population derived from *S. pennellii* LA0716 in which the heterozygote was the female parent (for higher recombination frequency) and in which all progeny expressed functional *ui6.1* (required for phenotyping *ui1.1*). Cultivar VF36 (LA0490) and *S. pennellii* LA0716 were used for *SLF* gene expression analysis. Candidate *ui1.1* genes were isolated from genomic DNA of LA0716. Tomato introgression line IL 6–1 (LA3500) was used for genetic transformations. Five accessions from cultivated tomato and from each related wild species were surveyed for the presence of *SLF-23* orthologs.

DNA Isolation and Genotyping. DNA was extracted from the mapping population in 96-well microtiter plates as previously described (19). Marker sequences and restriction enzymes used for revealing polymorphism are listed in *SI Appendix*, Table S1.

Gene Identification, Annotation, and Sequence Alignment. The tomato whole genome sequence (26) and the *S. pennellii* genome sequence (27) were searched for *SLF* coding genes. Candidate genes were annotated using GENSCAN (genes.mit.edu/GENSCAN.html) or manually annotated. Sequence alignments were performed on the Solgenomics website (solgenomics.net). Multiple sequences were aligned using ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2/).

Gene Expression Analysis. Expression of candidate *SLF* genes and transgenes was assayed by semiquantitative RT-PCR using gene-specific primers and restriction endonucleases, as needed, to distinguish endogenous genes from transgene products (*SI Appendix*, Table S2).

Gene Isolation and Plant Transformations. Candidate *SLF* genes were isolated from genomic DNA by long-distance PCR with Phusion high fidelity DNA polymerase (New England Biolabs) and cloned into a modified pCAMBIA1300 vector. Transgene constructs were inserted by Agrobacterium-mediated transformation into tomato line IL 6–1, which expresses functional *ui6.1*.

Sequencing *S-RNase* Genes. The *S-RNase* gene sequences of two allotriploid testers (GH266 and 10L2411) were obtained by 3′ rapid amplification of cDNA ends (RACE) as previously described (38).

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