Abstract Gametophytic self-incompatibility (GSI) typically “breaks down” due to polyploidy in many Solanaceaeous species, resulting in self-compatible (SC) tetraploid individuals. However, sour cherry (Prunus cerasus L.), a tetraploid species resulting from hybridization of the diploid sweet cherry (P. avium L.) and the tetraploid ground cherry (P. fruticosa Pall.), is an exception, consisting of both self-incompatible (SI) and SC individuals. Since sweet cherry exhibits GSI with 13 S-ribonucleases (S-RNases) identified as the stylar S-locus product, the objectives were to compare sweet and sour cherry S-allele function, S-RNase sequences and linkage map location as initial steps towards understanding the genetic basis of SI and SC in sour cherry.

S-RNases from two sour cherry cultivars that were the parents of a linkage mapping population were cloned and sequenced. The sequences of two S-RNases were identical to those of sweet cherry S-RNases, whereas three other S-RNases had unique sequences. One of the S-RNases mapped to the Prunus linkage group 6, similar to its location in sweet cherry and almond, whereas two other S-RNases were linked to each other but were unlinked to any other markers. Interspecific crosses between sweet and sour cherry demonstrated that GSI exists in sour cherry and that the recognition of common S-alleles has been maintained in spite of polyploidization. It is hypothesized that self-compatibility in sour cherry is caused by the existence of non-functional S-RNases and pollen S-genes that may have arisen from natural mutations.

Keywords Gametophytic self-incompatibility · S-ribonuclease · Polyploid

Introduction

Self-incompatibility (SI) is a common evolutionary strategy used by flowering plants to prevent self-fertilization and promote out-crossing (de Nettancourt 1977). In gametophytic self-incompatibility (GSI), SI is determined by a highly multi-allelic locus, called the S-locus, in which the compatibility of a cross is determined by the haploid genome of the pollen and the diploid genome of the pistil. In GSI, pollen tube growth is arrested if the pollen tube has an S-allele in common with one of the two S-alleles in the style. The S-locus has been classically described as a complex containing multi-allelic genes expressed by the pollen and style and tight linkage between these components. Because of the presence of at least two multi-allelic genes, the term “haplotype” has been used to describe variants of the S-locus and the term “allele” to describe variants of a given polymorphic gene at the S-locus (McCubbin and Kao 2000). In the Solanaceae and the Rosaceae, the gene controlling the pistil’s SI response is a ribonuclease (S-RNase) which is expressed only in the pistil (McClure et al. 1989; Sassa et al. 1992; Lee et al. 1994; Murfett et al. 1994; Broothaerts et al. 1995; Boškovic and Tobutt 1996; Ishimizu et al. 1996; Sassa et al. 1996; Tomimoto et al. 1996; Tao et al. 1997, 1999; Burgos et al. 1998; Ushijima et al. 1998; Yamane et al. 1999). A second gene that is hypothesized to be expressed specifically in the pollen has yet to be determined from any GSI species. Additional modifier genes have also been demonstrated to be required for normal SI function (McClure et al. 1999).

GSI present in diploid species has been observed to “break down” due to polyploidy, with the tetraploid relatives frequently self-compatible (SC) (Livermore and Johnstone 1940; Crane and Lewis 1942; Stout and Chandler 1942; Brewbaker 1954; Pandy 1968; de Nettancourt et al. 1974; Ueda and Akimoto 2001). To explain this phenomenon, Lewis (1947) proposed that pollen containing two different S-loci loses its SI phenotype, resulting in SC polyploid individuals. Evidence ob-
tained from recent research in Solanaceous species supports this theory (Chawla et al. 1997; Entani et al. 1999; Golz et al. 1999; Luu et al. 2001). In contrast, the GSI diploid sweet cherry (Prunus avium L., 2n=2x=16) and the tetraploid sour cherry (P. cerasus L., 2n=4x=32) represent a natural diploid-tetraploid series where the tetraploid individuals can be either SI or SC.

Sweet cherry and the tetraploid ground cherry (P. fruticosa Pall., 2n=4x=32) are believed to be the parental species that gave rise to sour cherry multiple times via unreduced gametes from sweet cherry (Olden and Nybom 1968; Iezzoni and Hancock 1984; Brettin et al. 2000). Although the vast majority of sour cherry cultivars are SC, numerous SI cultivars exist in Eastern Europe, the center of diversity (Lech and Tylus 1983; Redalen 1984a, b; Iezzoni et al. 1990; Lansari and Iezzoni 1990). However, the SI phenotype is not limited to landrace cultivars as SI sour cherry selections can result from crosses between two SC sour cherry parents (Lansari and Iezzoni 1990). For example, a sour cherry linkage mapping population generated by crossing two SC sour cherry cultivars, ‘Rheinische Schattenmorelle’ (‘RS’) × ‘Erdi Botermo’ (‘EB’), segregates for SI and SC (Wang et al. 1998). Since any successful new sour cherry cultivar would have to be SC to avoid the production problems associated with providing pollinator trees, our goal was to determine the genetic basis of SI and SC in sour cherry to increase the likelihood of obtaining SC progeny in our sour cherry breeding program.

Sweet cherry exhibits classical GSI with 13 S-RNases identified and validated in crossing experiments (Matthews and Dow 1969; Boškovic and Tobutt 1996, 2001; Choi et al. 2000; Hauck et al. 2001; Wiersma et al. 2001). In contrast, there is only one study of SI in sour cherry that takes advantage of the ability to determine putative S-RNase genotypes. Yamane et al. (2001) recently cloned two S-RNases from ‘EB’; one matched the S-P-RNase previously cloned from sweet cherry and the second was a novel S-RNase not previously identified in sweet cherry. RFLP and PCR analysis of S-RNase alleles in a set of sour cherry cultivars identified an additional four S-RNases that are presumably identical to previously identified sweet cherry S-RNases, and an additional three putative novel S-RNases. The ‘RS’ and ‘EB’ mapping parents had the putative S-RNase designations, $S_p$ and $S_n$, respectively. Yamane et al. (2001) further compared the S-RNase allele composition of six SI with seven SC selections and found that all SI selections, similar to the SC selections, contained three or four different putative S-RNase alleles. This suggests that heteroallelic pollen alone may be insufficient to cause SC in tetraploid sour cherry.

Due to the evolutionary relatedness of sweet and sour cherry, and the potentially on-going gene flow between the two species, it is not surprising that S-RNases presumably identical to those found in sweet cherry were identified in sour cherry (Yamane et al. 2001). We further investigated SI and SC in sour cherry by taking advantage of the potential commonalities between these two species. The inheritance and linkage map locations of the putative S-RNases from ‘RS’ and ‘EB’ could also be determined and compared with information from other Prunus species. In sweet cherry and almond (Prunus dulcis), the S-locus has been mapped to the end of the Prunus linkage group 6 (Ballester et al. 1998; K. Tobutt, personal communication).

Our objectives were: (1) to determine if the S-alleles that appeared to be common between sweet and sour cherry exhibited the expected recognition reactions in the styles by making inter-specific crosses, (2) to determine the sequences of the S-RNases in ‘EB’ and ‘RS’ and compare the amino acid alignment with previously sequenced sweet cherry S-RNases, and (3) to determine the sour cherry linkage map locations for the S-RNase loci.

### Materials and methods

#### Plant material

The two SI sour cherry cultivars, ‘Crisana’ and ‘Tschernokorka’, were chosen for pollination with sweet cherry cultivars based on previous examination of their S-RNase profiles using PCR and RFLP analyses (Yamane et al. 2001). ‘Crisana’ contains three different S-RNases, one of which is presumably present in double dose. Two of the S-RNases produce RFLP and PCR fragment profiles identical to the $S_1$ and $S_4$ alleles in sweet cherry. The third S-RNase is not similar to any sweet cherry S-RNase, and is called $S_9$. ‘Tschernokorka’ also contains three different S-RNases, only one of which is identical to a sweet cherry S-RNase ($S_2$). The other two S-RNases are named $S_3$ and $S_5$. The three sweet cherry cultivars used were ‘Schmidt’ ($S_2S_4$), ‘Ranier’ ($S_3S_5$) and ‘Sato Nishiki’ ($S_1S_4$). ‘Crisana’, ‘Tschernokorka’, and ‘Schmidt’ are maintained at MSU Clarksdale Horticultural Experimental Station (CHES), Clarksdale, Mich. ‘Ranier’ is maintained at the North West Horticultural Research Station. Traverse City, Mich, while ‘Sato Nishiki’ pollen was collected from trees growing at the Experimental Farm of Kyoto University, Kyoto, Japan, dried and frozen at −20°C. All pollen samples were tested to verify pollen viability prior to the crossing experiments as described by Brewbaker and Kwack (1963).

A pseudo-testcross mapping population consisting of 85 progeny from the cross between two sour cherry cultivars, ‘RS’ × ‘EB’ (Wang et al. 1998) was maintained at CHES.

#### Analysis of pollen tube growth

Eight interspecific crosses were performed and pollen tube growth was observed to determine whether the crosses were compatible or incompatible. Styles from each of the sour cherry cultivars (‘Crisana’ and ‘Tschernokorka’) were pollinated with pollen from the sweet cherry cultivars ‘Sato Nishiki’ or ‘Ranier’. Styles from the sweet cherry cultivars (‘Ranier’ and ‘Schmidt’) were pollinated with ‘Crisana’ and ‘Tschernokorka’ pollen. Pollination tests were performed as described by Lansari and Iezzoni (1990) with slight modifications. Pollen from newly opened flowers was collected from each of the pollen parents. Pollen was dried for 24 h. For each of the styril parents, a branch with flowers at the balloon stage was brought into the laboratory and 20 flowers were emasculated. All other flowers were removed. Ten emasculated flowers were hand pollinated when receptive (24 h after emasculaion) with each of the pollen sources. The pollinated pistils were collected 72 h after pollination, placed in fixion solution [chloroform:glycerol:glacial acetic acid, 1:3:1 (v/v)] for 24 h, transferred into 100% ethanol, and stored at 4°C until used for observation. The pistils