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Self-(in)compatibility in sour cherry (*Prunus cerasus* L.). A minireview

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Summary: Sour cherry (*Prunus cerasus* L.) is an allotetraploid species derived from hybridisation of the diploid sweet cherry (*P. avium* L.) and the tetraploid ground cherry (*P. fruticosa* Pall.). Although numerous self-incompatible cultivars exist, the most sour cherry cultivars are self-compatible, which might be due to their tetraploid nature. This review is dedicated to show the limited information on the genetics of self-incompatibility in sour cherry accumulated during the last five years. Two different hypotheses (genomic arrangement of the alleles or the accumulation of non-functional *S*-haplotypes) are discussed. Heteroallelic sour cherry pollen was shown to be self-incompatible, which is counter to the *Solanaceae* where heteroallelic pollen frequently self-compatible due to a kind of competitive interaction between the two different alleles. This review highlights some inconsistencies in the hope that clarification will be achieved in the near future.

Key words: genomic segregation, polyploidy, *Prunus cerasus*, self-(in)compatibility, *S*-genotype, sour cherry

World sour cherry production reached the volume of 1 million tons in 2004, 70 % of which is produced in Europe (*Nyéki* et al., 2005). Sour cherry (*Prunus cerasus* L.) is an important tetraploid fruit species (*Darlington*, 1927), apparently an allotetraploid derived from hybridisation of the diploid sweet cherry, *P. avium* L. (2n=2x=16), and the tetraploid ground cherry, *P. fruticosa* Pall. (2n=4x=32) (*Olden & Nybom*, 1968). Although the most sour cherry cultivars are self-compatible, numerous self-incompatible cultivars exist in Eastern Europe, the centre of diversity (*Lech & Tylus*, 1983; *Redalen*, 1984a,b; *Lansari & Iezzoni*, 1990) and there are also reports of cross-incompatibility, reciprocal or unilateral incompatibility (*Hruby*, 1963; *Redalen*, 1984a; *Nyéki & Szabó*, 1996; *Nyéki* et al., 2000). However, the self-incompatible (SI) phenotype is not limited to landrace cultivars as SI sour cherry selections can result from crosses between two self-compatible (SC) sour cherry parents (*Lansari & Iezzoni*, 1990). For example, a sour cherry linkage mapping population generated by crossing two SC sour cherry cultivars, 'Rheinische Schattenmorelle' ('RS') and 'Érdi Bőtermő' ('ÉB'), segregates for self-incompatibility and self-compatibility (*Wang* et al., 1998). The transition from SI to SC is regarded as one of the most prevalent transitions in Angiosperm evolution, having profound impacts on the genetic structure of populations. As most diploid cherries are self-incompatible, the relatively rare occurrence of incompatibility in sour cherry is likely due to its tetraploid (2n=4x=32) genome.

First report on the self-incompatibility of sour cherry was published by *Yamane* et al. (2001), who carried out pollen tube growth analysis and two-dimensional polyacrylamide gel electrophoresis. This demonstrated the presence of *S*-ribonucleases (*S*-RNases) in styles of SI and SC selections of tetraploid sour cherry (*Prunus cerasus* L.). Based on self-pollen tube growth in the styles of 13 sour cherry selections, seven selections were SC, while six selections were SI. In the SI selections, the swelling of pollen tube tips, which is typical of SI pollen tube growth in gametophytic SI, was observed. Glycoproteins which had molecular weights and isoelectric points similar to those of *S*-RNases in other *Prunus* species were detected in all selections tested. These proteins had immunological characteristics and N-terminal amino acid sequences consistent with the *S*-RNases in other *Prunus*. Two cDNAs encoding glycoproteins from 'Érdi Bőtermő' were cloned. One of them had the same nucleotide sequence as that of S_4-RNase of sweet cherry (*Prunus avium* L.), while the amino acid sequence from the other cDNA encoded a novel *S*-RNase (named S_a-RNase in this study). This novel RNase contained two active sites of T2/*S* type RNases and five regions conserved among other *Prunus* *S*-RNases. Genomic DNA blot analysis using cDNAs encoding *S*-RNases of sweet cherry as probes indicated that three or four *S*-RNase alleles were present in the genome of each selection regardless of the incompatibility status. All of the selections tested seemed to have at least one *S*-allele that was also found in sweet cherry.

Hauck et al. (2002) compared 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 (*Ballester* et al., 1998), 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.

Later, S_{6m}-haplotype, a mutated S_6-haplotype with an altered HindIII cut site, of sour cherry was characterized (*Yamane* et al., 2003a). Inheritance and pollination studies of *S*-haplotypes from reciprocal crosses between 'Érdi Bőtermő' ($S_4S_{6m}S_a$) and 'Rheinische Schattenmorelle' (RS; $S_6S_aS_bS_c$) revealed that the S_{6m}-haplotype conferred unilateral incompatibility with a non-functional pistil component and a functional pollen component. Expression analyses of S_6-RNase and SFB_6, a candidate gene for pollen-*S*, in the S_{6m}-haplotype showed that SFB_6 was transcribed in ÉB pollen, but S_6-RNase was not transcribed in ÉB styles. These results were consistent with data from the inheritance and pollination studies. Inverse PCR for the flanking regions of S_6-RNase in the S_6- and S_{6m}-haplotypes revealed an approximately 2600 bp insertion present at approximately 800 bp upstream of the S_6-RNase in the S_{6m}-haplotype, which is responsible for the alternation of the HindIII cut site and a possible cause of inhibition of the transcription of S_6-RNase. SFB_6 was present downstream of S_6-RNase in both the S_6- and S_{6m}-haplotypes and expressed in the same way, supporting the idea that SFB is a good candidate for pollen-*S* in sour, which was previously in several *Prunus* species (*Entani* et al., 2003; *Ushijima* et al., 2003) and even in sweet cherry (*Yamane* et al., 2003b; *Ikeda* et al., 2004).

Another research group having profound experiences in the self-incompatibility research used test crossing, pollen tube growth and stylar ribonuclease analyses in order to understand the background of the SI/SC phenotype in sour cherry cultivars (*Tobutt* et al., 2004). Stylar extracts of 36 accessions of sour cherry were separated electrophoretically and stained for ribonuclease activity. The zymograms of most accessions showed three bands, some two or four. Of the ten bands seen, six co-migrated with bands that in sweet cherry are attributed to the incompatibility alleles S_1, S_3, S_4, S_6, S_9 and S_{13}. 'Èaèanski Rubin', 'Érdi Bőtermő B', 'Kőrös' and 'Újfehértói Fürtös', which showed bands apparently corresponding to S_1 and S_4, were test pollinated with the sweet cherry 'Merton Late' (S_1S_4). Monitoring pollen tube growth, and, in one case, fruit set, showed that these crosses were incompatible and that the four sour cherries indeed have the alleles S_1 and S_4. Likewise, test pollination of 'Marasca Piemonte', 'Marasca Savena' and 'Morello, Dutch' with 'Noble' (S_6S_{13}) showed that these three sour cherries have the alleles S_6 and S_{13}. S_{13} was very frequent in sour cherry cultivars, but is rare in sweet cherry cultivars, whereas with S_3 the situation was reversed. It was suggested that the other four bands are derived from ground cherry and one of these, provisionally attributed to S_B, occurred frequently in a small set of ground cherry accessions surveyed. Analysing some progenies from sour by sweet crosses by *S*-allele-specific PCR and monitoring the success of some sweet by sour crosses indicated mostly disomic inheritance, with sweet cherry *S*-alleles belonging to one locus and, presumably, the ground cherry alleles to the other, and helped clarify the genomic arrangement of the alleles and the interactions in heteroallelic pollen.

If inheritance is disomic in sour cherry, as indicated by the isoenzyme studies of *Beaver* & *Iezzoni* (1993), the diploid pollen grains of 'regular' sour cherries will each contain one *S*-allele from sweet cherry and one from ground cherry, if the latter indeed has *S*-alleles. Of course, this may not be the case in the pollen grains of 'sour cherries' that are the result of backcrossing to either parental species. Some of the complications that might be expected in the genetic control of incompatibility in tetraploids were pointed out by *Crane Lawrence* (1929) and *Lawrence* (1930) amplified these observations to offer an explanation for unilateral incompatibility between certain tetraploids. A significant advance in the understanding of the subject was the proposal by *Lewis & Modlibowska* (1942) that the self-compatibility of a tetraploid sport of pear could be attributed to the compatibility of the heteroallelic diploid pollen in the selfed style. Lewis discussed evidence from further species, especially *Oenothera organensis*, that in heteroallelic diploid pollen in some species, the two *S*-alleles may interact competitively, so that such pollen is not rejected in styles having one or both of the same *S*-alleles (*Lewis*, 1947; 1954), or else one allele can dominate the other, so that the pollen is rejected in styles having the dominant allele (*Lewis*, 1947; 1954). In sour cherry, no detailed explanations of the genetics of (in)compatibility have been offered.

The genotypes of the pollen grains in sour cherries depend not only on the genotype of the cultivar, but also on the genomic arrangement of the alleles. *Tobutt* et al. (2004) have deduced the genotype and arrangement of 'Érdi Bőtermő B' as $S_1S_4.S_BS_D$. Its self-compatibility indicates competitive interaction in at least one of the four types of pollen expected, S_1S_B, S_1S_D, S_4S_B and S_4S_D. 'Amarena di Verona P.C.', self-incompatible, and 'Montmorency', self-compatible, shared the phenotype $S_6S_BS_D$, and test crosses were carried out following the approach of *Lewis* (1943) in *O. organensis*. That pollen of 'Amarena di Verona P.C.' failed on sweet cherry 'Sasha' (S_3S_6) but succeeded on 'Merton Late' (S_1S_4) indicated that all the pollen behaved as S_6. In contrast, the success of pollen of 'Montmorency' on both sweet cherry cultivars indicated that some of the pollen did not behave as S_6. This is consistent with 'Amarena di Verona P.C.' having the genotype and arrangement $S_6S_6.S_BS_D$; the pollen produced would be S_6S_B and S_6S_D and, in the absence of competitive interaction, both types would express S_6. 'Montmorency' may have one of the eight other genotypes and arrangements possible, none of which gives rise to S_6 in all pollen grains. This also explains why 'Amarena di Verona P.C.' is self-incompatible, whereas 'Montmorency' is self-compatible. This approach could contribute to an understanding of the allelic interactions in heteroallelic

pollen of other sour cherries. *Bošković* et al. (2006) indicated that tetrasomic segregation may also occur in the progeny of a cross between 'Rheinische Schattenmorelle' and 'Érdi Bőtermő'. Whether knowledge of the genomic arrangements and the interactions of the various combinations of alleles will be sufficient to explain self-compatibility versus self-incompatibility in all cases could not be assessed.

Three progenies of sour cherry (*Prunus cerasus*) were analysed to correlate self-(in)compatibility status with *S*-RNase phenotype (*Bošković* et al., 2006). Self-(in)compatibility was assessed in the field and by monitoring pollen tube growth after selfing. The *S*-RNase phenotypes were determined by isoelectric focusing of stylar proteins and staining for RNase activity and, for the parents, confirmed by PCR. Seedling phenotypes were generally consistent with disomic segregation of *S*-RNase alleles. The genetic arrangements of the parents were deduced to be 'Köröser' (self-incompatible) $S_1S_4.S_BS_D$, 'Schattenmorelle' (self-compatible) $S_6S_{13}.S_BS_B$, and clone 43.87 (self-compatible) $S_4S_{13}.S_BS_B$, where "." separates the two homoeologous genomes. The presence of S_4 and S_6 alleles at the same locus led to self-incompatibility, whereas S_{13} and *S*B at homoeologous loci led to self-compatibility. The failure of certain heteroallelic genotypes in the three crosses or in the self-incompatible seedlings indicates that S_4 and S_6 are dominant to *S*B. However, the success of $S_{13}S_B$ pollen on styles expressing corresponding *S*-RNases indicates competitive interaction or lack of pollen-*S* components. In general, the universal compatibility of $S_{13}S_B$ pollen may explain the frequent occurrence of S_{13} and S_B together in sour cherry cultivars. Alleles S_B and S_D, that are presumed to derive from ground cherry, and S_{13}, presumably from sweet cherry, were sequenced.

First, it was only hypothesized by the American research group 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 (*Hauck* et al., 2002). Yet, the identity and function of mutations that result in the breakdown of SI in nature are not well understood. *Hauck* et al. (2006) provided further data of the genetic analyses of six natural sour cherry selections and identified seven independent, non-functional *S*-haplotypes with disrupted pistil component (stylar-*S*) and/or pollen component (pollen-*S*) function. A genetic model was developed and validated demonstrating that the breakdown of SI in sour cherry is due to the accumulation of a minimum of two non-functional *S*-haplotypes within a single individual.

These data indicate that the breakdown of GSI in sour cherry is caused by the accumulation of stylar-part and pollen-part mutants affecting multiple *S*-haplotypes. In sour cherry, four functional (S_4, S_6, S_9, and S_{26}) and seven non-functional *S*-haplotypes (S_1', S_{6m}, S_{6m2}, S_{13}', S_a, S_d, and S_{null}) (*Hauck* et al., 2002; *Yamane* et al., 2003a; *Tobutt* et al., 2004; *Hauck* et al., 2006) have been identified. A comparison of the SI and SC selections revealed that the SI selections carried only one non-functional *S*-haplotype, whereas the SC selections carried two to four non-functional *S*-haplotypes. From this, *Hauck* et al. (2006) developed the "one-allele match" model, in which a match between a functional pollen-*S* gene product in the pollen and its cognate functional *S*-RNase in the style would result in an incompatible reaction. A similar reaction would occur regardless of whether the pollen contained a single functional pollen-*S* gene, or two different functional pollen-*S* genes. The absence of a functional match would result in a compatible reaction; thus, for successful self-fertilization, pollen must contain two non-functional *S*-haplotypes. To test this model, *Hauck* et al. (2006) genotyped 92 seedlings from four crosses among five sour cherry selections. All seedlings that contained only one non-functional *S*-haplotypes (n=17) were SI and all the seedlings that contained two or more non-functional and non-complementary *S*-haplotypes (n=75) were SC. Since the non-functional S_a- and S_d-haplotypes likely represent different mutations of a common *S*-haplotype, they hypothesized that S_a and S_d had complementary pistil-*S* and pollen-*S* mutations, resulting in a functional *S*-haplotype. Therefore, they concluded that these results validated the one-allele-match model for the genetic control of SC and SI in sour cherry.

Their finding that sour cherry is SI when only one non-functional *S*-haplotype is present has significant evolutionary implications since non-functional *S*-haplotypes would be maintained in the population without causing an abrupt shift to SC. Furthermore, they demonstrated that heteroallelic sour cherry pollen was self-incompatible, which is counter to the well-documented phenomena in the *Solanaceae* where SC accompanying polyploidization is frequently due to the SC of heteroallelic pollen (*Lewis*, 1947; 1954), suggesting that the pollen-*S* genes of *Prunus* and the *Solanaceae* may differ.

As two research groups are working in this field, some discrepancies in the allele labelling systems and in other issues occurred. For example S_a and S_c of Tao's laboratory corresponds to S_B and S_{13} of Tobutt's laboratory, respectively. In consequence, the *S*-genotype of 'Érdi Bőtermő' given as $S_4S_{6m}S_a$ (*Yamane* et al., 2003a) could be specified as $S_4S_{6m}S_B$ according to the English labelling system.

S_{6m} is a self-compatible mutant of S_6-allele in which the self-compatibility function is attributed to the inactivation of corresponding *S*-RNase but presence of an active pollen-*S* component (*Yamane* et al., 2003a). *Bošković* et al. (2006) detected the S_{14} and S_{13} in 'Bruine Waalse' and 'Montmorency,' respectively, by PCR but not by activity staining, which may be attributable to similar inactivation. A conflict, however, was not addressed by either of the research groups: *Tobutt* et al. (2004) described the genotype of 'Érdi Bőtermő' as $S_1S_4S_BS_D$ on the basis of stylar ribonuclease zymograms. Four distinct *S*-ribonuclease enzymes present in the styles of 'Érdi Bőtermő' is inconsistent with the results of *Yamane* et al. (2003a) detecting a mutated, non-functional S_{6m}-RNase in 'Érdi Bőtermő B' if the two accessions indeed represent the same cultivar and the results and their interpretations are not burdened with mislabelling or other

problems. Comprehensive experiments are needed to clarify all doubtful points regarding the transition of SI to SC in sour cherries.

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