The International Journal of Plant Reproductive Biology 8(1) Jan 2016, pp.1-0

DOI 10.14787/ijprb.2016 8.1.1-0

Self-incompatibility system in polyploid fruit tree species- A review

N. Makovics-Zsohár & J. Halász

Corvinus University of Budapest, Faculty of Horticultural Science, Department of Genetics and Plant Breeding, 1118 Budapest, Ménesi út 44., Hungary

***e-mail :** julia.halasz@uni-corvinus.hu

Received : 13.04.2015; Accepted and published on line: 01.07.2015

ABSTRACT

Self-incompatibility (SI) is a genetic mechanism that prevents self-fertilization (inbreeding) by enabling the pistil to reject self-pollen. Fertilization of rosaceous fruit tree species is governed by gametophytic SI, which has a major effect on commercial fruit production by regulating fruit set proportion. The GSI systems are homomorphic type, which means that there are no morphological differences in the structure of flowers; the recognition of the genetically related individual is based on the interaction between pollen- and pistil-expressed genes. It is controlled by a single multi-allelic locus, called the *S*-locus. A female determinant gene encodes glycoprotein with ribonuclease (*S*-RNase) activity in the pistils, and the male determinant gene expresses a specific F-box protein only in pollen. Cultivars sharing different *S*-alleles must be interplanted in orchards; therefore information on the *S*-genotypes is necessary for commercial fruit growing and breeding. Breakdowns in the pollen or pistil genes resulted in self-compatible genotypes. Studies of the rosaceous tree fruit *S*-locus are primarily focused on diploid species, while information is hardly available for polyploid fruit trees despite the fact that polyploidy is a prominent feature of plant genomes. Characteristics of the self-incompatibility system in three polyploid rosaceous species including sour cherry (*Prunus cerasus*, tetraploid), Chinese cherry (*P. pseudocerasus*, tetraploid) and European plum (*P. domestica*, hexaploid) are compared and discussed.

Keywords : self-incompatibility, polyploidy, *Prunus*, *S-RNase*, *F-box*

INTRODUCTION

Flowering plants (over 200,000 documented species) are among the most successful taxa on the planet (Mora *et al*. 2011). One of the major reasons for the great variability of angiosperms is supposed to be self-incompatibility. Angiosperms display diverse reproductive strategies to enhance outcrossing and produce genetic diversity (McClure *et al*. 2011). Self-incompatibility (SI) is a genetic mechanism that prevents self-fertilization (inbreeding) by enabling the pistil to reject self-pollen (Kao & McCubbin 1996). Most SI systems are of homomorphic type, which means that there are no any morphological differences in the structure of flowers, instead, the recognition of the genetically related individual is based on the interaction between pollen- and pistil-expressed genes (Busch *et al.* 2014). It is controlled by a single multi-allelic locus, called the *S*-locus (named after the term, "sterility") (Lawrence *et al*. 1985, Zavada & Tayler 1986). Gametophytic self-incompatibility represents the most prevalent form of self-incompatibility existing in many flowering plant families (Ebert *et al*. 1989). Two main genes in the *S*-locus were clarified to be responsible for male and female specificities. The female determinant gene encodes a glycoprotein with ribonuclease (*S*-RNase) activity in the pistils (McClure *et al*. 1989), and the male determinant gene expresses a specific F-box

protein (*SFB*) only in pollen (Lai *et al*. 2002, Ushijima *et al*. 2003). The term *S*-haplotype means the variants of the *S*-locus, while *S*-alleles are used to characterize the gene variants (pollen or pistil). The physical distance between the tightly linked *SFB* and *S-RNase* varies from 380 bp to 30 kb in different *S*-haplotypes (Entani *et al*. 2003, Yamane *et al*. 2003a). Allele names are labelled by different numbers or letters. Number of *S*-alleles is dependent upon the population size (N) and the mutation rate (Busch *et al.*, 2014). Fertilization of rosaceous fruit tree species is governed by GSI (de Nettancourt 1997), which mainly determines the commercial fruit production by effecting fruit set proportion. Cultivars sharing different *S*-alleles (belonging to different cross-incompatibility groups) must be interplanted in orchards; therefore, the information of *S*-genotypes is necessary for fruit growing and breeding (Yamane & Tao 2009).

Background of pollen-pistil interactions in diploid fruit tree species— *S-RNases* have been shown to have five conserved regions, named C1 to C5. Ushijima *et al*. (1998) explained that positions and sequences of the regions C1, C2, C3 and C5 in the rosaceous gene were similar to those of the solanaceous *S-RNases*, but sequence motif related to the solanaceous C4 region was not identified. The *Rosaceae*-specific conserved region, named RC4 (the "R"-letter refers to the name of the *Rosaceae* family), was identified in a position similar to that of the solanaceous C4. The hypervariable RHV region is important for *S*-allele recognition and for initiating the self-incompatibility response. In stone fruit, the allelic variants of *S-RNase* contain two introns (Fig.1.) and both of them unveiled considerable length polymorphism (Tao *et al.* 1999).

First intron is located between the signal peptide (SP) and the conservative region C1 while its 2nd intron is located within the hypervariable region (RHV). Generally, molecular techniques detected that sizes of the second intron (approx. 110 to 3100 bp) were higher than the sizes of the first intron (approx. 100-450 bp) in rosaceous *S-RNases* (Sonneveld *et al.* 2003).

The *S-haplotype-specific F-box* gene (*SFB*) was identified by sequencing the *S*-locus region of several *Prunus* species, such as almond (Ushijima *et al.* 2003), Japanese apricot (Entani et al. 2003; Yamane et al. 2003b), and both sweet and sour cherries (Yamane *et al*. 2003a). These genes, termed *SLF* (*S-locus F-box*) or *SFB* (*S-haplotype specific F-box*), fulfilled the conditions for a pollen *S*-determinant gene: (a) they are tightly linked to the *S-RNase* gene in the *S*-locus, (b) show high *S*-haplotype-specific polymorphism, and (c) are specifically expressed in pollen. The primary

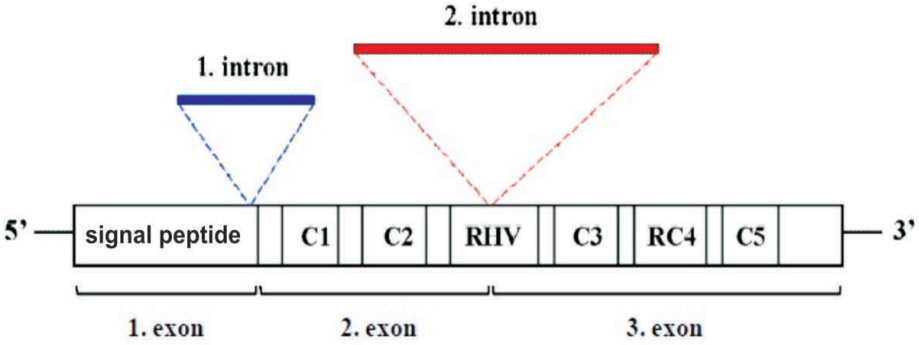

Fig. 1—Structure of a *Prunus S-RNase*. It contains five conserved regions (C1, C2, C3, C5 and RC4) and a hypervariable region (RHV) that exhibits evidence of positive selection (Sonneveld *et al*. 2003).

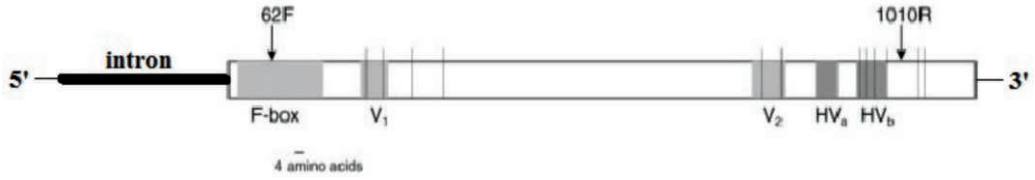

Fig. 2—Schematic structure of the *SFB* gene. The F-box, variable regions V_1 and V_2, and hypervariable regions HV_a and HV_b are shown according to Ikeda *et al*. 2004, Romero *et al.* 2004 and Nunes *et al*. 2006.

structural features of *Prunus SFB*s showed the presence of one F-box motif, two variable regions (V_1 and V_2) and two hypervariable regions (HV_a and HV_b) in the amino acid sequence (Fig.2) (Ikeda *et al*. 2004).

The incompatibility reactions take place between the pistil *S*-ribonuclease and the pollen-expressed *S*-haplotype specific F-box proteins within the pollen tubes. Based on the modified inhibitor model, SFB proteins inhibit the degradation of self *S*-RNases. It means that pollen RNA can be degraded and pollen tube growth is stopped (Sonneveld *et al.* 2005).

Breakdowns in the pollen or pistil *S*-gene resulted in self-compatible genotypes. Fruit breeders and growers appreciate SC mutants because they could overcome cross-pollination requirements. Nowadays, several SC cultivars are available for growers due to induced and/or naturally occurring mutation in case of cherries (Sonneveld *et al.* 2006, Marchese *et al.* 2007), almond (Boškovic *et al.* 1999, Kodad & Socias i Company, 2008), peach (Hegedûs *et al.* 2006, Tao *et al.* 2007) and apricot (Burgos *et al.* 1998, Halász *et al.* 2007). A total of 27 non-functional *S*-haplotypes have been identified and characterized, most (24) of which formed as a consequence of natural mutations. In the Prunoideae, 50 % of haplotypes proved to be pollen-part mutants, while 36 % are stylar-part mutants, one haplotype shows both mutations, and molecular changes for two haplotypes still have not been clarified (Hegedûs *et al.* 2012).

Study on the *S*-locus of polyploid fruit tree species—Crop reliability of fruit species depends on external factors and genetically controlled mechanisms. Among such, sexual incompatibility is in the focus of this review. Scientific achievements are primarily limited to diploid species like peach, almond and sweet cherry, while information is hardly available for polyploid species despite the fact that polyploidy is a prominent feature of plant genomes. It had an important role in evolution but complex genome structure makes understanding difficult (Dufresne *et al.* 2014). A polyploid cell nucleus contains more than two genomes. Two basic groups of polyploids are known: autopolyploids, which contain more than two genetically identical genomes, originating after genome doubling within single species; and allopolyploids, which combine genomes from more than one ancestral species (Leitch & Benett 1997).

In case of *Rosaceae* fruit trees, self-incompatibility locus of three polyploid species were analysed until now: sour cherry (*Prunus cerasus*, tetraploid), Chinese cherry (*P. pseudocerasus*, tetraploid) and European plum (*P. domestica*, hexaploid).

Sour cherry (*Prunus cerasus* L.)— Sour cherry (*Prunus cerasus* L.) is a tetraploid species with 32 chromosomes in sporophytic cells. It is an allotetraploid spontaneous hybrid of sweet cherry (*Prunus avium* L.) ($2n=2x=16$) and ground cherry, *Prunus fruticosa* Pall. ($2n=4x=32$) (Olden & Nybom 1968, Brown *et al.* 1996). It means that allele pools of sweet and sour cherry are overlapping. *S*-haplotypes present in sweet cherry (S_1, S_4, S_6, S_9, S_{12}, S_{13}, S_{14} and S_{16}) have been shown to occur in sour cherry, as well. The origin of the long-time dubious S_{34}-allele has been recently clarified to be transmitted by the sweet cherry parent because this allele was identified in a wild cherry collected around the Black Sea coast (Szikriszt *et al.* 2013). Previously, Tsukamoto *et al.* (2008) hypothesized that this allele together with S_{33} and S_{35} might have been derived from ground cherry (*P. fruticosa*). Most of the sour cherry cultivars are self-compatible but self-incompatible genotypes are also known (Lansari & Iezzoni 1990). Yamane *et al.* (2001) demonstrated for the first time the presence of *S*-ribonucleases in styles of both the SC and SI cultivars. All the 13 tested genotypes contained at least one sweet cherry *S*-allele. The presence of S_1, S_4, S_6, S_9 and S_{12} sweet cherry alleles was proved and they found new *S*-alleles labelled by letters until the confirmation of their novelty (S_a to S_e). Correspondence between self-compatibility and certain allelic combinations could not have been resolved. In contrast to some solanaceous species, heteroallelic pollen alone does not cause self-compatibility in sour cherry. Analyses of fertility properties in three progenies of sour cherry in the field and by monitoring pollen tube growth after selfing showed that seedling phenotypes correlated with disomic segregation of the *S-RNase* alleles (Tobutt *et al*. 2004, Boškovic *et al.* 2006).

Hauck *et al*. (2002) compared sweet and sour cherry *S*-allele function, *S-RNase* sequences and linkage map location as initial steps towards the understanding of 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 the S_4 and S_6-*RNases* in sour cherry were identical to those of sweet cherry *S-RNases*, whereas three other *S-RNases* had unique sequences. Segregation analysis in a progeny from the cross 'Rheinische Schattenmorelle' (S_a S_b S_c S_6) × 'Érdi Bõtermõ' (S_a S_4 S_{6m}) were optimal to understand the SI mechanism in sour cherry (Yamane *et al*. 2001). It was proved that the S_{6m}-RNase is not functionally active; however, its pollen component is functional. The expression analyses of the S_6-*RNase* and the pollen-part gene, SFB_6 helped to clarify what kind of mutation conferred the SC phenotype. The first described non-functional sour cherry allele was S_{6m}-*RNase* in 'Érdi Bõtermõ' derived from its pollen parent, 'Nagy Angol' and was ascribed to a natural mutation (Yamane *et al*. 2003a,b). In the S_{6m} haplotype transcription of the *RNase* gene could not take place, because of a 2,715 bp insertion in the upstream region of the *S-RNase* gene. Sequence alignment highlighted a 1 bp deletion and 2 bp substitutions in the S_{6m2}-*RNase* compared to the wild type S_6. Deletion of a guanine at the nucleotide position +555 resulted in a frame shift that led to a premature stop codon (Tsukamoto *et al.* 2006). Sequence analysis of S_1-haplotype revealed that SFB_1' was longer than the wild-type SFB_1, indicating the presence of a 615 bp insertion located +225 bp from the translation start site. The insert has 11-bp inverted repeats at the termini that are flanked by 8-bp direct repeats. Both the terminal inverted repeat and the direct repeat were characteristic of Ds transposable elements. This insertion generates a premature in-frame stop codon that would result in a putative truncated SFB_1 containing only 75 of the 375 amino acids present in the wild-type SFB_1 (Hauck *et al.* 2006a). Mobile element insertion is an evolutionary force contributing to the breakdown of GSI. Most of the natural mutations leading to self-compatibility in *Prunus* were clarified to be associated with transposable elements. Recently, a *Prunus*-specific non-autonomous Mutator element was identified in apricot and other stone fruits that has been named *Falling Stones (FaSt*) (Halász et al., 2014a). The insertion of one or two copies of such *FaSt* elements into the *SFB* gene of apricot resulted in the breakdown of self-incompatibility. S_{13}-haplotype in some sour cherry cultivars were also determined to be non-functional. When the nucleotide sequence for the 'Montmorency' S_{13m}-*RNase* was aligned with the wild-type S_{13}-*RNase* sequence, two deletions were identified in S_{13m}-*RNase*. The amino acid sequence of S_{13m}-RNase differed from that of the wild-type allele in position 130, resulting in a putatively truncated protein. Moreover, comparison of nucleotide sequences for the SFB_{13} and SFB_{13}' alleles revealed a 2-bp difference. At position +523, the adenine of SFB_{13} was substituted by a guanine in $SFB_{13'}$ resulting in an amino acid change. At position +733, the guanine of SFB_{13} was substituted by a thymine in $SFB_{13'}$, resulting in a premature stop codon. Due to these changes, the SFB_{13}' is unlikely to function as the pollen-*S* (Tsukamoto *et al.* 2006).

The S_{36}-haplotype has four different variants and all the S_{36} variants are nonfunctional. The S_{36a}-*RNase* diverged from the S_{36b}-*RNase* by one nucleotide mismatch in their second intron. SFB_{36a} and SFB_{36b} differed by eight nucleotides but these molecular alternations may not be linked to loss of specificity. A comparison of the S_{36a} and S_{36b}-RNases with other functional cherry *S*-RNases did not reveal any molecular alterations that are known to disrupt *S*-RNase activity. The S_{36b2}-*RNase* variant differed from the S_{36b}-*RNase* by a 1-bp substitution in the conserved region C2, causing a premature stop codon. But sequence analyses have not explored an unequivocal defect that might explain the loss of function for the S_{36a}, S_{36b}, and S_{36b3} haplotypes. It can be possible that replacement of the amino acid residue at position 188 (from tyrosine to phenylalanine) of the SFB protein caused the transition from SI to SC or another explanation can be the presence of a non-autonomous *Helitron* element 38 bp downstream of the stop codon of *SFB* in all four S_{36} variants (Tsukamoto *et al.* 2010).

The loss of SI in sour cherry is genotype-dependent and caused by the accumulation of non-functional *S*-haplotypes (Fig.3). These haplotypes can be classified as pollen-part mutants, labelled as a prime symbol (') or stylar-part mutants, signed by "m" following the *S*-haplotype number. This system named as 'one allele-match model': pollen rejection occurs if one or both of the fully functional *S*-haplotypes in the 2× sour cherry pollen grain matches an *S*-haplotype in the style (Hauck *et al.* 2006b). Therefore, a sour cherry selection is SC if it has a minimum of two non-functional *S*-haplotypes. It is different in the *Solanaceae* family because pollen grains

containing two different pollen-*S* alleles (heteroallelic pollen) are compatible, regardless of the *S*-RNase composition of the style. If pollen grains contain two copies of the same allele (homoallelic pollen), pollen tube growth is arrested when a cognate *S*-RNase is present in the style (Luu *et al*. 2001).

Tsukamoto *et al*. (2008, 2010) designed useful PCR markers to detect non-functional *S*-haplotypes enabling discrimination between SC and SI genotypes. Allele-specific primers and CAPS-markers (cleaved amplified polymorphic sequence) can distinguish between the mutant and wild-type alleles in case of S_1/S_1'; $S_6/S_{6m}/S_{6m2}$; $S_{13}/S_{13m}/S_{13}$' and $S_{36}/S_{36a}/S_{36b}/S_{36b2}/S_{36b3}$. This kind of CAPS system was also very effective in apple *S*-genotyping (Kim *et al.* 2009). The markers are based on length and sequence polymorphisms (restriction enzyme cut sites) and are useful for early detection at the seedling stage in sour cherry breeding programs resulting cost and time savings. The progenies must be screened for multiple alleles to decide whether the genotype is SC or SI.

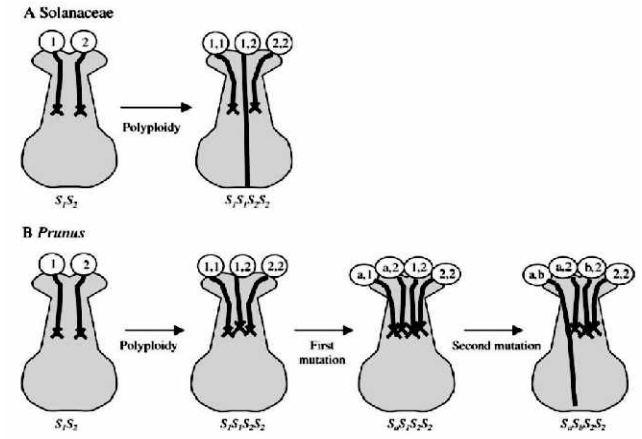

Fig.3–Comparison of the polyploidy effect on GSI in (A) the *Solanaceae* family and (B) *Prunus* genus. In the *Solanaceae*, SC phenotype formed directly by polyploidy due to the compatibility of heteroallelic pollen. In *Prunus* species it is not polyploidy *per se*, which causes the breakdown of SI. The absence of any functional match results in a compatible combination. Self-fertilization requires two non-functional *S*-haplotypes in 2× pollen (After Hauck *et al*. 2006b).

Chinese cherry (*Prunus pseudocerasus* L.)— *Prunus pseudocerasus* Lindl. is commonly known as Chinese sour cherry, and belongs to the family *Rosaceae*. It is an insect-pollinated, perennial species with a long history of cultivation in Asian countries. It originates from Southwest China and is widely distributed in the temperate zone of Northern Hemisphere (Li *et al*. 2009). Similarly to *P. cerasus*, Chinese cherry is also an allotetraploid species (Zhang *et al*. 2010) and it had been found to be naturally self-compatible (Huang *et al*. 2008). Chinese cherry is very useful as a model plant for the study of self-compatibility, and much of this information will be valuable for further work on fertility properties of other polyploid fruit trees.

For the first time, Huang *et al*. (2008) isolated four *PpsS*-haplotypes comprising at least two genes, i.e., *PpsS-RNase* and *PpsSFB* in *P. pseudocerasus* Lindl. cv. Nanjing Chuisi ("NC") for which the *S*-genotype was determined as $S_1S_3'S_5S_7$. No mutations leading to dysfunction of *S*-haplotype were found in their sequences, except for $PpsS_3'$-haplotype which was not amplified by PCR. These four *S*-haplotypes showed tetrasomic inheritance. Diploid pollen grains with *S*-genotypes S_7S_1, S_7S_5 and S_1S_5 could have been growing in the style after self-fertilization, while pollen grains with $S_3'S_7$, $S_3'S_1$ and $S_3'S_5$ were stopped. The results suggest that *PpsS*-haplotypes-*1, -5* and -*7* are functional, and that competitive interaction between two of them confer self-compatibility on cultivar "NC". Furthermore, in terms of recognition specificity, diploid pollen grains carrying $PpsS_3$'-haplotype are equal to monoploid pollen grains carrying the other functional *S*-haplotype. Later, Gu *et al*. (2010, 2012) isolated nine *S*-RNase alleles from cultivars of Chinese cherry by PCR amplification from genomic DNA and stylar cDNA. Amino acid sequences revealed five novel *S*-alleles (S_2, S_4, S_6, S_8, and S_9). Zhang *et al*. (2010) described two functional *S-RNases* and two non-functional *S-RNases*, which could be considered stylar-part mutants and labelled as S_{3m} and S_{4m}, and hence it might explain the self-compatibility of Chinese cherry consistently with the system written for sour cherry. In the meantime, Yu *et al.* (2010) characterized four *SFB*s. Of the four *SFB*s, three showed typical characteristics of *SFB*s from other *Prunus* species and functioned as *SFB* genes (these were termed SFB_a, SFB_b and SFB_c). The fourth *SFB*, termed SFB_d, has a 100-bp deletion that resulted in the transcript for SFB_d lacking the F-box domain. Genetic analysis of the *S*-genotypes showed that the pollen grains were hetero-diploid (Gu *et al*., 2013). The distributions of single *S*-haplotypes expressed in self- and cross-pollinated progenies were in

disequilibrium. The abnormal segregation ratios of pollen-*S* indicate that a few hetero-diploid pollen grains could inactivate self-stylar *S*-RNase inside the pollen tube and grow better into the self-ovaries than others. It indicates that other factors can regulate this phenomenon, which must be further investigated (Gu *et al*., 2014) and this time the occurrence of SC in Chinese cherry is not enough clear.

European plum (*Prunus domestica* L.)—Plums belong to the genus *Prunus* of the family *Rosaceae* and thereby all species show gametophytic self-incompatibility system (Hegedûs & Halász 2006). Plums are a diverse group of plants with many species adapted to a broad range of eco-geographic conditions and have been cultivated for centuries (Ayanoglu *et al.* 2007). This group contains 20-40 species (Szabó 2001) that are distributed in different parts of the world. The genus has a wide range of ploidy levels; the Japanese plum (*Prunus salicina* Lindl.), the myrobalan plum (*P. cerasifera* Ehrh.) and the American plums (*P. americana* Marsh., *P. munsoniana* Wight and *P. angustifolia* Marsh) are diploid (2*n*=2*x*=16), compared to the tetraploid sloe *P. spinosa* L. (2*n*=4*x*=32) and the hexaploid European species (2*n*=6*x*=48) (Shimada *et al.* 1999).

Domestic plum (*P. domestica* L.) is a hexaploid (2*n* = 6*x* = 48) allopolyploid species (Botu *et al*. 2002). *P. domestica* is assumed to be a relatively young species and its wild state is unknown (Das *et al.* 2011). Luther Burbank reported Caucasus Mountains near the Caspian Sea as the place of origin for *P. domestica* and its ancestors (Tóth & Surányi 1980, Malcolm 2006). A number of hypotheses have been put forward to explain the origin of European plum. It is assumed that domestic plum is a naturally formed hybrid that originated about 2000 years ago from the parents of the diploid myrobalan (*P. cerasifera*) and tetraploid sloe (*P. spinosa*). Molecular marker data analyses supported the hybrid origin of plum because *P. domestica*, *P. cerasifera* and *P. spinosa* shared common alleles. However, the possibility of secondary hybridisation with other species, cannot be ruled out. (Zohary *et al.* 1992, Zohary & Hopf 2000, Horvath *et al.* 2011). European plum is a less-investigated species in the *Prunoideae* subfamily, partly due to its complex structure of genome.

Information on the *S*-genotype of European plum cultivars is rather limited, there are only a few work available for the characterisation of germplasm collections. Until now, no cross-incompatibility groups were determined and knowledge about the number of plum *S*-alleles is rather limited. Based on the early controlled self- and cross-pollination experiments, "fully" self-compatible, "partially" self-compatible and self-incompatible cultivars were described (Rawes 1921). Since self-incompatibility is general in plum, cross-pollination is essential for most cultivars. Male sterility may also occur in some domestic plum cultivars and this further complicates the optimal orchard design (Szabó *et al*. 1999).

The first reports on the molecular analysis of plum *S*-alleles were published by Sutherland *et al.* (2004a, b). Analyses of trans-specific evolution of different *Prunus* (including plum) alleles revealed the evolution of new allele specificities, taxonomy of the genus and speciation. Altogether three plum *S*-alleles (S_5, S_6 and S_9) were sequenced and characterized (Sutherland *et al.* 2008). Since there are no available specific markers of plum *S*-alleles in comparison with other *Prunus* species, primers designed for the conservative regions of *S-RNase* and *F-box* genes can be used for gaining information about hexaploid plums. Six *S*-locus-specific markers were tested for the characterization of 33 European plum cultivars (Kota & Lâcis 2013). The average observed heterozygosity proved to be quite high; 14–37 alleles were amplified in case of different primer combinations. All plum cultivars were characterized by unique *S*-genotypes. The markers used did not show differences according to self-compatible or –incompatible phenotype. Halász *et al.* (2014b) scored 18 different alleles in 16 cultivars indicating high genetic diversity. These alleles were labelled using alphabetical codes from S_A to S_S. A total of 16 different *S*-genotypes were assigned, and discrimination of all plum cultivars was successful based on their unique *S*-genotypes. However, further research is required to reliably identify the *S*-alleles based on their DNA sequence and clarify complete *S*-genotypes.

Due to the lack of the complete gene sequences of the European plum *S*-haplotypes, currently there are no data on the emergence of self-compatibility in this species. If the "one-allele-match" model of self-incompatibility in tetraploid sour cherry is also valid in the hexaploid European plum cultivars, then SC requires

the loss-of-function for a minimum of three *S*-haplotype-specificity components (Fig.4), but there is no direct evidence of this to date.

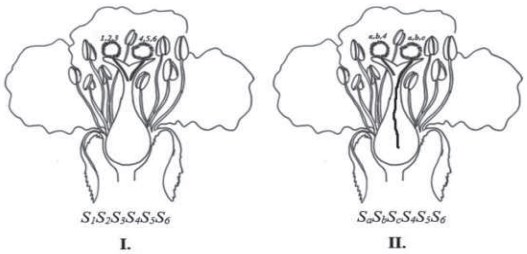

Fig. 4—Putative function of gametophytic self-incompatibility for the hexaploid *Prunus domestica*. SC requires the loss-of-function for a minimum of three *S*-haplotype-specificity components (a,b,c).

Acknowledgements—This work was funded by the OTKA K 112554 project and also supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (J. Halász).

LITERATURE CITED

Ayanoglu H, Bayazit S, Inan G, Bakir M, Akpinar AE, Kazan K & Ergul A 2007. AFLP analysis of genetic diversity in Turkish green plum accessions (*Prunus cerasifera* L.) adapted to the Mediterranean region. *Sci. Horti*. **114** 263-267.

Boškovic R, Tobutt KR, Duval H, Batlle I, Dicenta F & Vargas FJ 1999. A stylar ribonuclease assay to detect self-compatible seedlings in almond progenies. *Theor Appl Genet.* **99** (5) 800-810.

Boškovic RI, Wolfram B, Tobutt KR, Ceroviæ R & Sonneveld T 2006. Inheritance and interactions of incompatibility alleles in the tetraploid sour cherry. *Theor Appl Genet*. **112** 315–326.

Botu M, Sarpe C & Cosmulescu S 2002. The genetic control of pollen fertility, pollenizing and fruit set for the *Prunus domestica* L. plum cultivars. *Acta Hort*. **577** 139–145.

Brown SK, Iezzoni AF & Fogie HW 1996. Cherries 213-255 In: Janick J & Moore JN (eds.) Fruit breeding. Vol. 1. *Tree and tropical fruits*. John Wiley&Sons, Inc., New York.

Burgos L, Pérez-Tornero O, Ballester J & Olmos E 1998. Detection and inheritance of stylar ribonucleases associated with incompatibility alleles in apricot. *Sexual Plant Reprod.* **11** (3) 153-158.

Busch JW, Witthuhn T & Joseph M. 2014. Fewer *S* alleles are maintained in plant populations with sporophytic as opposed to gametophytic self incompatibility. *Plant Species Biology*, **29**(1) 34-46.

De Nettancourt D 1997. Incompatibility in angiosperm. *Sex Plant Reporod*. **10** 185-199.

Das B, Ahmed N & Singh P 2011. *Prunus* diversity-early and present development: A review. *Inter. J. Biodi. & Conser.* **3** (14) 721-734.

Dufresne F, Stift M, Vergilino R & Mable BK 2014. Recent progress and challenges in population genetics of polyploid organisms: an overview of current state of the art molecular and statistical tools. *Mol. Ecol*. **23** (1) 40-69.

Ebert PR, Anderson MA, Bernatzky R, Altshuler M & Clarke AE 1989. Genetic Polymorphism of Self-Incompatibility in Flowering Plants. *Cell.* **56** 255-262.

Entani T, Iwano M, Shiba H, Che F-S, Isogai A & Takayama S 2003. Comparative analysis of the self-incompatibility (S-)locus region of *Prunus mume*: identification of a pollen expressed F-box gene with allelic diversity. *Genes Cells* **8** 203–213.

Gu C, Zhang SL, Huang SX, Heng W, Liu QZ, Wu HQ & Wu J 2010. Identification of S-genotypes in Chinese cherry cultivars (*Prunus pseudocerasus* Lindl.). *Tree Genetics & Genomes.* **6** 579–590.

Gu C, Wu J, Zhnag SJ, Yang Y, Wu HQ, Tao ST & Zhang SL 2012. Characterization of the S-RNase genomic DNA allele sequence in *Prunus speciosa* and *P. pseudocerasus*. *Scientia Horticulturae.* **144** 93-101.

Gu C, Liu QZ, Yang YN, Zhang SJ, Khan MA, Wu J & Zhang SL 2013. Inheritance of Hetero-Diploid Pollen S-Haplotype in Self-Compatible Tetraploid Chinese Cherry (*Prunus pseudocerasus* Lindl). *PLOS ONE*. Vol**. 8** (4) e61219.

Gu C, Liu QZ, Khan AW, Wu J & Zhang SL 2014. Hetero-diploid pollen grains that represent self-

compatibility are incompatible with non-self receptors in tetraploid Chinese cherry (*Prunus pseudocerasus* Lindl). *Tree Genetics & Genomes*. **10** 619–625.

Halász J, Pedryc A & Hegedûs A 2007. Origin and dissemination of the pollen-part mutated SC haplotype which confers self-compatibility in apricot (*Prunus armeniaca*). *New Phytologist.* **176** (4) 792-803.

Halász J, Kodad O & Hegedûs A 2014a. Identification of a recently active *Prunus*-specific non-autonomous Mutator element with considerable genome shaping force. *The Plant Journal* **79** (2) 220-231.

Halász J, Kurilla A & Hegedûs A 2014b. Preliminary characterization of the self-incompatibility genotypes of European plum (*Prunus domestica* L.) cultivars. *Inter. J. Hort. Sci.* **20** (3-4) 23-26.

Hauck NR, Yamane H, Tao R & Iezzoni AF 2002. Self-compatibility and incompatibility in tetraploid sour cherry (*Prunus cerasus* L.). *Sex. Plant Reprod*. **15** 39–46.

Hauck NR., Ikeda K, Tao R & Iezzoni, AF 2006a. The mutated S1-Haplotype in sour cherry has an altered S-Haplotype-specific F-box protein gene. *J. Hered.* **97** (5) 514–520.

Hauck NR, Yamane H, Tao R & Iezzoni AF 2006b. Accumulation of nonfunctional S-Haplotypes results in the breakdown of gametophytic selfincompatibility in tetraploid *Prunus*. *Genetics.* **172** (2) 1191–1198.

Hegedûs A & Halász J 2006. Self-incompatibility in plums (*Prunus salicina* Lindl., *Prunus cerasifera* Ehrh. and *Prunus domestica* L.) *Inter. J. HortScience.* **12** (2) 137–140.

Hegedûs A, Szabó Z, Nyéki J, Halász J & Pedryc A 2006. Molecular analysis of S-haplotypes in peach, a self-compatible *Prunus* species. *J. Am. Soc. Hort. Sci.* **131** (6) 738-743.

Hegedûs A, Lénárt J & Halász J 2012. Sexual incompatibility in *Rosaceae* fruit tree species: molecular interactions and evolutionary dynamics. *Biologia Plantarium* **56** (2) 201-209.

Horvath A, Balsemin E, Barbot JC, Christmann H, Manzano G, Reynet P, Laigret F & Mariette S 2011. Phenotypic variability and genetic structure in plum (*Prunus domestica* L.), cherry plum (*P. cerasifera* Ehrh.) and sloe (*P. spinosa* L.). *Scientia Horticulturae* **129**(2) 283-293.

Huang SX, Wu HQ, Li YR, Wu J, Zhang SJ, Heng W & Zhang SL 2008. Competitive interaction between two functional S-haplotypes confer self-compatibility on tetraploid Chinese cherry (*Prunus pseudocerasus* Lindl. CV. Nanjing Chuisi). *Plant Cell Reprod.* **12** 1075-1085.

Ikeda K, Igic B, Ushijima K, Yamane H, Hauck NR, Nakano R, Sassa H, Iezzoni AF, Kohn JR & Tao R 2004. Primary structural features of the S haplotype-specific F-box protein, SFB, in Prunus. *Sex Plant Reprod*.**16** 235-243.

Kao TH & McCubbin AG 1996. How flowering plants discriminate between self and non-self pollen to prevent inbreeding. *Proc. Natl. Acad. Sci.* Vol. **93** 12059-12065.

Kim H, Kakui H, Kotoda N, Hirata Y, Koba T & Sassa H 2009. Determination of partial genomic sequences and development of a CAPS system of the S-RNase gene for the identification of 22 S haplotypes of apple (*Malus*× *domestica* Borkh.). *Molecular Breeding*. **23** (3) 463-472.

Kodad O & Socias i Company R 2008. Fruit set evaluation for self-compatibility selection in almond. *Scientia Horticulturae*. **118** (3) 260-265.

Kota ID & Lâcis G 2013. Evaluation of self-incompatibility locus diversity of domestic plum (*Prunus domestica* L.) using DNA-based *S*-genotyping. *Pro. Latvian Acad. Sci.* **67**(2) 109.115.

Lai Z, Ma W, Han B, Liang L, Zhang Y, Hong G & Xue Y 2002. An F-boksz gene linked to the self-incompatibility (*S*) locus of *Antirrhinum* is expressed specifically in pollen and tapetum. *Plant Mol. Biol.* **50** (1) 29–42.

Lansari A & Iezzoni A 1990. A preliminary analysis of self-incompatibility in sour cherry. *HortSience* **25** (12) 1636-1638.

Lawrence MJ, Marshall DF, Curtis VE & Fearon CH 1985. Gametophytic self-incompatibility re-examined: a reply. *Heredity* **54** 131-138.

Leitch IJ & Bennett MD 1997. Polyploidy in angiosperms. *Trends in Plant Sci.* **2** (12) 470-476.

Li MM, Cai YL, Qian ZQ & Zhao GF 2009. Genetic diversity and differentiation in Chinese sour cherry *Prunus pseudocerasus* Lindl., and its implications for conservation. *Genet Resour Crop Evol.* **56** 455-464.

Luu DT, Qin X, Laublin G, Yang Q, Morse D & Cappadocia M 2001. Rejection of S-heteroallelic pollen by a dual-specific S-RNase in *Solanum chacoense* predicts a multimeric SI pollen component. *Genetics* **159** 329–335.

Malcolm P 2006. History of plum trees and their hybrids. www.The phantom writers.com.

Marchese A, Boškoviæ RI, Caruso T, Raimondo A, Cutuli M & Tobutt KR 2007. A new self-compatibility haplotype in the sweet cherry 'Kronio', S52 , attributable to a pollen-part mutation in the SFB gene. *J. Exp. Bot.* **58** (15-16) 4347-4356.

McClure, B, Cruz-García, F & Romero C 2011. Compatibility and incompatibility in S-RNase-based systems. *Annals of botany*, **108**(4) 647-658.

Mora C, Tittensor DP, Adl S, Simpson AGB & Worm B 2011. How Many Species Are There on Earth and in the Ocean? *PLOS Biolo*gy. **9** (8) e1001127.

Nunes MDS, Santos RAM, Ferreira SM, Vieira J & Vieira P 2006. Variability patterns and positively selected sites at the gametophytic selfincompatibility pollen SFB gene in a wild self-incompatible *Prunus spinosa* (*Rosaceae*) population. *New Phytol*. **172** 577–587.

Olden EJ & Nybom N 1968. On the origin of *Prunus cerasus* L. *Hereditas*. **59** 327–345.

Rawes AN 1921. Pollination in orchards (IV.). Self-fertility and self-sterility in plums. *J. Roy. Hort. Soc.* **39** 353–356.

Romero C, Vilanova S, Burgos L, Martínez-Calvo J, Vicente M, Llácer G & Badenes1 ML 2004. Analysis of the S-locus structure in *Prunus armeniaca* L. Identification of S-haplotype specific S-RNase and F-box genes. *Plant Molecular Bio.* **56** 145–157.

Shimada T, Hayama H, Haji T, Yama-guchi M & Yoshida M 1999. Genetic diversity of plums characterized by random amplified polymorphic DNA (RAPD) analysis. *Euphytica*. **109** 143-147.

Sonneveld T, Tobutt KR & Robbins TP 2003. Allele-specific PCR detection of sweet cherry self-incompatibility (*S*) alleles S_1 to S_{16} using consensus and allelespecific, primers. *Theor. Appl. Genet*. **107** 1059–1070.

Sonneveld T, Tobutt KR, Vaughan SP & Robbins TP 2005. Loss of pollen-S function in two self-compatible selections of *Prunus avium* is associated with deletion/mutation of an S haplotype- specific F-box gene. *Plant Cell*. **17** 37–51.

Sonneveld T, Robbins TP & Tobutt KR 2006. Improved discrimination of self-incompatibility S-RNase alleles in cherry and high throughput genotyping by automated sizing of first intron polymerase chain reaction products. *Plant Breeding*. **125** (3) 305-307.

Sutherland BG, Robbins TP & Tobutt KR 2004a. Primers amplifying a range of *Prunus S*-alleles. *Plant Breed*. **123** 582–584.

Sutherland BG, Tobutt, KR & Robbins TP 2004b. Molecular genetics of self-incompatibility in plums. *Acta Hort.* **663** 557–562.

Sutherland BG, Tobutt KR & Robbins TP 2008. Trans-specific S-RNase and SFB alleles in *Prunus* self-incompatibility haplotypes. *Molecular Genetics and Genomics* **279**(1), 95-106.

Szabó Z, Nyéki J, Andrasfalvy A & Soltész M 1999. Association of European plum varieties in the orchards. *Inter. J. Hort. Sci.* **5** 21–24.

Szabó Z 2001. Szilva. In: *Gyümölcsészet.* Edit:.Tóth M. PRIMOM Sz-Sz-B. Megyei Vállalkozástélénkító Alapítvány Vállalkozói Központ. Pp. 216-242.

Szikriszt B, Doðan A, Ercisli S, Akcay ME, Hegedûs A & Halász J 2013. Molecular typing of the self-incompatibility locus of Turkish sweet cherry genotypes reflects phylogenetic relationships among cherries and other *Prunus* species. *Tree Genet. Genomes.* **9** (1) 155-165.

Tao R, Yamane H, Sugiura A, Murayama H, Sassa H & Mori H 1999. Molecular typing of S-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. *J. Am. Soc. Horti. Sci.* **124** 224–233.

Tao R, Watari A, Hanada T, Habu T, Yaegaki H, Yamaguchi M & Yamane H 2007. Self-compatible peach (*Prunus persica*) has mutant versions of the S haplotypes found in self-incompatible *Prunus* species. *Plant Mol. Biol.* **63** (1) 109-123.

Tobutt KR, Boškoviæ R, Ceroviæ R, Sonneveld T & Ružiæ Đ 2004. Identification of incompatibility alleles in the tetraploid species sour cherry. *Theor. Appl. Genet*. **108** (5) 775-785.

Tóth E & Surányi D 1980. *Szilva. Mezõgazda Kiadó.* Budapest.

Tsukamoto T, Hauck NR, Tao R, Jiang N & Iezzoni AF 2006. Molecular characterization of three non-functional Shaplotypes in sour cherry (*Prunus cerasus*). *Plant Mol. Biol.* **62** 371–383.

Tsukamoto T., Potter D., Tao R., Vieira CP., Vieira, J. & Iezzoni, AF 2008. Genetic and molecular characterization of three novel S-haplotypes in sour cherry (*Prunus cerasus* L.). *J. Exp. Bot.* **59**(11) 3169-3185.

Tsukamoto T., Hauck NR., Tao R., Jiang N. & Iezzoni AF 2010. Molecular and genetic analyses of four nonfunctional S haplotype variants derived from a common ancestral S haplotype identified in sour cherry (*Prunus cerasus* L.). *Genetics* **184**(2) 411-427.

Ushijima K, Sassa H, Tao R, Yamane H, Dandekar AM, Gradziel TM & Hirano H 1998. Cloning and characterization of cDNAs encoding *S*-RNases from almond (*Prunus dulcis*): primary structural features and sequence diversity of the *S*RNases in *Rosaceae*. *Mol. Gen. Genet.* **260** 261–268.

Ushijima K, Sassa H, Dandekar AM, Gradziel TM, Tao R & Hirano H 2003. Structural and transcriptional analysis of the self-incompatibility locus of almond. *Plant Cell* **15** 771–781.

Yamane H & Tao R 2009. Molecular basis of self-(in) compatibility and current status of S-genotyping in Rosaceous fruit trees. *J. Japanese Soc. Horti. Sci.* **78**(2) 137-157.

Yamane H, Tao R, Sugiura A, Hauck NR & Iezzoni, AF 2001. Identification and characterization of *S*-RNases in tetraploid sour cherry (*Prunus cerasus*). *J. Am. Soc. Horti. Sci.* **126** 661–667.

Yamane H, Ikeda K, Hauck NR, Iezzoni AF & Tao R 2003a. Self-incompatibility (*S*) locus region of the mutated *S*6- haplotype of sour cherry (*Prunus cerasus*) contains a functional pollen *S* allele and a non-functional pistil *S* allele. *J. Exp. Bot*. **54** 2431–2437.

Yamane H, Ikeda K, Ushijima K, Sassa H & Tao R 2003b. A pollen-expressed gene for a novel protein with an F-box motif that is very tightly linked to a gene for *S*-RNase in two species of cherry, *Prunus cerasus* and *P. avium*. *Plant Cell Physiol*. **44** 764–769.

Yu P, Chen X, Meng Q, Zheng Y, Shen X & Chen X 2010. Three nonfunctional S-haplotypes in self-compatible tetraploid Chinese cherry (*Prunus pseudocerasus* L. cv. Taixiaohongying). *Euphytica* **174** 143–151.

Zavada MS & Tayler TN 1986. The role of self-incompatibility and sexual selection in the gymnosperm-angiosperm transition: a hypothesis. *The American Naturalist*. **128** 4.

Zhang XY, Chen XS, Meng QW, Jiang YM, Zheng Y & Chen XL 2010. Transcript and Sequence Analysis of *S-RNases* in Self-Compatible Tetraploid Chinese Cherry (*Prunus pseudocerasus* L.). *Agricultural Sciences in China* **9** (6) 792-798.

Zohary D 1992. Is the European plum, *Prunus domestica* L., a *P. cerasifera* Ehrh. × *P. spinosa* L. allo-polyploid? *Euphytica*. **60** 75–77.

Zohary D & Hopf M 2000. Domestication of plants in the old world the origin and spread of cultivated plants in west Asia, Europe and the Nile Valley. Oxford: Oxford University Press. Pp. 316.