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Self-incompatibility in pears (*Pyrus communis* L., *Pyrus serotina* Rehd. and *Pyrus ussuriensis*) Review

Halász, J.[1] & Hegedűs, A.[2]

[1]*Corvinus University of Budapest, Faculty of Horticultural Science, Department of Genetics and Plant Breeding, 1118 Budapest, Ménesi út 44., Hungary*
[2]*Corvinus University of Budapest, Faculty of Food Science, Department of Applied Chemistry, 1118 Budapest, Villányi út 29., Hungary E-mail: julia.halasz@stud.uni-corvinus.hu*

Summary: Self-incompatibility system and allele pool of three different pear species, European pear (*Pyrus communis*), Japanese pear (*P. serotina*) and Chinese pear (*P. ussuriensis*) are displayed. Several inconsistencies and the absence of the harmonization of three different allele series are revealed in the European pears. By collecting data from several reports eight incompatibility groups of Japanese pear cultivars could be established. A self-compatible genotype is analysed in details and shown to be a stylar-part mutant. As Japanese pear was the first fruit tree species from which *S*-ribonucleases were identified, the history of *S*-genotyping from the beginning to the latest achievements and technical developments can be also monitored from the experiments enumerated. In Chinese pears, seven *S*-alleles and one incompatibility group could be identified.

Key words: pear, *Pyrus* spp., self-incompatibility, *S*-genotype, incompatibility group

In 2005 pear production in the world totalled at a level of 19,513,699 Mt (*Faostat*, 2005). Most of the commercial pear and quince cultivars are self-incompatible (*Szabó* et al., 1999; *Nyéki & Soltész*, 2003), which is a gametophytic trait controlled by the so-called *S*-locus. This locus is a multigene complex: an *S*-RNase gene is expressed in pistil and an *S*-haplotype-specific F-box gene in the pollen tubes. Self/nonself recognition process and the consequent acceptance or rejection takes place between the protein products of these genes. Similarly to apple, some pear cultivars are also predisposed to parthenocarpous fruit set (*Nyéki & Soltész*, 2003), thereby the evaluation of seed content of fruits resulting from controlled crosses must be taken into consideration when self-(in)compatibility properties are analyzed through the classical fruit set studies (see below).

Fertility properties of European pear cultivars (*Pyrus communis* L.)

Most pear cultivars have been traditionally considered as completely or almost completely self-incompatible (*Crane & Lewis*, 1942), while some of them can be partially self-compatible, depending on the environment (*Griggs & Iwakiri*, 1954; *Nyéki* et al., 2000). The firs report available on the *S*-genotypes of European pear cultivars was published by *Tomimoto* et al. (1996). In eight cultivars nine *S*-alleles were identified by 2D-PAGE (*Table 1*).

In most breeding programs developed in the last decades 'Williams' and 'Coscia' cultivars were often used as parental lines (*Morettini*, 1957; *Bellini* et al., 2000). This fact resulted in an increase in the frequency of the *S*-alleles from these two cultivars within the new hybrids and it increased the cases of cross-incompatibility in pear. *Sanzol & Herrero* (2002) developed a reliable *in vivo* method to test pollen-pistil incompatibility in pear: pollen tube performance was studied along the pistil following self- and cross pollination. Their results show that ovule observation at the microscope for the presence of pollen tube in the nucellus is a proper method to test incompatibility in this crop, in contrast to pollen tube growth in the style, which may be an unclear test. The *S*-allele constitution of six commercial pear cultivars was determined on the basis of the compatibility relationships and their parentage relationship. The alleles allocated to these cultivars were named as S_1, S_2, S_3 and S_4. However, it must be mentioned that these results are not reconciled with the previous allele designation used by *Tomimoto* et al. (1996), therefore these allele labels must only be considered as showing the interrelationships among the given six cultivars used in the study. The authors found two crosses inter-incompatible in both ways: 'Agua de Aranjuez' × 'Butirra Precoz Morettini' and 'Santa Maria Morettini × 'S(AA×W)7' (*Table 1*).

Zuccherelli et al. (2002) determined the *S*-locus composition of ten European pear cultivars *via* *S*-PCR molecular assay using apple primers, and a subsequent digestion with restriction endonucleases. The identified

Table 1. *S*-allele constitution of several European pear cultivars

Cultivar	S-genotype	Reference
Grand Champion	S_1S_2	*Tomimoto* et al., 1996
Alexandle Doullard	S_1S_2	*Tomimoto* et al., 1996
Doyenne du Comice	S_3S_4	*Tomimoto* et al., 1996
Bartlett	S_5S_6	*Tomimoto* et al., 1996
Flemish Beauty	S_5S_6	*Tomimoto* et al., 1996
Le France	S_5-	*Tomimoto* et al., 1996
Le Lectier	S_7S_8	*Tomimoto* et al., 1996
General Leclerc	S_2S_9	*Tomimoto* et al., 1996
Agua de Aranjuez	S_1S_3	*Sanzol & Herrero*, 2002*
Butirra Precoce Morettini	S_1S_3	*Sanzol & Herrero*, 2002*
Santa Maria Morettini	S_2S_3	*Sanzol & Herrero*, 2002*
S(AA×W)7	S_2S_3	*Sanzol & Herrero*, 2002*
Williams	S_1S_2	*Sanzol & Herrero*, 2002*
Coscia	S_3S_4 (S_bS_k)	*Sanzol & Herrero*, 2002* *Zisovich* et al., 2004
Tosca	S_1S_4	*Sanzol & Herrero*, 2002*
Abbé Fétel	S_aS_b	*Zuccherelli* et al., 2002
Doyenne du Comice	S_aS_b	*Zuccherelli* et al., 2002
Cascade	S_bS_e	*Zuccherelli* et al., 2002
Max Red Bartlett	S_e-	*Zuccherelli* et al., 2002
Bartlett	S_e-	*Zuccherelli* et al., 2002
Beurré Hardy	S_cS_d	*Zuccherelli* et al., 2002
Eletta Morettini	S_aS_c	*Zuccherelli* et al., 2002
Passe Crassane	S_a-	*Zuccherelli* et al., 2002
Conference	S_dS_h	*Zuccherelli* et al., 2002
Beurré Bosc	S_c-	*Zuccherelli* et al., 2002
Dr.Jill Guyot	S_aS_j	*Zisovich* et al., 2004a
Red Clapp	S_dS_j	*Zisovich* et al., 2004a
Gentile	S_iS_j	*Zisovich* et al., 2004a
Spadona	S_jS_k	*Zisovich* et al., 2004a
Bon Rouge	S_jS_l	*Zisovich* et al., 2004a
Forelle	S_jS_n	*Zisovich* et al., 2004a
Spadochina	S_kS_l	*Zisovich* et al., 2004a
Lawson	S_mS_o	*Zisovich* et al., 2004a

*Allele designation is not reconciled with that used by *Tomimoto* et al. (1996)

alleles were called S_a, S_b, S_c, S_d, S_e and S_h, and then all the six *S*-allele fragments were sequenced. Different degrees of fertility when crossing compatible pear cultivars were suspected to be the consequence of the action of modifier genes (*Zuccherelli* et al., 2002). The action of the modifier genes are hypothesized in cherry (*Wünsch & Hormaza*, 2004) and peach (*Hegedűs* et al., 2005), as well.

Zisovich et al. (2004a) identified seven new alleles by PCR and sequencing and determined the compatibility relationships among nine cultivars. The comparison of these alleles with each other exposed a high degree of similarity among them. The results revealed that 'Spadona' (S_jS_k), the main pear cultivar in Israel was semi-compatible with its pollinators 'Coscia', 'Gentile' and 'Spadochina' (*Table 1*) and thereby it can explain the reason for relatively low yields. A new cultivar 'Lawson' being considered for introduction seemed to be fully compatible with 'Spadona'. In another study, the same research group (*Zisovich* et al., 2004b) showed that the deduced amino acid sequences of S_n-RNase and S_i-RNase alleles have an identical hypervariable (RHV) region. However, S_n-RNase does not prevent fertilization by S_i pollen-haplotype, thus presenting a case in which RHV is not required for the determination of specific pollen rejection by *S*-RNase. The mode of the allele-specific recognition still waits for clarification in this case; however, several regions of the *S*-RNase gene may be implicated in it.

Compatibility relationship of Japanese pear cultivars (*Pyrus serotina* Rehd. syn.: *Pyrus pyrifolia* Nakai)

Many data about the self-incompatibility system were obtained in Japanese pear, as this was the first species within the *Rosaceae* family in which stylar ribonucleases were detected (*Sassa* et al., 1992). As a matter of curiosity, we mention that from the report of another Japanese research group published in the same year, low correspondence was obtained between protein bands on IEF gels and *S*-alleles, mainly due to the improper separation at the basic part of the gel. Almost all cultivars of Japanese pear exhibit self-incompatibility. *Table 2* demonstrates *S*-genotypes that have been determined for a number of cultivars (*Terami* et al., 1946; *Kajiura* et al., 1967; *Machida*, 1972). The only self-compatible cultivar is 'Osa-Nijisseiki', which is a mutant derived from the cultivar 'Nijisseiki' (*Hirata*, 1989). It was revealed that the style is unable to arrest self pollen tubes, which is attributed to a mutation of the S_4-allele (labelled as S_4^{sm}; sm= stylar-part mutant) based on genetical analyses of progenies (*Sato* et al., 1988). It means that the pollen function is unaltered thereby 'Osa-Nijisseiki' as a male parent will show unilateral incompatibility to cultivars with an S_2S_4 genotype. *Sassa* et al. (1992) found that the S_4-related RNase band from 'Osa-Nijisseiki' was much less intense than that in the original cultivar. After silver staining of the proteins in IEF gels, the intensity of the S_4-related band was also much reduced in cv. 'Osa-Nijisseiki', which indicated a reduced level of expression in the self-compatible genotype. *Hiratsuka* et al. (1995) studied the expression and inheritance of the S_2- and S_4-alleles using progenies of self-compatible 'Osa-Nijisseiki', and totally confirmed the above described results. However, no difference was observed in the S_2-protein band between these two cultivars.

Norioka et al. (1996) cloned cDNAs for the S_4 from a stylar cDNA library, while S_4^{sm} was neither amplified by PCR nor cloned from the library, confirming that the mutation resulted in a failure of expression of S_4-RNase. Later, the S_4-RNase could not be detected in the mutant cultivar by genomic Southern blot, indicating that the mutant lacks the gene (*Sassa* et al., 1997). The extent of deletion in the mutant was estimated to be more than 4 kbp, which spans the entire length of the S_4-RNase gene. *Hiratsuka* et al. (1999) have obtained results inconsistent with the previous findings: even at a lower level, S_4-protein was expressed in 'Osa-Nijisseiki' styles. Mechanism of self-compatibility seemed to be similar to the low levels of *S*-proteins and weak incompatibility in immature styles of self-incompatible

'Nijisseiki'. The authors concluded that part of this protein repression might be regulated during post-transcriptional events. Later it was confirmed by showing that the depressed growth of unilateral-compatible pollen tubes ('Osa-Nijisseiki' × 'Kikusui') and self-pollen-tubes in 'Osa-Nijisseiki' is due to this small amount of biologically active S_4-RNase (*Zhang & Hiratsuka*, 2005).

Later, stylar RNases associated with self-incompatibility genes were further characterized by 2D-PAGE and N-terminal sequencing (*Sassa* et al., 1993). The same approach was also used to determine the amino acid sequences of S_1 to S_7 (*Ishimizu* et al., 1996). Using these sequence information, and by aligning them with other rosaceous sequences, the same research group was the first to describe primary structural features of rosaceous *S*-RNases (*Ishimzu* et al., 1998b), and to predict topology of secondary and tertiary structures of *S*-RNases and identify putative regions for *S*-allele-specific recognition (*Ishimzu* et al., 1998a). A crystal structure of the S_3-RNase was determined at 1.5 Å resolution, the structural features consisting 8 helices and 7 β-strands confirmed again the location and molecular role of hypervariable regions (*Matsuura* et al., 2001). Aphylogenetic tree of rosaceous *S*-RNases showed that *S*-RNase polymorphism predated the divergence of *Pyrus* and *Malus* (*Ishimzu* et al., 1998b).

Hiratsuka & Okada (1995) investigated the S_3-protein because of its abundance and clear separation upon isoelectric focusing. They described that this protein was present only as a soluble form in the mature style. The younger styles also contain soluble S_3-protein only, suggesting that the *S*-protein is not bound chemically to cell wall and membrane in younger styles but produced gradually in the developing styles. Its concentration was the highest in the upper part of the style. The sum of two allelic *S*-proteins was found to correlate positively with the strength of SI in the cultivars (*Zhang & Hiratsuka*, 1999).

The S_4- and S_5-RNases could be successfully PCR-amplified by apple primers (*Sassa* et al., 1996). The first PCR-based method for identifying *S*-genotypes of Japanese pear cultivars was performed by *Ishimizu* et al. (1999). It was based on primers designed from conserved regions of several apple and Japanese pear *S*-RNases and it was evaluated to be a rapid and reliable method. PCR amplification was followed by a subsequent digestion of the PCR fragments with *S*-allele-specific restriction endonucleases. Using this method, unknown *S*-genotypes of nine cultivars were determined and self-compatible genotypes were selected from the offsprings of 'Osa-Nijisseiki'.

Newer self-incompatibility RNases were described, the S_8 by *Carlos* et al. (2001) and the S_9 by *Sawamura* et al. (2002). This latter allele was further characterized by *Takasaki* et al. (2004) and a PCR-RFLP system was applied to distinguish S_1 to S_9; and another for the selection of self-compatible cultivars from the progeny of the cross of 'Osa-Nijisseiki' and self-incompatible cultivars (*Kim* et al., 2004). The same authors have isolated an additional *S*-allele, S_{10}, from the cultivar 'Chengsilri'.

Table 2 Currently available *S*-genotypes of Japanese pear cultivars arranged to eight incompatibility groups

Cultivars	S-Genotype*	Cultivars	S-Genotype*
Self-compatible		Group VII	
Akibae	$S_4^{sm}S_5$	Aikansui	S_4S_5
Osa-Nijisseiki	$S_2S_4^{sm}$	Asahi	S_4S_5
Group I		Hakko	S_4S_5
Doitsu	S_1S_2	Kisui	S_4S_5
Hayatama	S_1S_2	Kiyozumi	S_4S_5
Group II		Kogiku	S_4S_5
Suisei	S_1S_4	Kosui	S_4S_5
Yakumo	S_1S_4	Seiryu	S_4S_5
Group III		Shinsui	S_4S_5
Ichiharawase	S_1S_5	Taihaku	S_4S_5
Meigetsu	S_1S_5	Waseaka	S_4S_5
Group IV		Group VIII	
Gion	S_2S_4	Amanogawa	S_4S_9
Rokugatsu	S_2S_4	Nangetsu	S_4S_9
Wasechojuro	S_2S_4	Nansui	S_4S_9
Kikusui	S_2S_4	Shinsei**	S_4S_9
Nijisseiki	S_2S_4	Shinkou	S_4S_9
Group V		Unique genotypes	
Chikusui	S_3S_4	Chojuro	S_2S_3
Ohgon-nashi	S_3S_4	Hogetsu	S_1S_7
Seigyoku	S_3S_4	Imamuraaki	S_1S_6
Shinseiki	S_3S_4	Niitaka	S_3S_9
Group VI		Okusankichi	S_5S_7
Housui	S_3S_5	Shinsetsu	S_5S_6
Tanzawa	S_3S_5	Yasato	S_2S_5

*Data compiled from the following reports: *Carlos* et al. (2001); *Ishimizu* et al. (1999); *Hiratsuka* et al. (1998); *Nakanishi* et al. (1992); *Sassa* et al. (1992); *Sawamura* et al. (2002); *Takasaki* et al. (2004)

**Cv. 'Shinsei' in a diallele cross among the cultivars from the same incompatibility group showed 20-39.8% fruit set, but contained only 1.1 seed/fruit, which indicates the parthenocarpic ability of 'Shinsei'

Self-incompatibility of Chinese pear (*Pyrus ussuriensis*)

The only available data on the *S*-allele composition of Chinese pear cultivars were provided by *Tomimoto* et al. (1996). By 2D-PAGE, they have genotyped eight cultivars and found seven incompatibility alleles, described as S_1-S_7. Full *S*-genotypes were determined for five cultivars (involving one cross-incompatibility group) and partial *S*-genotype for three others (*Table 3*).

Table 3 *S*-genotypes of Chinese pear cultivars deduced from 2D-PAGE (After Tomimoto et al., 1996)

Cultivars	S-genotype
Lai yang ci li	S_3S_4
Ao ao li	S_3S_4
Xin qing li	S_1S_2
Hong xiao li	S_5S_7
Yuan ba li	S_2S_7
Ya li	S_1–
Ping zi li	S_6–
Zhu zhi li	S_7–

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