Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Identification of Self Incompatibility (*S*) Alleles in Turkish Apple Gene Sources using Allele-specific PCR

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ARTICLE INFO
Research Article
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Received: 11 May 2021 / Revised: 26 April 2022 / Accepted: 07 May 2022 / Online: 18 January 2023

Cite this article
KARATAŞ M D, HAZRATİ N, ÖZMEN Y C, HASANZADEH M, ALTINTAŞ S, AKÇAY E, ERGÜL A (2023). Identification of Self Incompatibility (*S*) Alleles in Turkish Apple Gene Sources using Allele-specific PCR. *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 29(1):287-297. DOI: 10.15832/ankutbd.930238

ABSTRACT
Self-incompatibility (SI) is a genetic mechanism in many flowering plants by which generative reproduction is prevented. The self-incompatibility caused by the genetic functions of the cell is controlled by genes called S genes or self-incompatibility genes. Self-incompatibility results in decreased pollination and ultimately yield loss. In apple (*Malus domestica* L.), self-incompatibility is controlled by multi-allelic S-locus. Approaches in the S-glycoprotein profiles and allele-specific PCR methods using the gene profiles and S-glycoprotein profiles for determination of the incompatibility levels are of great importance. In current study, the self-incompatibility status of 192 apple genotypes (such as, Amasya, Hüryemez, Şah elması, Tokat, Demir elması etc.) obtained from the National Collection of Atatürk Horticultural Central Research Institute, Yalova, Turkey, has been determined. For this purpose, genotype-specific allele status and compatibility levels were screened via PCR (Polymerase Chain Reaction) using 4 different S-alleles (Sd, Sf, S26 and S9). 181 genotypes containing at least 1 S-allele were identified as 'Partially Incompatible' and 12 genotypes involving 4 S-alleles were assigned 'Totally Incompatible'. No S-alleles were observed in 2 genotypes (Pancarlık and Hüryemez) which exhibited 'Compatibility' status.

Keywords: *Malus domestica* L., Self-incompatibility, Anatolian gen resourses, *S*-alleles

1. Introduction

Apple (*Malus domestica* Borkh), belonging to the family *Rosaceae*, originates from the temperate countries of the Western Asia, between Black Sea and Caspian Sea. Apple trees are medium-sized, defoliating trees (Nour et al. 2010; Shaheen et al. 2017) and have economic significance worldwide (Shulaev et al. 2008). In addition to the commercial varieties production in the world, identification of individual species superior to natural gene sources is important for the production and improvement of the new variety candidates. Turkey possesses very rich apple gene sources. A National Collection including about 200 apple genotypes has been established within the Atatürk Horticultural Central Research Institute (Yalova, Turkey). No studies have been conducted yet about the self-incompatibility status of the genotypes/varieties in this collection.

Self-incompatibility is one of the cellular functions that protect intraspecific genetic diversity by preventing/decreasing self-pollination in flowering plants (Silva & Goring 2001). In most plant species, self-incompatibility is typically regulated by a gene locus containing several alleles (*S*-locus), on which at least two genes, pistil S and pollen S, are located and pollen tube inhibition occurs when the specificity of the same "*S*-allele" is expressed by both pollen and pistil. Gametophytic Self-Incompatibility (GSI) is the most common type of self-incompatibility (Franklin-Tongand & Franklin 2003; Abdallah et al. 2019) detected in large numbers of flowering plant species (Ma et al. 2018) so that, whenever the *S*–haplotype of pollen is homogeneous with one of the pistil *S*-haplotypes, pollen tube fails to grow in the style. In the apple GSI, the *S-RNase* gene and an *F-box* gene called *SFBB* (*S*-locus F-box brothers) act as pistil and pollen factors, respectively (Broothaert et al. 1995; De Franceschi et al. 2012). At the same chromosomal locus, a series of *SLF* (*S*-locus F-box protein) genes are aligned with *S-RNase*, which have been presumably obtained by gene exchange and duplication. The product of the pistil is *S-RNase*, which is considered an extracellular and polymorphic ribonuclease encoded by *S* gene (de Nettancourt 2001), whereas, the pollen *S* gene encodes a protein including F-box motif, called S haplotype-specific F-box protein (SFB). Later investigations have shown that *Rosaceae* SI system consists

of two distinct mechanisms, for example, in *Prunus* from *Amygdaleae* tribe, the SFB recognizes self *S-RNase*, through a self-recognition manner whereas, *Pyrus* and *Malus* from tribe *Pyreae* exhibit a non-self-recognition system in which a subset of non-self *S-RNases* are recognized specifically by various SFBB proteins of the SI system. Additional biological and biochemical description of the *S*-locus genes, along with the other SI-related genes located elsewhere than *S* locus, could elucidate the evolution, origin and molecular mechanisms of *Rosaceae* SI system (Sassa 2016).

In pollination of distantly related species, the SI ratio is considered to be one of the most important determinants of the diversity in evolutionary development of flowering plants (Whitehouse 1951). Pollinators are required for commercial production of many self-incompatible species and use of inappropriate pollinators results in economic loss.

Many fruit species (including *Malus* and *Pyrus*) in the family *Rosaceae* exhibit typical GSI (Shulaev et al. 2008). In terms of molecular mechanism of self-incompatibility in apple, *S*-allele genes have been isolated and characterized (Broothaerts et al. 1995). In many studies, apple specific self-incompatibility alleles were identified and *S*-genotypes were determined by using allele-specific PCR applications which are fast and useful methods (Sakurai et al. 1997, 2000; Verdoodt et al. 1998; Matsumoto & Kitahara 2000; Broothaerts &Van Nerum 2003; Broothaerts et al. 2004).

There are also a number of researches on proteins of self-incompatibility alleles. S-glycoproteins have been studied in Japanese pear (*Pyrus pyrifolia*) and it has been revealed that *S-RNase* (S_1, S_3, S_5, S_6, and S_7) regions that break the pollen tube growth were similar in *Pyrus* and *Malus* (Sassa et al. 1994; Janssens et al. 1995; Ishimizu et al. 1998; Van Nerum et al. 2001). Recent studies have shown that *S-RNases* interact with a conserved protein (MdROP) in the pistil of apple (Meng et al. 2014). Also, it is specified that, in both types of SI, programmed cell death (PCD) which is known as an active and genetically mechanism for the controlled elimination of targeted cells, plays a key role in the rejection of self-incompatible pollen (Serrano et al. 2015). In this regard, nitric oxide (NO) and reactive oxygen species (ROS), have been found as important regulators that are required for PCD in plants (Sadhu et al. 2019). In this sense, the H_2O_2 to NO ratio, determines the activation time of cell death (Delledonne et al. 2001) and ROS, which is produced from the degradation of O_3 in the apoplast is involved in both the initiation and progression of cell death (Overmyer et al. 2003; Sharma et al. 2012; Serrano et al. 2015).

In order to investigate the possible involvement of polyamines (PAs) and transglutaminase (TGase) in the reproduction of *Pyrus communis* L. plants, Mandrone et al. (2019) studied the content of free, soluble-conjugated and insoluble-bound PAs as well as the activity, abundance and immunolocalization of TGase. Results clearly indicate that during the SI response, TGase activity is increased, resulting in the accumulation of PAs conjugated to hydroxycinnamic acids and other small molecules. Li et al. (2018) also reported that treating with self *S-RNases*, leads to a marked growth inhibition in apple pollen tubes, as well as a decrease in endogenous soluble pyrophosphatase activity (MdPPa) and elevated levels of inorganic pyrophosphate (PPi). *S-RNase* binding to two variable regions of MdPPa leads to silencing of MdPPa expression and results in a reduction in pollen tube growth.

In the current study, compatible/incompatible allele profiles of 192 apple genotypes obtained from the National Collection of Atatürk Horticultural Central Research Institute, Yalova, Turkey, have been determined using 4 different *S*-alleles. Due to the successful amplification results and widely usage in *S*-allele screenings of the different geographic apple populations; S_d (Matsumoto & Kitahara 2000; Sakurai et al. 2000), S_f (Matsumoto & Kitahara 2000; Sakurai et al. 2000), S_{26} (Janssens et al. 1995; Halász et al. 2011; Brancher et al. 2020) and S_9 (Janssens et al. 1995; Halász et al. 2011; Brancher et al. 2020) *S*-allels were employed in this study to screen Turkish apple gene sources. The genotypes were classified according to compatibility levels and the possible correlations between compatibility and genetic similarities of the genotypes were revealed.

2. Material and Methods

2.1. Plant material

In this study, 192 apple genotypes obtained from Atatürk Horticultural Central Research Institute, Yalova, Turkey, were used as plant material. Genomic DNA was extracted from apple leaves using Lefort et al. (1998) method. DNA quantification was performed with Nanodrop ND-100 spectrometer and the DNA was visualized on 1% agarose gel.

2.2. PCR reactions

Allele-specific primers (S_9, S_{26}, S_f, S_d) were used to identify single alleles. Primers and their nucleotide sequences were used as described by Sakurai et al. (2000) for S_{26}, S_f and S_d alleles and Janssens et al. (1995) for S_9 allele (Table 1). The optimized PCR reactions for the mentioned primers were performed using 5 pmol primers, 25 mM $MgCl_2$, 100 µM dNTP mix, 5X PCR Buffer, 5U Taq polymerase and 50-250 ng genomic DNA in a total volume of 15 µL. Negative control was used to monitor contamination in each PCR reaction. TouchDown PCR program was applied in BioRad T100TM brand thermocycler as: 3-min pre-denaturation at 94 °C followed by 1-min denaturation at 94 °C, 1-min 45-sec annealing at annealing temperature of each primer pairs and 2-min extension at 72 °C (10-min final extension at 72 °C).

2.3. Evaluation of S-Alleles by band profiles

Amplified PCR products were run using 2% agarose gel electrophoresis with 100 bpDNA Marker (Solis Byodyne) at 100V for 1 hour and then visualized using agarose gel imaging system (Gene Genius Bio Imaging System).

Table 1- Oligonucleotide sequences for allele-specific PCR

Primer name	*Primer sequences*	*Expected length (bp)*
MalusS_{26}*	GAAGATGCCATACGCAATGG ATGAATTCTTAATACCGAATATTGGCC	193bp
MalusS_9**	CAGCCGGCTGTCTGCCACTT CGGTTCGATCGAGTACGTTG	343bp
MalusS_d*	ATCGAACTGATCATGTAGGC TATCGTGAACCTTGTGGTGG	355bp
MalusS_f*	CAATCGAAACGATCATGAAG TCCGTGTATAGGCCATCGAC	493bp

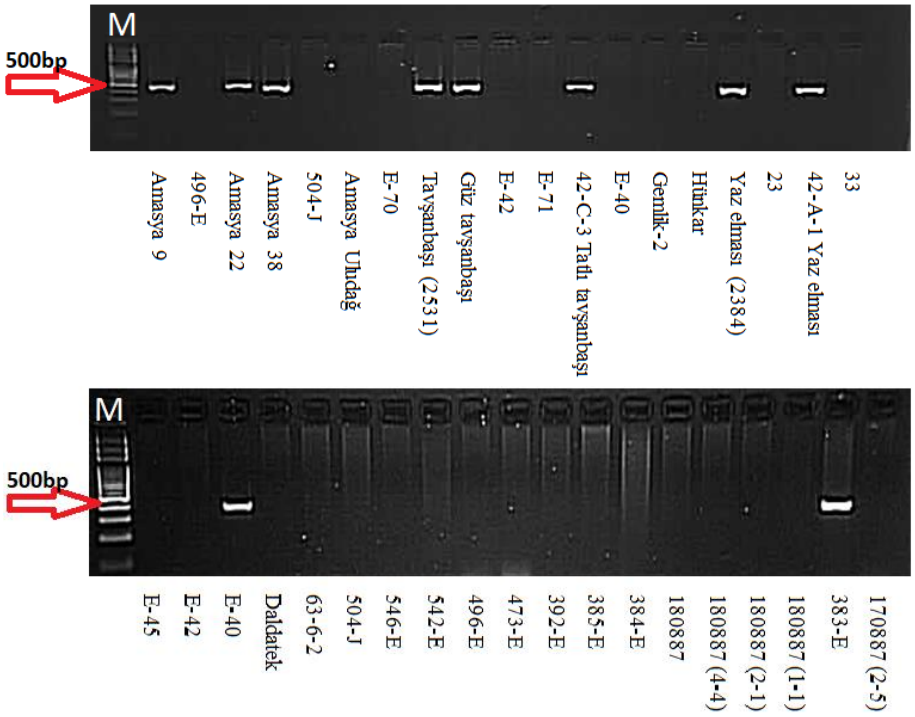
*: Sakurai et al. (2000), **: Janssens et al. (1995)

After agarose gel imaging, genotypes containing *Malus* S_{26} allele with193 bp, genotypes containing *Malus* S_9 allele with 343 bp, genotypes containing *Malus* S_d allele with 355 bp, and genotypes containing *Malus* S_f allele with 493 bp were found to be self-incompatible with the corresponding *S*-alleles. The results were evaluated based on the allele sharing criteria described by Broothaerts et al. (1996) and Ishimizu et al. (1999). Genotypes containing at least 1, 2, and 3 out of 4 *S*-alleles studied were partially incompatible, the genotypes in which these regions could not be amplified were compatible, and those in which all regions could be amplified by PCR were totally incompatible.

3. Results

3.1. Identification of S-Alleles

Four different *S*-alleles (S_9, S_{26}, S_f, S_d) were successfully amplified by PCR in 192 apple genotypes and after agarose gel electrophoresis, visualized using agarose gel imaging system (Figure 1). 181 out of 192 genotypes containing at least 1 *S*-allele were 'Partially Incompatible' and of the remaining, 12 genotypes which contained 4 *S*-alleles were 'Totally Incompatible'. No *S*-alleles were observed in 2 genotypes (Pancarlık and Hüryemez) which exhibited 'Total Compatibility' contrary to the rest of the genotypes (Table 2).



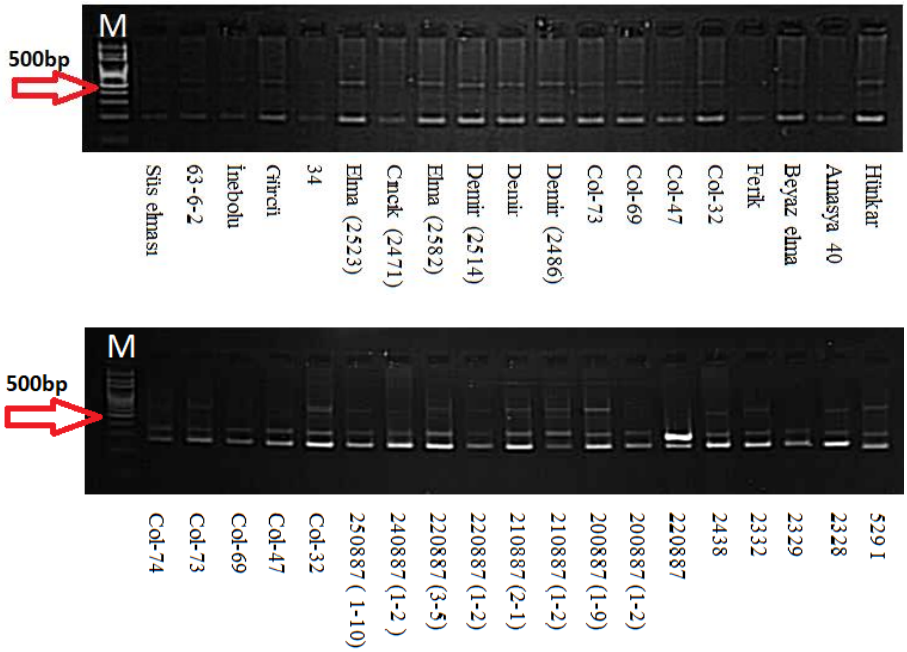


Figure 1- *S*-allele-specific PCR analysis (S_f, S_d, S_{26} and S_9, respectively, M: 100 bp DNA Marker)

Table 2- *S*-allele compositions of apple genotypes

NO	*Genotype*	*S-allele*	*Compatibility status*	*Region*
1	Amasya 9	S_fS_{26}	Semi compatible	Central B.S
2	Amasya 21	S_fS_{26}	Semi compatible	Central B.S
3	Amasya 22	S_fS_{26}	Semi compatible	Central B.S
4	Amasya 38	$S_9S_fS_{26}$	Semi compatible	Central B.S
5	Amasya 50	S_fS_{26}	Semi compatible	Central B.S
6	Amasya Uludağ	$S_{26}S_{26}$	Semi compatible	Central B.S
7	Şah elması	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
8	Tavşanbaşı (2531)	$S_9S_fS_{26}S_d$	Incompatible	Unknown
9	Güz tavşanbaşı	$S_9S_fS_{26}$	Semi compatible	Unknown
10	Yaz tavşanbaşı	$S_9S_fS_{26}$	Semi compatible	Unknown
11	42-KP-1 Mayhoş tavşanbaşı	$S_9S_fS_{26}$	Semi compatible	Unknown
12	42-C-3 Tatlı tavşanbaşı	S_fS_{26}	Semi compatible	Unknown
13	Tokat-1	S_fS_{26}	Semi compatible	Unknown
14	Tokat-2	$S_9S_fS_{26}$	Semi compatible	Unknown
15	Tokat-4	$S_9S_fS_{26}$	Semi compatible	Unknown
16	Yaz elması (2384)	S_fS_{26}	Semi compatible	Unknown
17	Yaz elması (2563)	S_fS_{26}	Semi compatible	Unknown
18	42-A-1 Yaz elması	$S_{26}S_{26}$	Semi compatible	Unknown
19	Kaba elma (42-E-6)	$S_9S_fS_{26}$	Semi compatible	Central A.
20	130887	$S_9S_fS_{26}$	Semi compatible	Aegean
21	130887 (2-3)	$S_9S_fS_{26}$	Semi compatible	Aegean
22	130887 (3-4)	$S_9S_fS_{26}S_d$	Incompatible	Aegean
23	170887 (2-5)	$S_9S_fS_{26}$	Semi compatible	Aegean
24	180887	$S_9S_fS_{26}$	Semi compatible	Aegean
25	180887 (1-1)	$S_9S_fS_{26}$	Semi compatible	Aegean
26	180887 (2-1)	$S_9S_fS_{26}$	Semi compatible	Aegean
27	180887 (4-4)	$S_9S_fS_{26}$	Semi compatible	Aegean
28	180887 (5-4)	$S_9S_fS_{26}S_d$	Incompatible	Aegean
29	372-E	S_fS_{26}	Semi compatible	Unknown
30	383-E	$S_9S_fS_{26}S_d$	Incompatible	Unknown
31	384-E	$S_9S_fS_{26}$	Semi compatible	Unknown
32	385-E	$S_9S_fS_{26}$	Semi compatible	Unknown
33	392-E	$S_9S_fS_{26}$	Semi compatible	Unknown
34	473-E	$S_9S_fS_{26}$	Semi compatible	Unknown
35	496-E	S_9S_{26}	Semi compatible	Unknown
36	542-E	$S_9S_fS_{26}$	Semi compatible	Unknown
37	546-E	$S_9S_fS_{26}$	Semi compatible	Unknown
38	504-J	$S_{26}S_{26}$	Semi compatible	Unknown
39	63-6-2	S_9S_f	Semi compatible	Marmara
40	Daldatek	$S_{26}S_{26}$	Semi compatible	Unknown

Table 2 (Continue)- *S*-allele compositions of apple genotypes

NO	*Genotype*	*S-allele*	*Compatibility status*	*Region*
41	E-70	$S_{26}S_{26}$	Semi compatible	Unknown
42	E-42	$S_{26}S_{26}$	Semi compatible	Unknown
43	E-71	$S_{26}S_{d}$	Semi compatible	Unknown
44	E-40	$S_{9}S_{26}S_{d}$	Semi compatible	Unknown
45	E-45	$S_{9}S_{26}$	Semi compatible	Unknown
46	55	$S_{9}S_{26}$	Semi compatible	Marmara
47	52	$S_{9}S_{26}$	Semi compatible	Marmara
48	51	$S_{9}S_{26}$	Semi compatible	Marmara
49	60	$S_{9}S_{26}$	Semi compatible	Marmara
50	12	$S_{9}S_{26}$	Semi compatible	Marmara
51	49	$S_{9}S_{26}$	Semi compatible	Marmara
52	61	$S_{9}S_{26}$	Semi compatible	Marmara
53	82	$S_{9}S_{26}$	Semi compatible	Marmara
54	57	$S_{9}S_{26}$	Semi compatible	Marmara
55	62-1	$S_{26}S_{26}$	Semi compatible	Marmara
56	78	$S_{9}S_{26}$	Semi compatible	Marmara
57	66	$S_{9}S_{26}$	Semi compatible	Marmara
58	47	$S_{9}S_{26}$	Semi compatible	Marmara
59	62-2	$S_{9}S_{26}$	Semi compatible	Marmara
60	56	$S_{9}S_{26}$	Semi compatible	Marmara
61	37	$S_{9}S_{26}S_{d}$	Semi compatible	Marmara
62	63	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
63	41	$S_{9}S_{26}$	Semi compatible	Marmara
64	81	$S_{9}S_{26}S_{d}$	Semi compatible	Marmara
65	9	$S_{9}S_{26}$	Semi compatible	Marmara
66	76	$S_{9}S_{26}$	Semi compatible	Marmara
67	48	$S_{9}S_{26}$	Semi compatible	Marmara
68	67	$S_{9}S_{26}$	Semi compatible	Marmara
69	73	$S_{26}S_{26}$	Semi compatible	Marmara
70	17	$S_{9}S_{26}$	Semi compatible	Marmara
71	72	$S_{9}S_{26}S_{d}$	Semi compatible	Marmara
72	65	$S_{9}S_{26}$	Semi compatible	Marmara
73	29	$S_{9}S_{26}S_{d}$	Semi compatible	Marmara
74	21	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
75	24	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
76	14	$S_{9}S_{f}S_{26}S_{d}$	Incompatible	Marmara
77	20	$S_{9}S_{26}$	Semi compatible	Marmara
78	13	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
79	Candır	$S_{9}S_{f}S_{26}S_{d}$	Incompatible	Unknown
80	19	$S_{9}S_{26}$	Semi compatible	Marmara
81	25	$S_{9}S_{26}$	Semi compatible	Marmara
82	11	$S_{9}S_{26}$	Semi compatible	Marmara
83	32	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
84	458 S (Ciğit)	$S_{9}S_{f}S_{26}$	Semi compatible	Unknown
85	23	$S_{9}S_{26}$	Semi compatible	Marmara
86	33	$S_{9}S_{26}$	Semi compatible	Marmara
87	15	$S_{9}S_{26}S_{d}$	Semi compatible	Marmara
88	7	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
89	2	$S_{9}S_{26}S_{d}$	Semi compatible	Marmara
90	1	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
91	6	$S_{9}S_{26}S_{d}$	Semi compatible	Marmara
92	34	$S_{9}S_{9}$	Semi compatible	Marmara
93	4	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
94	18	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
95	31	$S_{9}S_{26}$	Semi compatible	Marmara
96	Gemlik-2	$S_{9}S_{26}$	Semi compatible	Marmara
97	Gemlik-3	$S_{9}S_{f}S_{26}$	Semi compatible	Marmara
98	Almıla (42-BS-9)	$S_{9}S_{26}$	Semi compatible	Central A.
99	Hanım teni (42-E-3)	$S_{9}S_{26}$	Semi compatible	Central A.
100	Karapınar elması (42KP-3)	$S_{9}S_{26}$	Semi compatible	Central A.
101	Hünkar	$S_{9}S_{26}$	Semi compatible	Unknown
102	Amasya 40	$S_{9}S_{26}$	Semi compatible	Central B.S
103	Beyaz elma	$S_{9}S_{26}$	Semi compatible	Eastern B.S
104	Ferik	$S_{9}S_{26}$	Semi compatible	Marmara
105	Bey elması (2477)	$S_{9}S_{26}$	Semi compatible	Eastern B.S
106	Gelin elması (2475)	$S_{9}S_{26}S_{d}$	Semi compatible	Eastern B.S

Table 2 (Continue)- *S*-allele compositions of apple genotypes

NO	*Genotype*	*S-allele*	*Compatibility status*	*Region*
107	Göbek (2475)	$S_{26}S_{26}$	Semi compatible	Eastern B.S
108	Altınok elması (2490)	S_9S_{26}	Semi compatible	Aegean
109	Demir (2486)	S_9S_{26}	Semi compatible	Unknown
110	Demir	S_9S_{26}	Semi compatible	Unknown
111	Demir (2514)	S_9S_{26}	Semi compatible	Unknown
112	Elma (2582)	S_9S_{26}	Semi compatible	Eastern B.S
113	Cıncık (2471)	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
114	Elma (2523)	S_9S_{26}	Semi compatible	Eastern B.S
115	Haşhaş elması (2596)	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
116	Gürcü	$S_{26}S_{26}$	Semi compatible	Eastern B.S
117	5	S_9S_9	Semi compatible	Marmara
118	38	S_9S_9	Semi compatible	Marmara
119	529 I	S_9S_{26}	Semi compatible	Unknown
120	2328	S_9S_{26}	Semi compatible	Unknown
121	2329	S_9S_{26}	Semi compatible	Unknown
122	2331	$S_{26}S_{26}$	Semi compatible	Unknown
123	2332	$S_9S_fS_{26}$	Semi compatible	Unknown
124	2438	S_9S_{26}	Semi compatible	Unknown
125	220887	S_9S_{26}	Semi compatible	Aegean
126	200887 (1-2)	$S_9S_fS_{26}$	Semi compatible	Aegean
127	200887 (1-9)	S_9S_{26}	Semi compatible	Aegean
128	210887 (1-2)	S_9S_{26}	Semi compatible	Aegean
129	210887 (2-1)	$S_9S_fS_{26}$	Semi compatible	Aegean
130	220887 (1-2)	S_9S_{26}	Semi compatible	Aegean
131	220887 (3-5)	S_9S_{26}	Semi compatible	Aegean
132	240887 (1-2)	$S_9S_fS_{26}$	Semi compatible	Aegean
133	250887 (1-10)	$S_9S_fS_{26}$	Semi compatible	Aegean
134	42-E-2 (Ankara güzeli)	$S_9S_fS_{26}$	Semi compatible	Central A.
135	Arpa elması (2482)	$S_9S_fS_{26}$	Semi compatible	Central B.S
136	Col-32	$S_9S_fS_{26}$	Semi compatible	Unknown
137	Col-47	$S_9S_fS_{26}$	Semi compatible	Unknown
138	Col-69	$S_9S_fS_{26}$	Semi compatible	Unknown
139	Col-73	$S_9S_fS_{26}$	Semi compatible	Unknown
140	Col-74	S_9S_{26}	Semi compatible	Unknown
141	Cidagut	$S_9S_fS_{26}S_d$	Incompatible	Unknown
142	El-23035 (Amasya)	$S_{26}S_{26}$	Semi compatible	Central B.S
143	Hüryemez	-	Compatible	Eastern B.S
144	J/5/4/59 Bel.	$S_{26}S_{26}$	Semi compatible	Unknown
145	Kadir-Hatice	$S_fS_{26}S_d$	Semi compatible	Unknown
146	Kalkandelen	S_fS_f	Semi compatible	Unknown
147	Karpuz	S_9S_f	Semi compatible	Unknown
148	Kavun (425 E)	S_fS_f	Semi compatible	Eastern B.S
149	Kış elması (2590)	$S_9S_fS_{26}S_d$	Incompatible	Eastern B.S
150	Laz elması (2570)	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
151	Mahsusa elması	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
152	Mektep elması (2565)	S_9S_f	Semi compatible	Eastern B.S
153	Niğde İngiliz	$S_9S_fS_{26}$	Semi compatible	Unknown
154	Oltu elması (2594)	S_9S_{26}	Semi compatible	Eastern B.S
155	Paşa elması	S_fS_{26}	Semi compatible	Eastern B.S
156	Petek (2577)	$S_{26}S_d$	Semi compatible	Eastern B.S
157	Petevrek elması (2566)	$S_fS_{26}S_d$	Semi compatible	Eastern B.S
158	Piraziz	S_fS_{26}	Semi compatible	Eastern B.S
159	Portakal	S_fS_{26}	Semi compatible	Unknown
160	Reçel elması (2506)	S_fS_{26}	Semi compatible	Eastern B.S
161	Rize demir	S_fS_{26}	Semi compatible	Eastern B.S
162	Sandık	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
163	Sarı elma	S_fS_{26}	Semi compatible	Unknown
164	Sinop	$S_9S_fS_{26}$	Semi compatible	Central B.S
165	Susuz elma	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
166	Şeker	S_fS_{26}	Semi compatible	Unknown
167	Tatlı elma (2492)	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
168	Tatlı elma (2511)	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
169	Uzun yorma	$S_9S_fS_{26}$	Semi compatible	Unknown
170	Yenişehir	$S_9S_fS_{26}$	Semi compatible	Unknown
171	42-E-7 Yıldızkıran	$S_9S_fS_{26}S_d$	Incompatible	Central A.
172	42-E-4 Mayhoş yıldızkıran	$S_9S_fS_{26}S_d$	Incompatible	Central A.

Table 2 (Continue)- *S*-allele compositions of apple genotypes

NO	*Genotype*	*S-allele*	*Compatibility status*	*Region*
173	Adsız	$S_9S_fS_{26}$	Semi compatible	Unknown
174	Orak	$S_9S_fS_{26}$	Semi compatible	Unknown
175	Yenice	$S_{26}S_{26}$	Semi compatible	Unknown
176	Süs elması	S_9S_f	Semi compatible	Eastern B.S
177	Amasya 37	$S_9S_fS_{26}$	Semi compatible	Central B.S
178	10	$S_9S_fS_{26}$	Semi compatible	Aegean
179	Söğüt elma	$S_9S_fS_{26}$	Semi compatible	Eastern B.S
180	Samsun	$S_9S_fS_{26}$	Semi compatible	Central B.S
181	528 J	$S_9S_fS_{26}$	Semi compatible	Unknown
182	YB-2	$S_9S_fS_{26}S_d$	Incompatible	Unknown
183	Yaz elması (2482)	$S_{26}S_{26}$	Semi compatible	Eastern B.S
184	Gelendost	S_9S_{26}	Semi compatible	Unknown
185	Pozmer 20	$S_9S_fS_{26}S_d$	Incompatible	Unknown
186	32-E-1	$S_9S_fS_{26}$	Semi compatible	Unknown
187	42-C-5	$S_{26}S_{26}$	Semi compatible	Unknown
188	Daldabir	S_fS_{26}	Semi compatible	Unknown
189	Pancarlık	-	Compatible	Unknown
190	359 (11)	$S_{26}S_d$	Semi compatible	Unknown
191	Cidagut	$S_9S_fS_{26}$	Semi compatible	Unknown
192	İnebolu	S_dS_d	Semi compatible	Unknown

-: Genotype does not carry any of the *S*-alleles. Central B.S: Central Black Sea; Eastern B.S: Eastern Black sea; Central A.: Central Anatolian.

S_{26} allele was the most frequent *S*-allele (49%) followed by S_9 allele (31%). S_d was found to be the least frequent *S*-allele (5%). In terms of *S*-allele combinations, the most common 2-allele combination was detected as "S_9-S_{26}" *S*-genotype (29%) out of total *S*-genotypes. The most common 3-allele combinations, was observed in "S_9-S_f-S_{26}" alleles (39%). 4-allele combination was only reported in 12 genotypes (6%).

In studied apple genotypes, there were 2 genotypes (Pancarlık and Hüryemez) (1%) containing none of the 4 alleles (S_9, S_{26}, S_f, S_d), 22 genotypes containing 1 type of *S*-alleles (11%), 81 genotypes containing 2 types of *S*-alleles (42%) and 75 genotypes containing 3 types of *S*-alleles examined (39%) and 12 genotypes (6%) contained a total of 4 *S*-alleles.

3.2. Relationship with Type-Clonal level similarity

Homonymous 8 Amasya apple genotypes (Amasya 9, Amasya 21, Amasya 22, Amasya 37, Amasya 38, Amasya 40, Amasya 50, Amasya Uludağ) contained mostly S_f and S_{26} *S*-alleles in 4 loci, homonymous 3 Demir apple genotypes (Demir, Demir (2486) and Demir (2514)) contained mostly S_9 and S_{26} alleles in 4 loci, homonymous 3 Tokat apple genotypes (Tokat-1, Tokat-2 and Tokat-4) contained mostly S_f and S_{26} alleles, homonymous 5 Tavşanbaşı apple genotypes (Tavşanbaşı (2531), Güz Tavşanbaşı, Yaz Tavşanbaşı, 42-KP-1 Mayhoş Tavşanbaşı and 42-C-3 Tatlı Tavşanbaşı) contained mostly S_9, S_f and S_{26} alleles, homonymous 4 Yaz apple genotypes (Yaz (2384), Yaz (2563), Yaz (2482) and 42-A-1 Yaz) contained mostly S_{26} allele and all of these genotypes were found to be 'Partially Incompatible' (Tavşanbaşı (2531)-Totally Incompatible).

3.3. Relationship with SSR based similarity

SSR based similarity rates of 58 binary comparisons have been formerly calculated (Burak et al. 2014). From which, 12 (20.6%) cases were totally similar (S_9S_{26}), 34 (20%) were similar in terms of 2 *S*-alleles (S_9S_{26} and S_fS_{26}), 1 (1.7%) was similar in terms of 3 *S*-alleles ($S_9S_fS_{26}$) and 22 (37.9%) were similar in terms of single *S*-allele (S_{26}) (Table 3).

Table 3- Genetic similarity rates based on SSR analysis

No	*Genotypes with similar genetic origin*	*Similarity rates %*
1	1 – 52	90.6
2	42-E-6 (Kaba elma) – 180887	
3	42-E-6 (Kaba elma) - 180887 (5-1)	
4	392-E – 496-E	
5	458 S – 76	
6	65 – 61	
7	65 – 62-1	
8	65 – 62-2	
9	65 – 11	
10	65 – Amasya 38	
11	65 – Amasya 40	
12	65 – 2329	
13	Amasya 50 – Amasya Uludağ	
14	Demir (2486) – Demir	
15	4 – 31	93.8
16	1 – 130887 (2-3)	
17	25 – Demir (2486)	
18	37 – 76	
19	55 – Göbek (2475)	
20	Amasya 50 – 180887 (5-4)	
21	Amasya 50 – Amasya 9	
22	Amasya 50 – Amasya 21	
23	Amasya 50 – Amasya 22	
24	Amasya 50 – Amasya 37	
25	Daldatek – 180887	96.9
26	180887 (5-4) – 62-1	
27	180887 (5-4) – 62-2	
28	180887 (5-4) – 11	
29	180887 (5-4) – Amasya 38	
30	180887 (5-4) – Amasya 40	
31	180887 (5-4) – 2329	
32	Amasya 9 – 62-1	
33	Amasya 9 – 62-2	
34	Amasya 9 – 11	
35	Amasya 9 – Amasya 38	
36	Amasya 9 – Amasya 40	
37	Amasya 9 – 2329	
38	Amasya 21 – 62-1	
39	Amasya 21 – 62-2	
40	Amasya 21 – 11	
41	Amasya 21 – Amasya 38	
42	Amasya 21 – Amasya 40	
43	Amasya 21 – 2329	
44	Amasya 22 – 62-1	
45	Amasya 22 – 62-2	
46	Amasya 22 – 11	
47	Amasya 22 – Amasya 22	
48	Amasya 22 – Amasya 38	
49	Amasya 37 – 62-1	
50	Amasya 37 – 62-2	
51	Amasya 37 – 11	
52	Amasya 37 – Amasya 38	
53	Amasya 37 – Amasya 40	
54	Amasya 37 – 2329	
55	Amasya 50 – Amasya 38	
56	Amasya 50 – Amasya 40	

3.4. Relationship with triploidy

Based on the genetic analysis of the same apple population, Burak et al. (2014) reported that 12 genotypes were potentially triploid. Of the triploid genotypes, except 1 genotype (Cidagut), remaining 11 genotypes contained at least 2 different incompatibility alleles and were identified as 'Partially Incompatible'. High diversity of incompatibility alleles can be attributed to triploid genotypes (Table 4).

Table 4- Incompatibility states of triploid genotypes identified through SSR analysis

Triploid genotypes identified through SSR analysis	*Incompatibility alleles*	*Incompatibility status*
25	S_9S_{26}	Semi compatible
37	$S_9S_{26}S_d$	Semi compatible
76	S_9S_{26}	Semi compatible
52	S_9S_{26}	Semi compatible
Beyaz Elma	S_9S_{26}	Semi compatible
1	$S_9S_fS_{26}$	Semi compatible
Mektep Elması (2565)	S_9S_f	Semi compatible
Susuz Elma	$S_9S_fS_{26}$	Semi compatible
20	S_9S_{26}	Semi compatible
240887(1-2)	$S_9S_fS_{26}$	Semi compatible
Col-32	$S_9S_fS_{26}$	Semi compatible
Cidagut	$S_9S_fS_{26}S_d$	Incompatible

4. Discussion

The apple reproductive mechanism is regulated genetically by the *S*-locus through the *S-RNase* based gametophytic self-incompatibility system (De Franceschi 2018). Accurate recognition of the *S*-genotypes through *S*-genotyping by *S-RNase* alleles is important for the economic and permanent apple production because in order to apple fertilization, at least two genotypes without or only with one common *S*-haplotype are required. Moreover, *S*-alleles are used for supporting new genotypes, and also help recognizing the parental ones (Kasajima et al. 2017; Matsumoto et al. 2018).

There are two different labelling of *S*-alleles: European labelling uses figures like S_1 and S_2, and Japanese labelling uses characters like S_a and S_b. S_a, S_b, S_c, S_d, S_f, S_g, S_i, S_h, and S_z are identical with S_2, S_3, S_9, S_7, S_1, S_{20}, S_{24}, S_{10}, and S_{25}, respectively, but the identity of S_e is not clear (Hegedűs 2006).

S-genotypes of 192 diploid, triploid or tetraploid Turkish genotypes were discriminated using four different *S*-alleles. In current study, most of the analysed apple genotypes (53%) having two *S*-alleles were diploid and 40% of which including three or four *S*-alleles were determined as triploid and tetraploid genotypes. The frequency of occurrence of the examined *S*-alleles displayed a wide variation in the apple germplasm. Two *S*-alleles (S_{26} and S_9) were very common among the evaluated genotypes, presumably as a result of the prevalent use of the same breeding parents, and two (S_d and S_f) alleles were very rare so that, there was almost a 10-fold difference in frequency between the most prevalent (S_{26}) and rare (S_d) alleles. In this regard, it could be said that rare alleles (S_d and S_f) may belong to non-commercial or old species.

Recently, in a study conducted by Broothaerts et al. (2004) over *S*-genotyping of 150 European, American and Japanese apple cultivars, S_3 allele was reported as the most prevalent allele, followed by S_2 and S_9 while the S_{26} allele was found as rare allele. The authors concluded that the high frequency of the S_3 allele is due to the large-scale utilization of 'Golden Delicious' (S_2S_3) and its descendants in many present and past breeding programs (Broothaerts et al. 2004). Hegedűs (2006) also reported that the S_2, S_3, S_5, S_7, S_9, and S_{10} alleles were the most common among the commercial apple cultivars, and the high abundance of these alleles is attributed mainly to the prevalent application of the 'Golden Delicious', 'Delicious', 'Jonathan', 'McIntosh', and 'Cox's Orange Pippin' genotypes in apple breeding programs worldwide. Whereas, Matsumoto et al. (2007) found S_1, S_7 and S_9 to be the most common alleles, Dreesen et al. (2010) identified S_2, S_3, S_5, and S_9 as the most frequent *S*-alleles among European apple cultivars. Larsen et al. (2016) reported a high frequency of S_3 allele (28%) among 432 *Malus* genotypes. Brancher et al. (2020) also found the most frequent allele to be S_3, followed by S_5, since the most genotypes studied were indirect or direct derivatives of the 'Golden Delicious' (S_2S_3), 'Gala' (S_2S_5), and 'Imperatriz' (S_3S_5) cultivars. In our study, the high frequency of S_9 allele may be derived from the 'Delicious', 'Cox's Orange' and/or 'Fuji' cultivars all having S_9 which are also ancestors to many American, European and Japanese cultivars.

As represented in Table 2, eight diploid incompatibility groups were identified while, theoretically it is possible to form 10 incompatibility groups out of 4 alleles. Besides, 190 out of 192 studied genotypes (98%) were fully genotyped with mentioned 4 *S*-alleles. Although a large number of *S*-alleles are available in apple, artificial selection or repeated use of the same genotypes as parents appears to significantly restrict the number of compatibility groups associated with commercial clones. Overall, it could be noted that the actual number of cross-incompatible groups is not already large enough therefore, many incompatibility problems are expected in natural environments.

In the current study, it is required to new markers for identifying the two genotypes with unknown *S*-genotypes (Hüryemez and Pancarlık). The *S*-allele used for this purpose should be selected from the alleles that have a lower frequency among the apple genotypes.

According to studies by Halász et al. (2011) and De Franceschi et al. (2016), alleles S_2, S_3 and S_5 were found to be associated with apple scab (*Venturia inaequalis*) resistance. Since, the S_{26} allele has been identified as a rare one among the European apple cultivars in numerous studies, this allele is not yet fully characterized in literature. A comprehensive study should be performed to identify possible association of the S_{26} allele with important traits in Turkish apple genotypes. For example, this allele may be related to the taste of the fruit, as in the case of "Jonica" cultivar (Mir et al. 2016), or it may be related to woolly apple aphid resistance or other important traits.

5. Conclusions

The assignment of *S*-genotypes of apple cultivars needs important consideration in selecting proper pollen donors in breeding practices and orchard management (Li et al. 2011). For most genotypes, evidences for the correct *S*-allele discrimination seems to be strong and is often supported by studies at the DNA and protein levels, as well as by functional assessments through pollinations. Furthermore, the selection of suitable pollinizers may now more accurately include the correct compatibility relationships in addition to other factors that need to be considered, such as the overlapping of the blooming periods.

The present study has provided the first comprehensive discrimination of *S*-allele genotyping of common Turkish apple genotypes and presented optimized methodologies for genetic studies which will be very helpful for future researches on apple incompatibility. Additional data was acquired for identification of Turkish apple gene sources and incompatible genotypes were determined. It is suggested that obtained data can be utilized in cultivation, breeding and pollinator selection activities regarding the studied apple genotypes.

References

Abdallah D, Baraket G, Perez V, Mustapha S B, Salhi-Hannachi A & Hormaza J I (2019). Analysis of self-incompatibility and genetic diversity in diploid and hexaploid plum genotypes. *Frontiers in Plant Science* 10: 896. https://doi.org/10.3389/fpls.2019.00896

Brancher T L, Hawerroth M C, Kvitschal M V, Manenti D C & Guidolin A F (2020). Self-incompatibility alleles in important genotypes for apple breeding in Brazil. *Crop Breeding and Applied Biotechnology* 20(4): e28652041. https://doi.org/10.1590/1984-70332020v20n4a54

Broothaerts W & Van Nerum I (2003). Apple self-incompatibility genotypes: an overview. In XXVI International Horticultural Congress: *Genetics and Breeding of Tree Fruits and Nuts* pp. 379-387, Canada.

Broothaerts W, Janssens G A, Proost P & Broekaert W F (1995). Cdna cloning and molecular analysis of two self-incompatibility alleles from apple. *Plant Molecular Biology* 27(3): 499-511. https://doi.org/10.1007/bf00019317

Broothaerts W, Neram I V & Keulemans J (2004). Update on and Review of the Incompatibility (*S*-) Genotypes of Apple Cultivars. *HortScience:* a publication of the *American Society for Horticultural Science* 39(5): 943-947. https://doi.org/10.21273/hortsci.39.5.943

Broothaerts, W, Verdoodt, L, Keulemans, J, Janssens, G A & Broekaert, W F (1996). The self-incompatibility gene in apple and determination of the *S*-genotype of apple cultivars by PCR. *Acta Horticulturae* 423: 103-109. https://doi.org/10.17660/ActaHortic.1996.423.13

Burak M, Ergül A, Kazan K, Akçay M E, Yüksel C, Bakir M & Ayanoğlu H (2014). Genetic analysis of Anatolian apples (Malus sp.) by simple sequence repeats. *Journal of Systematics and Evolution* 52(5): 580-588. https://doi.org/10.1111/jse.12099

De Franceschi P, Bianco L, Cestaro A, Dondini L & Velasco R (2018). Characterization of 25 full-length *S-RNase* alleles, including flanking regions, from a pool of resequenced apple cultivars. *Plant Molecular Biology* 97: 279-296. https://doi.org/10.1007/s11103-018-0741-x

De Franceschi P, Cova V, Tartarini S & Dondini L (2016). Characterization of a new apple *S-RNase* allele and its linkage with the *Rvi5* gene for scab resistance. *Molecular Breeding* 36: 1-11. https://doi.org/10.1007/s11032-015-0427-x

De Franceschi P, Dondini L & Sanzol J (2012) Molecular bases and evolutionary dynamics of self-incompatibility in the *Pyrinae* (*Rosaceae*). *Journal of Experimental Botany* 63(11): 4015-4032. https://doi.org/10.1093/jxb/ers108

Delledonne M, Zeier J, Marocco A & Lamb C (2001). Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proceedings of the National Academy of Sciences* 98: 13454-13459. De Nettancourt D (2001). Incompatibility and Incongruity in Wild and Cultivated Plants. Berlin/Heidelberg/New York: *Springer-Verlag*. 322 pp. 2nd ed.

Dreesen R S G, Vanholme B TM, Luyten K, Van Wynsberghe L, Fazio G, Roldán-Ruiz I & Keulemans J (2010). Analysis of Malus *S-RNase* gene diversity based on a comparative study of old and modern apple cultivars and European wild apple. *Molecular Breeding* 26: 693-709. https://doi.org/10.1007/s11032-010-9405-5

Franklin-Tong & Franklin F C H (2003). Gametophytic self incompatibility inhibits pollen tube growth using different mechanisms. *Trends Plant Science* 8: 598-605. https://doi.org/10.1016/j.tplants.2003.10.008

Halász J, Hegedűs A, György Z, Pállinger É & Tóth M (2011). *S*-genotyping of old apple cultivars from the Carpathian basin: methodological, breeding and evolutionary aspects. *Tree Genetics & Genomes* 7: 1135-1145. https://doi.org/10.1007/s11295-011-0401-7

Hegedűs A (2006). Review of the self-incompatibility in apple (Malus×domestica Borkh., syn.:Malus pumila Mill.). *International Journal of Horticultural Science* 12: 31-36. https://doi.org/10.31421/ijhs/12/2/632

Ishimizu T, Shinkawa T, Sakiyama F & Norioka S (1998). Primary structural features of rosaceous *S-rnases* associated with gametophytic self-incompatibility. *Plant Molecular Biology* 37(6): 931-941. https://doi.org/10.1023/a:1006078500664Ishimizu, T, Inoue, K, Shimonaka, M, Saito, T, Terai, O & Norioka, S (1999). PCR-based method for identifying the *S*-genotypes of Japanese pear cultivars. *Theoretical and Applied Genetics* 98: 961-967

Janssens G A, Goderis I J, Broekaert W F & Broothaerts W (1995). A molecular method for *S*-allele identification in apple based on allele-specific PCR. *Theoretical and Applied Genetics* 91(4): 691-698. https://doi.org/10.1007/bf00223298

Kasajima I, Kikuchi T & Yoshikawa N (2017). Rapid identification of apple (*Malus*×*domestica* Borkh.) *S*-alleles using sequencing-based DNA marker APPLid. *Plant Biotechnology* 34: 97-106. https://doi.org/10.5511/plantbiotechnology.17.0503a

Larsen B, Orgaard M, Toldam-Andersen T B & Pedersen C (2016). A high-throughput method for genotyping *S-RNase* alleles in apple. *Molecular Breeding* 36: 24. https://doi.org/10.1007/s11032-016-0448-0

Lefort F, Lally M, Thompson D & Douglas G C (1998). Morphological traits, microsatellite finger printing and genetic relatedness of a stand of elite oaks (Q. *robur* L.) at Tullynally, Ireland. *Silvae Genetica* 47(5): 257-261

Li M, Zhu K, Bai S, Liu Z & Li T (2011). Isolation and *S*-genotyping application of *S*-allelic polymorphic MdSLFBs in apple (*Malus domestica* Borkh.). *Molecular Breeding* 28: 171-180. https://doi.org/10.1007/s11032-010-9471-8

Li W, Meng D, Gu Z, Yang Q, Yuan H, Li Y, Chen Q, Yu J, Liu C & Li T (2018). Apple *S-RNase* triggers inhibition of tRNA aminoacylation by interacting with a soluble inorganic pyrophosphatase in growing self-pollen tubes in vitro. *New Phytol* 218: 579-593

Ma Q, Chen C, Zeng Z, Zou Z, Li H, Zhou Q, Chen X, Sun K & Li X (2018). Transcriptomic analysis between self- and cross-pollinated pistils of tea plants (*Camellia sinensis*). *BMC Genomics* 19: 289. https://doi.org/10.1186/s12864-018-4674-1

Mandrone M, Antognoni F, Aloisi I, Potente G, Poli F, Cai G, Faleri C, Parrotta L & Del Duca S (2019). Compatible and Incompatible Pollen-Styles Interaction in *Pyrus communis* L. Show Different Transglutaminase Features, Polyamine Pattern and Metabolomics Profiles. *Frontiers in Plant Science* 10: 741. https://doi.org/10.3389/fpls.2019.00741

Matsumoto S, Tianzhong L, Otagaki S, Yang L & Songling B (2018). Efficient breeding and cultivation of type2 Red-fleshed apple cultivars using a search system for suitable apple cultivar combination. *Horticultural Plant Journal* 4(6): 219-225. https://doi.org/10.1016/j.hpj.2018.06.002

Matsumoto S & Kitahara K (2000). Discovery of a new self-incompatibility allele in apple. *Hortscience* 35(7): 1329-1332. https://doi.org/10.21273/hortsci.35.7.1329

Matsumoto S, Eguchi T,Bessho H & Abe K (2007). Determination and confirmation of *S-RNase* genotypes of apple pollinators and cultivars. *Journal of Horticultural Science and Biotechnology* 82: 323-329. https://doi.org/10.1080/14620316.2007.11512236

Meng D, Gu Z, Wang A, Yuan H, Li W, Yang Q & Li T (2014). Screening and characterization of apple Rho-like gtpase (mdrops) genes related to *S-rnase* mediated self-incompatibility. *Plant Cell, Tissue and Organ Culture (PCTOC)* 117(3): 465-476. https://doi.org/10.1007/s11240-014-0457-9

Mir J I, Ahmed N, Singh D B, Sheemar G, Hamid A, Zaffer S & Shafi V (2016). Molecular identification of *S*-alleles associated with self-incompatibility in apple (Malus spp.) genotypes. *Indian Journal of Agricultural Sciences* 86(1): 78-81

Nour V, Trandafir I & Ionica M E (2010). Compositional characteristics of fruits of several apples (Malus domestica Borkh.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38: 228-233. https://doi.org/10.15835/nbha.43.2.10081

Overmyer K, Brosché M & Kangasjärvi J (2003). Reactive oxygen species and hormonal control of cell death. *Trends in Plant Science* 8: 335-342. https://doi.org/10.1016/S1360-1385(03)00135-3

Sadhu A, Moriyasu Y, Acharya K & Bandyopadhyay M (2019). Nitric oxide and ROS mediate autophagy and regulate Alternaria alternata toxin-induced cell death in tobacco BY-2 cells. *Scientific Reports* 9: 8973. https://doi.org/10.1038/s41598-019-45470-y

Sakurai K, Brown SK & Weeden N (2000). Self-incompatibility alleles of apple cultivars and advanced selections. *Hortscience* 35(1): 116-119. https://doi.org/10.21273/hortsci.35.1.116

Sakurai K, Brown SK & Weeden, NF (1997). Determining the self-incompatibility alleles of Japanese apple cultivars. *Hortscience* 32(7): 1258-1259. https://doi.org/10.21273/hortsci.32.7.1258

Sassa H (2016). Molecular mechanism of the *S-RNase*-based gametophytic self-incompatibility in fruit trees of Rosaceae. *Breeding Science* 66: 116-121. https://doi.org/10.1270/jsbbs.66.116

Sassa H, Mase N, Hirano H & Ikehashi H (1994). Identification of self-incompatibility-related glycoproteins in styles of apple (Malus x domestica). *Theoretical and Applied Genetics* 89(2): 201-205. https://doi.org/10.1007/bf00225142

Serrano I, Romero-Puertas M C, Sandalio L M & Olmedilla A (2015). The role of reactive oxygen species and nitric oxide in programmed cell death associated with self-incompatibility. *Journal of Experimental Botany* 66: 2869-2876. https://doi.org/10.1093/ jxb/erv083

Shaheen F A, Khan K A, Husain M, Mahmood R & Rafique M K (2017). Role of Honey bees (*Apis mellifera* L.) Foraging activities in increased fruit setting and production of Apples (*Malus domestica*). *Pakistan Journal of Agricultural Research* 30(1): 29-34.

Sharma P, Jha A B, Dubey R S & Pessarakli M (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany* https://doi.org/10.1155/2012/217037

Shulaev V, Korban S S, Sosinski B, Abbott A G & Aldwinckle H S (2008). Multiple models for Rosaceae genomics. *Plant Physiology* 47(3): 985-1003. https://doi.org/10.1104/pp.107.115618

Silva N F & Goring D R (2001). Mechanisms of self-incompatibility in flowering plants. *Cellular and Molecular Life Sciences* 58(14): 1988-2007. https://doi.org/10.1007/pl00000832

Van Nerum I, Geerts M, Van Haute A, Keulemans J & Broothaerts W (2001). Re-examination of the self-incompatibility genotype of apple cultivars containing putative 'new' *S*-alleles. *Theoretical and Applied Genetics* 103(4): 584-591. https://doi.org/10.1007/pl00002913

Verdoodt L, Van Haute A, Goderis I J, De Witte K, Keulemans J & Broothaerts W (1998). Use of the multi-allelic self-incompatibility gene in apple to assess homozygocity in shoots obtained through haploid induction. *Theoretical and Applied Genetics* 96(2): 294-300. https://doi.org/10.1007/s001220050739

Whitehouse H L K (1951). Multiple-allelomorph incompatibility of pollen and style in the evolution of the angiosperms. *Annals Botany New Series* 14: 198-216. https://doi.org/10.1093/oxfordjournals.aob.a083243

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