S-genotyping Supports the Genetic Relationships between Turkish and Hungarian Apricot Germplasm

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ABSTRACT. The *S*-genotypes of a set of Turkish and Hungarian apricot (*Prunus armeniaca* L.) cultivars were determined by polymerase chain reaction (PCR) amplification of their *S*-*RNase* intron regions. In addition, the *S*-genotyping method was extended to the *SFB* gene to detect the non-functional *S_C*-haplotype and hence reliably identify self-compatible apricot cultivars. We determined the complete *S*-genotype of 51 cultivars and the partial *S*-genotype of four cultivars. A total of 32 different *S*-genotypes were assigned to the 51 cultivars, and many of them (28) were classified into newly established cross-incompatibility groups III through XIV. Another 12 cultivars demonstrated unique incompatible genotypes and seven self-compatible cultivars were identified in the examined accessions. The fact that Turkish and Hungarian apricot cultivars carry 12 and five *S*-alleles, respectively, and all five alleles detected in Hungarian cultivars were also present in Turkish apricots furnished molecular evidence supporting the long-suspected historical connection between Hungarian and Turkish apricots. The connection between these two gene pools appeared to be relatively recent and associated with historical events dating back 300 years. Our results confirm that Turkish germplasm contributed considerably to the development of several desirable Hungarian apricot cultivars. Results suggest that the mutation rendering the *S_C*-haplotype non-functional might have occurred somewhere east of central Turkey.

Apricot is thought to have originated in China, from where it was disseminated to Europe through central Asia and Asia Minor (Faust et al., 1998). According to Kostina (1969), apricot cultivars are classified into four major eco-geographical groups: central Asian, Irano-Caucasian, European, and Dzhungar-Zailing (Tien-shan area). The central Asian and Irano-Caucasian (encompassing Turkish cultivars) groups show the richest phenotypic variability, while the European group (including cultivars grown in North America, Australia, and South Africa) is said to have the least diversity (Mehlenbacher et al., 1991). Apricot cultivars originating in the eastern Europe cultivar group can be clearly distinguished in their pomological characteristics from other cultivars within the European origin (Faust et al., 1998; Kostina, 1970).

The Irano-Caucasian apricots were described as predominantly self-incompatible (SI), while most European apricots are self-compatible (SC) (Halász et al., 2005; Kostina, 1970).

Cross-incompatibility, resulting in the mutual failure of fruit set between a pair of cultivars, occurs frequently in predominantly SI species. In apricot, the first cross-incompatibility group was described among the North American cultivars, Goldrich, Hargrand, and Lambertin-1 (Egea and Burgos, 1996), while the second group encompassed giant-fruited Hungarian apricots (Szabó and Nyéki, 1991).

Similar to other *Prunus* L. species, apricots reportedly demonstrate gametophytic self-incompatibility controlled by a single locus with multiple variants, termed *S*-haplotypes (de Nettancourt, 2001). The *S*-haplotype contains a gene (*S*-*RNase*) encoding for a ribonuclease enzyme (McClure et al., 1989), and the recently identified *S*-haplotype-specific *F*-box gene (Entani et al., 2003; Romero et al., 2004) responsible for pistil and pollen functions, respectively (Fig. 1).

To date, 21 *S*-*RNase* alleles have been described in European apricots, 20 of which (*S*₁–*S*₂₀) code for self-incompatibility and one (*S_C*) allowing self-compatibility (Burgos et al., 1998; Halász, 2007; Halász et al., 2005, 2007a). The *S_C*-haplotype was long suspected and recently confirmed to be a pollen-part mutant of the *S*₈-haplotype (Halász et al., 2007a) with a 358-bp insertion in the *SFB_C* gene (Vilanova et al., 2006). Additional *S*-alleles have been identified in Chinese cultivars (Wu et al., 2009).

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Fig. 1. Schematic structure of the *S*-locus in apricot and annealing sites of the consensus (above) and allele-specific (below) primers used for the PCR analysis. Boxes and lines are exons and introns, respectively (not to scale).

Microsatellite analyses suggested that Hungarian and European cultivars might have originated through hybridization among Irano-Caucasian genotypes (Maghuly et al., 2005). This assumption seems to be confirmed by historical and linguistic evidence, as well. During the one and a half centuries of Ottoman occupation in Hungary, abundant records exist to document the admission of Turkish graft-wood and other propagation materials to Hungary (Faust et al., 1998). Even the Hungarian word used for this fruit crop, “kajszi,” has a Turkish origin as “kaysi,” meaning superior, sweet-seeded, and grafted apricots in contrast with the world “zerdali,” used for old, local seedlings with smaller fruit and bitter seed (Ercisli, 2004).

Turkey dominates world apricot production with more than 700,000 Mt produced in 2008 (Food and Agriculture Organization of the United Nations, 2008). As considerable genetic variability exists in Turkish apricot germplasm, and this crop is of great economical significance, many detailed analyses have been performed in recent years on morphological and pomological characterization, as well as on fruit quality, yield, and fertilization characteristics of Turkish apricots (Akin et al., 2008; Asma and Ozturk, 2005; Gülcan et al., 2006). However, discrepancies between study years for orchard bagging studies and pollen tube growth analyses have led to uncertainty of the self-incompatibility genotypes for many Turkish cultivars (Misirli et al., 2006).

In this study, we used polymerase chain reaction (PCR) amplification of the *S-RNase* intron regions to determine their lengths and the *S*-genotypes of a set of Turkish apricot cultivars. In addition, the *S*-genotyping method was extended to the *SFB* gene to detect the non-functional *S_C*-haplotype and hence reliably identify *S_C* apricot cultivars. The information was then compared with *S*-genotypes of Hungarian apricots to establish a possible relationship between Turkish and Hungarian apricot germplasm.

Materials and Methods

PLANT MATERIAL. The 55 Turkish apricot cultivars used in the experiments were obtained as chance seedlings and were characterized by phenotypic diversity and geographic origin (Table 1). All cultivars were grown at Malatya Fruit Research Institute, Malatya, Turkey. Six cultivars with known *S*-genotypes [Bergeron (*S_{2S_C}*), Ceglédi óriás (*S_{8S₉}*), Goldrich (*S_{1S₂}*), Mari de Cenad (*S_CS₁₉*), Moniqué (*S_{2S₆}*), and Sunglo (*S_{2S₃}*) (Burgos et al., 1998; Halász, 2007; Halász et al., 2005, 2007a)] were used as controls and were obtained from the orchard of the Corvinus University of Budapest, Department of Genetics and Plant Breeding, Szigetcsép, Hungary.

DNA EXTRACTION. Genomic DNA was extracted from fully expanded young leaves using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The quantity and quality of DNA were

analyzed by spectrophotometer (NanoDrop™ ND-1000; Bio-Science, Budapest, Hungary).

GENOMIC PCR WITH *S-RNase* AND *SFB*-SPECIFIC PRIMERS. PCR was conducted according to Sutherland et al. (2004) using the degenerate primers EM-PC2consFD and EM-PC3consRD for the amplification of the second intron region of

the *S-RNase* gene (Fig. 1). To amplify the first intron, the fluorescently labeled (_{JOE}) forward primer SRc-F (Romero et al., 2004) was used in combination with the reverse primer SRc-R (Vilanova et al., 2005).

For the identification of the *S_C*-haplotype, a two-step approach was used. An allele-specific reverse primer, AprSC8R (CTAATAACTAAATGCTAAAGAGCA) was designed to selectively amplify the *S_C/S₈-RNase* allele (Fig. 1) and was used in combination with PaConsl F (Sonneveld et al., 2003). The amplification was carried out using a temperature profile with an initial denaturing of 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 55 °C for 1.5 min and 72 °C for 2 min, and a final extension of 72 °C for 5 min. *SFB_C/SFB₈*-specific primers, AprFBC8-F (5'-CATGGAAAAAGCTGACTTATGG-3') and AprFBC8-R (5'-GCCTCTAATGTCATCTACTCTTAG-3') were designed based on the V2 and HVb variable region of the *SFB_C* allele (Halász et al., 2007a) (Fig. 1). The amplification was carried out as described for the *S_C/S₈-RNase*-specific primers.

PCR was carried out in a thermocycler (PTC 200; MJ Research, Budapest, Hungary). For amplification of the *S-RNase* first and second introns, we used the programs originally described for the primers (Sutherland et al., 2004; Vilanova et al., 2005). About 20 to 80 ng of genomic DNA was used for PCR amplification in a 25-μL reaction volume, containing 1 × PCR buffer (Sigma, Budapest, Hungary) with final concentrations of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of dNTPs, 0.4 μM of each primer, and 0.625 U of *Taq* DNA polymerase (Sigma).

The PCR products were separated on 2% TAE agarose gels at 100 V for 2 h and DNA bands were stained with ethidium bromide. Fragment sizes were estimated by comparison with the 1 kb + DNA ladder (Promega, Madison, WI). For exact size determination of *S-RNase* first intron region fragments smaller than 500 bp, the fluorescently labeled products were run in an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary) using the GENOTYPER 3.7 software and GS500 LIZ size standard (Applied Biosystems).

Results

Determination of the *S*-genotypes of 55 Turkish apricot cultivars was carried out using the SRc-F and SRc-R consensus primers (Vilanova et al., 2005) for the first intron and the EM-PC2consFD and EM-PC3consRD primers (Sutherland et al., 2004) for the second intron analysis. The sizes of the PCR products obtained were compared with those previously published by Vilanova et al. (2005) and Halász (2007). Primers designed from conserved coding regions flanking the second intron yielded two fragments ranging from 310 to 1980 bp, except in the cases of 17 cultivars where only one fragment was amplified (Fig. 2). Four cultivars did not show PCR products at all.

Table 1. Name of apricot cultivars collected from different Turkish regions, as well as their ripening time, skin color, fruit firmness, size, use, self-incompatibility status, and *S*-genotype.

	Cultivar	Region	Ripening time	Skin color	Firmness	Size	Use	Self-(in) compatibility ^z	<i>S</i> -genotype
1.	Adilcevaz 1	Bitlis	Medium	Yellow	Firm	Small	Fresh	<u>SI</u>	<i>S</i> ₈ <i>S</i> ₁₉
2.	Adilcevaz 3	Bitlis	Medium	Yellow	Firm	Medium	Fresh	<u>SI</u>	<i>S</i> ₁₃ <i>S</i> ₁₉
3.	Adilcevaz 5	Bitlis	Medium	Yellow	Very firm	Medium	Fresh	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₁₃
4.	Agerik	Igdir	Medium	White	Firm	Big	Fresh	<u>SI</u>	<i>S</i> ₇ <i>S</i> ₁₃
5.	Akkadag Günay	Malatya	Medium	Yellow	Firm	Small	Dried	<u>SI</u>	<i>S</i> ₃ <i>S</i> ₉
6.	Alioglu 49	Unknown	Medium	Yellow	Medium	Medium	Fresh	<u>SI</u>	<i>S</i> ₈ <i>S</i> ₁₂
7.	Alyanak	Izmir	Medium	Yellow	Firm	Big	Fresh	<u>SI</u>	<i>S</i> ₂ <i>S</i> ₈
8.	Artvin P.A	Artvin	Medium	Yellow	Soft	Medium	Fresh	<u>SI</u>	<i>S</i> ₂ <i>S</i> ₇
9.	Canakkale	Canakkale	Medium	Yellow	Firm	Medium	Fresh	<u>SC</u>	<i>S</i> _C <i>S</i> _C
10.	Catologlu	Malatya	Medium	Yellow	Medium	Medium	Dried	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₉
11.	Cekirge 52	Bursa	Medium	Light Orange	Firm	Medium	Fresh	<u>SI</u> ^e	<i>S</i> ₉ <i>S</i> ₂₀
12.	Cigli	Izmir	Medium	Yellow	Firm	Small	Fresh	<u>SI</u>	<i>S</i> ₇ <i>S</i> ₁₉
13.	Cologlu	Malatya	Medium	Yellow	Firm	Small	Dried	<u>SI</u>	<i>S</i> ₈ <i>S</i> ₉
14.	Dörtüol 2	Hatay	Medium	Yellow	Medium	Medium	Fresh	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₇ ^x
15.	Dörtüol 4	Hatay	Medium	Light Orange	Soft	Medium	Fresh	<u>SI</u>	<i>S</i> ₂ <i>S</i> ₁₉
16.	Ethembey	Edirne	Medium	Light Orange	Firm	Medium	Fresh	<u>SC</u>	<i>S</i> _C <i>S</i> ₈
17.	Gec Aprikoz	Igdir	Medium	Yellow	Firm	Big	Fresh	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₁₁
18.	Güz Aprikozu	Malatya	Late	Yellow	Medium	Big	Fresh	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₇ ^x
19.	Haci Haliloglu	Malatya	Medium	Yellow	Firm	Medium	Dried	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₁₃
20.	Hacikiz	Malatya	Medium	Yellow	Very firm	Medium	Dried	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₈
21.	Hasanbey	Malatya	Medium	Yellow	Very firm	Medium	Fresh-Dried	<u>SI</u>	<i>S</i> ₂ <i>S</i> ₉
22.	Imrahor	Izmir	Medium	Yellow	Medium	Medium	Fresh	<u>SI</u> ^y	<i>S</i> ₃ <i>S</i> ₇ ^x
23.	Iri Bitirgen	Tekirdag	Medium	Light Orange	Medium	Medium	Fresh	<u>SI</u>	<i>S</i> ₂ <i>S</i> ₆
24.	Ismailaga	Malatya	Medium	Yellow	Firm	Medium	Dried	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₁₁
25.	Kabaasi	Malatya	Medium	Yellow	Firm	Medium	Dried	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₁₃
26.	Kadioglu	Malatya	Medium	Orange	Medium	Small	Dried	<u>SI</u>	<i>S</i> ₈ <i>S</i> ₉
27.	Kamelya	Izmir	Medium	Light Orange	Firm	Medium	Fresh	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₁₃
28.	Karacabey	Bursa	Medium	Orange	Very firm	Medium	Fresh	<u>SC</u>	<i>S</i> _C <i>S</i> ₂
29.	Kayisi Erigi	Nigde	Medium	Yellow	Firm	Medium	Fresh	<u>SI</u>	<i>S</i> ₁₁ <i>S</i> _{plum} ^w
30.	Kayseri P.A	Kayseri	Medium	Yellow	Very firm	Small	Fresh	<u>SI</u>	<i>S</i> ₃ <i>S</i> ₈
31.	Kurukabuk	Malatya	Medium	Light Orange	Medium	Medium	Dried	<u>SI</u>	<i>S</i> ₇ <i>S</i> ₉
32.	Levent	Malatya	Very late	Yellow	Medium	Medium	Fresh	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₁₉
33.	Mahmudun Erigi	Erzincan	Medium	Yellow	Very firm	Medium	Fresh	<u>SI</u>	<i>S</i> ₁₃ <i>S</i> ₇ ^x
34.	Mektep	Izmir	Medium	Yellow	Medium	Medium	Fresh	<u>SC</u>	<i>S</i> _C <i>S</i> ₈
35.	No 1 Zerdali	Van	Medium	Yellow	Soft	Medium	Fresh	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₁₂
36.	No 2 Zerdali	Konya	Medium	Yellow	Medium	Medium	Fresh	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₁₃
37.	Ordubat	Igdir	Medium	Yellow	Very soft	Small	Dried	<u>SI</u>	<i>S</i> ₇ <i>S</i> ₁₂
38.	Ozal	Malatya	Very late	Yellow	Soft	Small	Fresh	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₉
39.	Pasa Mismisi	Malatya	Medium	Yellow	Medium	Big	Dried	<u>SC</u> ^y	<i>S</i> _C <i>S</i> ₈
40.	Sakit 1	Hatay	Medium	Yellow	Medium	Medium	Fresh	<u>SI</u> ^y	<i>S</i> ₆ <i>S</i> ₁₉
41.	Sakit 3	Hatay	Medium	Light Orange	Soft	Medium	Fresh	<u>SI</u>	<i>S</i> ₃ <i>S</i> ₁₉
42.	Shalakh (Aprikoz)	Igdir	Medium	Yellow	Soft	Medium	Fresh	<u>SI</u>	<i>S</i> ₁₁ <i>S</i> ₁₃
43.	Sam	Izmir	Medium	Yellow	Soft	Medium	Fresh	<u>SC</u>	<i>S</i> _C <i>S</i> ₂
44.	Sebbiyiki	Malatya	Medium	Yellow	Firm	Medium	Dried	<u>SI</u>	<i>S</i> ₂ <i>S</i> ₁₉
45.	Seftalioglu	Malatya	Medium	Yellow	Firm	Medium	Dried	<u>SI</u>	<i>S</i> ₈ <i>S</i> ₉
46.	Sekerpare	Erzincan	Medium	Yellow	Soft	Medium	Dried	<u>SI</u>	<i>S</i> ₃ <i>S</i> ₆
47.	Soganci	Malatya	Medium	Yellow	Very firm	Medium	Dried	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₉
48.	Tokaloglu Izmir	Izmir	Medium	Light Orange	Medium	Medium	Fresh	<u>SI</u>	<i>S</i> ₃ <i>S</i> ₁₉
49.	Turfanda Izmir	Izmir	Early	Yellow	Medium	Medium	Fresh	<u>SI</u>	<i>S</i> ₇ <i>S</i> ₈
50.	X1 Zerdali	Malatya	Medium	Yellow	Firm	Medium	Fresh	<u>SI</u>	<i>S</i> ₆ <i>S</i> ₁₂
51.	X2 Zerdali	Malatya	Medium	Yellow	Firm	Small	Fresh	<u>SI</u>	<i>S</i> ₇ <i>S</i> ₁₂
52.	X3 Zerdali	Malatya	Medium	Yellow	Firm	Small	Fresh	<u>SI</u>	<i>S</i> ₉ <i>S</i> ₂₀
53.	Yegen	Malatya	Medium	Light Orange	Very soft	Medium	Dried	<u>SI</u>	<i>S</i> ₈ <i>S</i> ₁₁
54.	Yerli Izmir	Izmir	Medium	Yellow	Medium	Medium	Fresh	<u>SC</u>	<i>S</i> _C <i>S</i> ₇
55.	Ziraat Okulu	Malatya	Medium	Yellow	Medium	Big	Fresh	<u>SI</u>	<i>S</i> ₂ <i>S</i> ₈

^zSI = self-incompatible, SC = self-compatible; self-(in)compatibility phenotypes supported by fruit set ratios after self-pollination (Gülcan et al., 2006) are underlined.

^yThe proposed self-(in)compatibility phenotype is not supported by fruit set ratios after self-pollination (Gülcan et al., 2006).

^xUnknown alleles were labeled as *S*₇.

^w“Kayisi Erigi” is a natural hybrid of *Prunus armeniaca* and *Prunus salicina*; hence, its unidentified *S*-allele was transmitted from the plum parent.

Fig. 2. PCR products (in negative) in 50 Turkish and three control apricot cultivars using the second intron consensus primers of *Prunus S-RNase* gene [M = 1 kb + DNA ladder, Mq = 'Moniqui' (S_2S_6), Sg = 'Sunglo' (S_2S_3), MC = 'Mari de Cenad' ($S_C S_{19}$)]. Numbers refer to cultivars shown in Table 1.

For nine Turkish cultivars (Alyanak, Artvin P.A., Dörtöl 4, Hasanbey, Iri Bitirgen, Karacabey, Sam, Sebbiyiki, and Ziraat Okulu) and two control cultivars (Moniqui and Sunglo) a fragment of ≈ 900 bp was detected that indicated the presence of allele S_2 . A fragment of ≈ 310 bp occurred in six Turkish cultivars (Akcadag Günay, Imrahör, Kayseri P.A., Sakit 3, Sekerpare, and Tokaloglu İzmir) and Sunglo, confirming this allele as S_3 . Twelve cultivars (Cataloglu, Hacikiz, Gec Aprikoz, Güz Aprikoz, Iri Bitirgen, Levent, Ozal, No 1 Zerdali, Sakit 1, Sekerpare, Soganci, and X1 Zerdali) yielded a fragment of ≈ 1300 bp, similar to 'Moniqui' (S_2S_6) and hence this allele was labeled S_6 . The allele S_7 occurred in eight cultivars (Agerik, Artvin P.A., Cigli, Kurukabuk, Ordubat, Turfanda İzmir, X2 Zerdali, and Yerli İzmir) as a fragment of ≈ 820 bp.

An ≈ 500 -bp fragment was detected in 18 cultivars (Adilcevaz 5, Akcadag Günay, Cataloglu, Cekirge 52, Cologlu, Dörtöl 2, Haci Haliloglu, Hasanbey, Ismailaga, Kabaasi, Kadioglu, Kamelya, Kurukabuk, No 2 Zerdali, Ozal, Seftalioglu, Soganci, and X3 Zerdali), which could be attributed to allele S_9 or S_{20} . Determination of the sizes of the first intron region would be required to distinguish between these alleles. An ≈ 1700 -bp fragment appeared in five cultivars (Gec Aprikoz, Ismailaga, Kayisi Erigi, Shalakh, and Yegen), indicating the presence of S_{11} -allele. A fragment size characteristic for allele S_{12} was observed in five cultivars (Alioglu 49, No 1 Zerdali, Ordubat, X1 Zerdali, and X2 Zerdali). A band of ≈ 1250 bp appeared in nine cultivars (Adilcevaz 3, Adilcevaz 5, Agerik, Haci Haliloglu, Kabaasi, Kamelya, Mahmudun Erigi, No 2 Zerdali, and Shalakh), suggesting that the S_{13} -allele is common to all. The allele S_{19} has a second intron region (≈ 1980 bp), which was found in nine cultivars (Adilcevaz 1, Adilcevaz 3, Cigli, Dörtöl 4, Levent, Sakit 1, Sakit 3, Sebbiyiki, and Tokaloglu İzmir).

The identity of this allele was further supported by using a control cultivar, Mari de Cenad ($S_C S_{19}$).

'Kayisi Erigi' is a hybrid of *P. armeniaca* and *Prunus salicina* Lindl. Its S_{11} -allele is derived from the apricot parent, but the other allele differs from all apricot alleles and must have originated from the plum (*P. salicina*) parent. The 1270- and 1950-bp sizes of the second intron region in 'Güz Aprikoz' and 'Imrahör', respectively, do not match the sizes of any known alleles. The novelty of these alleles must be verified by DNA sequencing.

To support the *S*-genotype determinations, precise first intron lengths were also determined for all cultivars using fluorescently labeled primers and automated sizing of the first intron regions. Based on the second intron region analysis, 18 cultivars remained undistinguished with regard to their S_9 - or S_{20} -alleles. However, the first intron sizes of these two alleles differ from each other: S_9 is characterized by a fragment of 203 bp, while the size of S_{20} is 222 bp. Determination of the precise fragment sizes revealed that all 18 cultivars carried the S_9 -allele, and two of them (Cekirge 52 and X3 Zerdali) also harbored the S_{20} -allele.

In the study of Vilanova et al. (2005), the accurate size of fragments amplified by the SRc-F primer pair from S_3 - and S_6 -alleles was not determined. Using 'Sunglo' (S_2S_3) and 'Moniqui' (S_2S_6) as control cultivars, the sizes of the first intron region of the S_3 - and S_6 -alleles were determined in the present study as 269 and 423 bp, respectively.

Two peaks were observed for 51 accessions, while four cultivars (Canakkale, Ethembey, Mektep, and Pasa Mismisi) had only one fragment of 355 bp. These four cultivars were also similar in that they did not give amplification in the second intron region. The 355-bp fragment was also present in 14 other cultivars. This fragment size was previously attributed to the

S_C - and S_8 -*RNase* alleles (Halász et al., 2007a). To confirm that no other unidentified alleles could give rise to a fragment with a first intron region size identical to S_C and S_8 , a specific primer (AprSC8) was designed to anneal exclusively within the second intron region of the S_C - and S_8 -*RNase* alleles. This primer pair selectively amplified a fragment of 547 bp only in the case of S_8/S_C -alleles, as demonstrated by using control cultivars Ceglédi óriás (S_8S_9) and Bergeron (S_CS_2). In contrast, there was no amplification in ‘Goldrich’ (S_1S_2) when used as a negative control (Fig. 3A). The presence of S_8/S_C -alleles was indeed confirmed among the tested 18 Turkish cultivars.

Because coding regions of the S_8 - and S_C -*RNase* alleles are identical, discrimination of SI from SC cultivars could not be achieved in this analysis. The S_8 - and S_C -haplotypes differ only in the *SFB* gene in that an insertion of 358 bp can be found in the *SFB_C*, resulting in a truncated protein and the consequent breakdown of self-incompatibility. Using a new primer pair (AprFBC8), we could distinguish between the SI and SC cultivars because genotypes carrying the *SFB_C*-allele showed an amplification product of ≈ 500 bp, while genotypes carrying the *SFB_8*-allele showed a fragment of ≈ 150 bp (Fig. 3B). Additionally, the primer worked as a codominant marker because homo- and heterozygotes could be unequivocally differentiated. Altogether, seven cultivars (Canakkale, Ethembey, Kayisi Erigi, Mektep, Pasa Mismisi, Sam, and Yerli Izmir) proved to be self-compatible, six were heterozygotes, and only one homozygote, ‘Canakkale’ (S_CS_C), was identified. Three cultivars shared the S_CS_8 -genotype (Ethembey, Mektep, and Pasa Mismisi) as demonstrated by two fragments of appropriate sizes in the agarose gel (Fig. 3B).

Finally, we determined complete *S*-genotypes of 51 cultivars and partial *S*-genotypes of four cultivars by combining the results obtained in two rounds of PCR (Table 1). Twelve previously described *S*-alleles were identified among the Turkish cultivars. S_9 was the most frequent *S*-allele in the tested Turkish germplasm (occurring in 18 cultivars), followed by S_8 (14), S_6 (12), S_2 , S_{13} and S_{19} (9), S_7 (8), S_C (7), S_3 (6), S_{11} , and S_{12} (5), while S_{20} -allele was only found in two cultivars.

Discussion

***S*-GENOTYPES OF TURKISH CULTIVARS.** While the *S*-genotypes of many North American and European apricot cultivars are

known, this is the first study to examine *S*-genotype diversity of apricots native to Turkey, the leading apricot producer in the world. As such, this information can be used directly by producers in making correct selections of pollination partners in new orchard plantings, as well as by researchers interested in clarification of the evolutionary history of this crop.

Complete *S*-genotypes were given for 51 apricot cultivars. For four others, only partial *S*-genotypes could be determined. A total of 32 different *S*-genotypes were assigned to the 51 cultivars with complete *S*-genotypes. Many of them (28) were proposed to form new cross-incompatibility groups, III–XIV. Previously, two groups were described, group I for North American cultivars (Egea and Burgos, 1996) and group II for Hungarian giant-fruited cultivars (Szabó and Nyéki, 1991). The limited number of groups identified in former studies might be associated with the fact that most of the tested European cultivars were SC (Halász et al., 2007a; Vilanova et al., 2005). In other SI *Prunus* species, more groups have been described; currently, 22, 36, and 19 groups are known for almond [*Prunus dulcis* (Mill.) D.A. Webb], sweet cherry (*Prunus avium* L.), and japanese plum (*P. salicina*), respectively (Guerra et al., 2009; Halász et al., 2007b, 2010; Ortega et al., 2006; Schuster et al., 2007; Tobutt et al., 2005). Self-incompatibility of some of the *S*-genotyped cultivars was previously confirmed by fruit set analysis in Turkey (Gülcan et al., 2006; Misirli et al., 2006).

Another 12 cultivars demonstrated unique incompatible genotypes, which can be used as universal pollen donors because they are mutually compatible with all 14 cross-incompatibility groups. Among the 55 cultivars, only seven self-compatible genotypes were determined. Six of these cultivars were also checked and confirmed for self-fruitful phenotype in fruit set analyses (Gülcan et al., 2006). The S_CS_C homozygote ‘Canakkale’ showed outstanding levels (over 30%) of fruit set after self-pollination over 2 years.

Based on fruit set analysis after self-pollination, Gülcan et al. (2006) determined fruit set ratios exceeded 5% for another seven cultivars of those tested here and proposed to be SI in this study. Considering that fruit set ratios were only slightly higher than 5% for four cultivars and data were somewhat inconsistent between years, our study confirmed that results from open-field fruit set analyses can only be regarded as reliable if consistent results are available from several years. The remaining three cultivars (Cekirge-52, Imrahör, and Sakit-1) were reported to produce fruit set ranging from 14% to 34% (Gülcan et al., 2006). However, these cultivars do not carry the S_C -haplotype. If the self-fruitful phenotype of these cultivars and the identity of plant materials can be confirmed, mutation would be a possibility that renders the *S*-locus non-functional in these genotypes.

CROP EVOLUTIONARY PERSPECTIVES. A gradually decreasing allele number was detected in apricot landraces from China to western Europe, with some allelic exclusivity occurring in certain geographic areas (Halász, 2007). For example, alleles S_{10} – S_{14} were only detected in apricots of Armenian origin (Halász

Fig. 3. PCR detection and reliable differentiation of S_C - and S_8 -haplotypes in Turkish apricots. Allele-specific PCR reaction (A) was used to control the presence of the $S_{8/C}$ -*RNase* alleles and a selective amplification of the *SFB* gene (B) was used to differentiate between *SFB_C* and *SFB_8* alleles [M = 1 kb + DNA ladder, Co = ‘Ceglédi óriás’ (S_8S_9), Bg = ‘Bergeron’ (S_2S_C), Gd = ‘Goldrich’ (S_1S_2)]. Numbers 1 through 50 refer to cultivars shown in Table 1.

et al., 2005), while alleles S_8 , S_9 , S_{19} , and S_{20} were only found in Hungarian apricots (Halász, 2007; Halász et al., 2007a). In the present study, S_{11} – S_{13} alleles were also detected in Turkish apricots, most frequently in those originating from the eastern part of the country, near the Turkish-Armenian border.

In addition, each allele that was previously detected only in Hungarian cultivars was relatively frequent in Turkish apricots. Even cross-incompatibility group II, encompassing the giant fruited Hungarian cultivars, expanded with the addition of the Turkish apricots (Table 2). The fact that Turkish and Hungarian apricot cultivars carry 12 and five S -alleles, respectively, and all five alleles detected in Hungarian cultivars were also present in Turkish apricots furnished molecular evidence to support historical records that apricots were intensively transferred to Hungary from Turkey during the 15th and 16th centuries (Faust et al., 1998). After the Ottoman retreat, apricot orchards were abandoned and sometimes devastated. However, apricot trees could survive these intervals from seeds or as escaped individuals. It has been shown that selection may lead to significant genetic gain in apricot (Ledbetter, 2008). The imported Turkish material was likely subjected to natural and human selection pressures that resulted in some landrace cultivars being well adapted to Hungarian ecological conditions and having valuable pomological characteristics. It also explains why present-day Hungarian and Turkish apricot cultivars differ from each other in many respects.

Most of the present Hungarian cultivars were selected four or five decades ago in regions overlapping with the former Turkish estates and orchards (Faust et al., 1998). For example, a Hungarian cultivar Korai piros carries the rare S_{20} -allele that was also identified in two Turkish landrace cultivars. ‘Korai piros’ was found in the area formerly occupied and controlled by the Turks for more than 100 years (Harsányi, 1981). Genetic relatedness between Hungarian and Irano-Caucasian apricots was also demonstrated by simple sequence repeat (SSR)

analysis (Maghuly et al., 2005). However, the connection between these germplasm sources seems to be relatively recent and associated with historical events only 300 years ago rather than primary dissemination routes of apricot from central Asia to Europe dating back 2000 years, as previously believed (Faust et al., 1998; Maghuly et al., 2005).

We must highlight that the same S_C -haplotype associated with the SC phenotype of all European cultivars has been detected in Turkish apricots as well. It is interesting to realize that most of the SC cultivars originated at the western or central part of the country, while no SC cultivars could have been found among the cultivars from eastern Turkey (Fig. 4). However, the non-mutated, wild-type of the S_C -haplotype (S_8) was quite frequent in that region. It may indicate that the mutation rendering the S_C -haplotype non-functional could have occurred somewhere close to the region east from central Turkey. However, this hypothesis requires further support.

If the S_C -haplotype had been brought to Hungary, it would have allowed self-fertilization and reliable fruit set. The Hungarian Magyarkajszai cultivars are well known in central Europe, and all share the $S_C S_8$ genotype. Among the Turkish cultivars, three have the same $S_C S_8$ genotype (Ethembey, Mektep, and Pasa Mismisi). These cultivars are characterized by medium or large fruit size, and two of them are used for fresh consumption (Table 1) similar to the Hungarian Magyarkajszai cultivars. It would be interesting to know whether these cultivars or their ancestors contributed to the formation of the present Hungarian Magyarkajszai cultivars. Additional molecular analyses (SSR or SNP) might be used to control this possibility.

Conclusions

Our results show that most Turkish apricots are SI, hence, S -genotyping may help to achieve reliable fruit set in commercial

Table 2. Cross-incompatibility groups of apricot. Cultivars with previously determined S -genotypes (Egea and Burgos, 1996; Halász et al., 2005) are set in italics.

Cross-incompatibility groups	Cultivars	S -genotype
I	<i>Goldrich, Hargrand, Lambertin-1</i>	$S_1 S_2$
II	<i>Cologlu, Kadioglu, Seftalioglu, Ceglédi óriás, Ligeti óriás</i>	$S_8 S_9$
III	<i>Iri Bitirgen, Moniqui</i>	$S_2 S_6$
IV	<i>Artvin P.A, Priana</i>	$S_2 S_7$
V	<i>Alyanak, Ziraat Okulu</i>	$S_2 S_8$
VI	<i>Dörtöl 4, Sebbiyiki</i>	$S_2 S_{19}$
VII	<i>Sakit 3, Tokaloglu Izmir</i>	$S_3 S_{19}$
VIII	<i>Cataloglu, Ozal, Soganci</i>	$S_6 S_9$
IX	<i>No 1 Zerdali, X1 Zerdali</i>	$S_6 S_{12}$
X	<i>Ordubat, X2 Zerdali</i>	$S_7 S_{12}$
XI	<i>Adilcevaz 5, Haci Haliloglu, Kabaasi, Kamelya, No 2 Zerdali</i>	$S_9 S_{13}$
XII	<i>Shalakh (Aprikoz), Voski</i>	$S_{11} S_{13}$
XIII	<i>Levent, Sakit 1^z</i>	$S_6 S_{19}$
XIV	<i>Cekirge 52^z, X3 Zerdali</i>	$S_9 S_{20}$
0: universal pollen donors	Canakkale ($S_C S_C$), Ethembey ($S_C S_8$), Karacabey ($S_C S_2$), Mektep ($S_C S_8$), Pasa Mismisi ($S_C S_8$), Sam ($S_C S_2$), Yerli Izmir ($S_C S_7$) Adilcevaz 1 ($S_8 S_{19}$), Adilcevaz 3 ($S_{13} S_{19}$), Agerik ($S_7 S_{13}$), Akcadag Günay ($S_3 S_9$), Alioglu 49 ($S_8 S_{12}$), Cigli ($S_7 S_{19}$), Gec Aprikoz ($S_6 S_{11}$), Hacikiz ($S_6 S_8$), Hasanbey ($S_2 S_9$), Ismailaga ($S_9 S_{11}$), Kayseri P.A ($S_3 S_8$), Kurukabuk ($S_7 S_9$), Sekerpare ($S_3 S_6$), Turfanda Izmir ($S_7 S_8$), Yegen ($S_8 S_{11}$)	

^zFurther analyses are required to confirm self-incompatibility phenotype.

Fig. 4. Distribution of *S*-alleles detected in apricot cultivars from different growing areas in Turkey. West of the broken line, self-compatible cultivars were detected, while east of the line, self-compatible cultivars did not occur.

orchards of the world's largest apricot-producing country. It is also highlighted by the fact that 12 new cross-incompatibility groups have been proposed among Turkish apricots in contrast with only two cross-incompatibility groups previously described among European and North American cultivars. Our results support the hypothesis that Turkish germplasm contributed considerably to the formation of precious Hungarian apricot cultivars. The realization of the shared *S*-allele pools of the Irano-Caucasian and European apricots confirms previous crop evolutionary hypotheses and phylogenetic data, and also holds possibilities for breeding in Turkey and other European countries.

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