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).
Microsatellite analyses suggested that Hungarian and European cultivars might have originated through hybridization among Iranian-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 introduction 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 to 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., 2004; 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-genootypes 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 SC 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_S_S), Ceglédí oris (S_S_S), Goldrich (S_S), Mari de Cenad (S_S_S), Moniqui (S_S_S), and Sunglo (S_S)] (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, Szeged, 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 S Rc-F (Romero et al., 2004) was used in combination with the reverse primer S Rc-R (Vilanova et al., 2005).

For the identification of the S_c-haplotype, a two-step approach was used. An allele-specific reverse primer, AprS c8R (CTAATAACTAAATGCTAAAGAGCA) was designed to selectively amplify the S_c/S_RNase allele (Fig. 1) and was used in combination with PaCons 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. S FBR/S FBR-specific primers, AprFBc8-F (5′-CATGGAAAAGTCTGATTTAGG-3′) and AprFBc8-R (5′-GCCTCATTTGAATCGATCTCTTAG-3′) were designed based on the V2 and HVb variable region of the S FBR allele (Halász et al., 2007a) (Fig. 1). The amplification was carried out as described for the S_c/S_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 S Rc-F and S Rc-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.

**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).**

![S-RNase Structure](image-url)
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 | S-genotype |
|------------------|----------------|---------------|-------------|----------|-----------|----------|------------------------|------------|
| Adilcevaz 1      | Bitlis         | Medium        | Yellow      | Firm     | Small     | Fresh    | SI                     | S<sub>y</sub> |
| Adilcevaz 3      | Bitlis         | Medium        | Yellow      | Firm     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Adilcevaz 5      | Bitlis         | Medium        | Yellow      | Very firm | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Agerik           | Iğdır          | Medium        | White       | Firm     | Big       | Fresh    | SI                     | S<sub>y</sub> |
| Akcadag Günay    | Malatya        | Medium        | Yellow      | Firm     | Small     | Dried    | SI                     | S<sub>y</sub> |
| Alioglu 49       | Unknown        | Medium        | Yellow      | Medium   | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Alyanak          | İzmir          | Medium        | Yellow      | Firm     | Big       | Fresh    | SI                     | S<sub>y</sub> |
| Artvin P.A       | Artvin         | Medium        | Yellow      | Soft     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Canakkale        | Canakkale      | Medium        | Yellow      | Firm     | Medium    | Fresh    | SC                    | S<sub>y</sub> |
| Cataloglu        | Malatya        | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Cekirge 52       | Bursa           | Medium        | Light Orange| Firm     | Medium    | Fresh    | SF                    | S<sub>y</sub> |
| Cigli            | İzmir          | Medium        | Yellow      | Firm     | Small     | Fresh    | SI                     | S<sub>y</sub> |
| Cologlu          | Malatya        | Medium        | Yellow      | Firm     | Small     | Dried    | SI                     | S<sub>y</sub> |
| Dörrtyol 2       | Hatay          | Medium        | Yellow      | Medium   | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Dörrtyol 4       | Hatay          | Medium        | Light Orange| Soft     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Ethembev         | Edirne         | Medium        | Light Orange| Firm     | Medium    | Fresh    | SC                    | S<sub>y</sub> |
| Gec Aprikoz      | Iğdır          | Medium        | Yellow      | Firm     | Big       | Fresh    | SI                     | S<sub>y</sub> |
| Güz Aprikoz      | Malatya        | Late          | Yellow      | Medium   | Big       | Fresh    | SI                     | S<sub>y</sub> |
| Haci Hallioglu   | Malatya        | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Hackiz           | Malatya        | Medium        | Yellow      | Very firm | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Hasanbey         | Malatya        | Medium        | Yellow      | Very firm | Medium    | Fresh-Dried | SI                     | S<sub>y</sub> |
| Imrahor          | İzmir          | Medium        | Yellow      | Firm     | Small     | Dried    | SI                     | S<sub>y</sub> |
| Iri Bitirgen     | Tekirdag       | Medium        | Light Orange| Medium   | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Ismailaga        | Malatya        | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Kabasli          | Malatya        | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Kadioglu         | Malatya        | Medium        | Orange      | Small    | Dried    | SI                     | S<sub>y</sub> |
| Kamelya          | İzmir          | Medium        | Light Orange| Firm     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Karacabey        | Bursa           | Medium        | Orange      | Very firm | Medium    | Fresh    | SC                    | S<sub>y</sub> |
| Kayisi Erigi     | Nigde          | Medium        | Yellow      | Firm     | Firm      | Fresh    | S<sub>y</sub> | S<sub>y</sub> |
| Kayseri P.A      | Kayseri        | Medium        | Yellow      | Firm     | Firm      | Fresh    | S<sub>y</sub> | S<sub>y</sub> |
| Kurukubuk        | Malatya        | Medium        | Light Orange| Medium   | Medium    | Dried    | SI                     | S<sub<y</sub> |
| Levent           | Malatya        | Very late     | Yellow      | Medium   | Medium    | Fresh    | SC                    | S<sub>y</sub> |
| Mahmudun Erigi   | Erzincan       | Medium        | Yellow      | Very firm | Medium    | Fresh    | S<sub<y</sub> |
| Mkctep           | İzmir          | Medium        | Yellow      | Medium   | Medium    | Fresh    | SC                    | S<sub>y</sub> |
| No 1 Zerdali     | Van            | Medium        | Yellow      | Soft     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| No 2 Zerdali     | Konya          | Medium        | Yellow      | Very late| Soft      | Fresh    | SI                     | S<sub>y</sub> |
| Ordubat          | Iğdır          | Medium        | Yellow      | Very soft| Small     | Dried    | SI                     | S<sub>y</sub> |
| Ozal             | Malatya        | Very late     | Yellow      | Soft     | Small     | Fresh    | SI                     | S<sub>y</sub> |
| Pasa Mismisi     | Malatya        | Medium        | Yellow      | Medium   | Big       | Dried    | SC                    | S<sub>y</sub> |
| Sakit 1          | Hatay          | Medium        | Yellow      | Medium   | Medium    | Fresh    | SI<sup>+</sup> | S<sub>y</sub> |
| Sakit 3          | Hatay          | Medium        | Light Orange| Soft     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Shalakh (Aprikoz)| Iğdır          | Medium        | Yellow      | Soft     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Sam              | İzmir          | Medium        | Yellow      | Soft     | Medium    | Fresh    | SC                    | S<sub>y</sub> |
| Sebbiyiki        | Malatya        | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Sealatoglu       | Malatya        | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Sekerpare        | Erzincan       | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Soganci          | Malatya        | Medium        | Yellow      | Firm     | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Tokaloglu Izmir  | İzmir          | Medium        | Light Orange| Firm     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| Tufanda İzmir    | İzmir          | Early         | Yellow      | Medium   | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| X1 Zerdali       | Malatya        | Medium        | Yellow      | Firm     | Medium    | Fresh    | SI                     | S<sub>y</sub> |
| X2 Zerdali       | Malatya        | Medium        | Yellow      | Firm     | Small     | Fresh    | SI                     | S<sub>y</sub> |
| X3 Zerdali       | Malatya        | Medium        | Yellow      | Firm     | Small     | Fresh    | SI                     | S<sub>y</sub> |
| Yegen            | Malatya        | Medium        | Light Orange| Firm     | Very soft | Medium    | Dried    | SI                     | S<sub>y</sub> |
| Yerli İzmir      | İzmir          | Medium        | Yellow      | Medium   | Medium    | Fresh    | SC                    | S<sub>y</sub> |
| Ziraat Okulu     | Malatya        | Medium        | Yellow      | Medium   | Medium    | Big      | Fresh    | SI                     | S<sub>y</sub> |

<sup>SI</sup> = self-incompatible, <sup>SC</sup> = self-compatible; self-(in)compatibility phenotypes supported by fruit set ratios after self-pollination (Gülcen et al., 2006) are underlined.

<sup>1</sup>The proposed self-(in)compatibility phenotype is not supported by fruit set ratios after self-pollination (Gülcen et al., 2006).

<sup>2</sup>Unknown alleles were labeled as S<sub>y</sub>.

<sup>3</sup>"Kayisi Erigi" is a natural hybrid of <i>Prunus armeniaca</i> and <i>Prunus salicina</i>; hence, its unidentified S<sub>y</sub>-allele was transmitted from the plum parent.
For nine Turkish cultivars (Alyanak, Artvin P.A., Dörtl yol 4, Hasanbey, Iri Bitirgen, Karacabey, Sam, Sebbiyiki, and Ziraat Okulu) and two control cultivars (Moniquía 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 Güney, Imrahor, Kayseri P.A., Sakit 3, Sekerpare, and Tokaloglu Izmir) and Sunglo, confirming this allele as $S_3$. Twelve cultivars (Cataloglu, Hacikiz, Gec Aprikoz, Güz Aprikozu, Iri Bitirgen, Levent, Özal, No 1 Zerdali, Sakit 1, Sekerpare, Soganci, and X1 Zerdali) yielded a fragment of ≈1300 bp, similar to ‘Moniquía’ ($S_2 S_6$) and hence this allele was labeled $S_6$. The allele $S_7$ occurred in eight cultivars (Agerik, Artvin P.A., Cigli, Kurukabuk, Ordubat, Turfanda Izmir, X2 Zerdali, and Yerli Izmir) as a fragment of ≈820 bp.

An ≈500-bp fragment was detected in 18 cultivars (Adilecvaž 5, Akcadag Güney, Cataloglu, Cekirge 52, Cologlu, Dört yol 2, Haci Haliloglu, Hasanbey, Ismailağa, Kabası, Kadioglu, Kamelya, Kurukabuk, No 2 Zerdali, Özal, Seftaloglu, 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, Ismailağa, Kayısı Eriği, Shalak, 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 (Adilecevaz 3, Adilecevaz 5, Agerik, Haci Haliloglu, Kabası, Kamelya, Mahmudun Eriği, No 2 Zerdali, and Shalak), 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 (Adilecevaz 1, Adilecevaz 3, Cigli, Dörtl yol 4, Levent, Sakit 1, Sakit 3, Sebbiyiki, and Tokaloglu Izmir).

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

‘Kayısı Eriği’ 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 Aprikozu’ and ‘Imrahor’, 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’ ($S_2 S_3$) and ‘Moniquía’ ($S_2 S_6$) 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...
SC- and S8-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 SC and S8, a specific primer (AprSC8) was designed to anneal exclusively within the second intron region of the SC- and S8-RNase alleles. This primer pair selectively amplified a fragment of 547 bp only in the case of S8/SC-alleles, as demonstrated by using control cultivars Ceglédi óriás and Bergeron (SC, S2). In contrast, there was no amplification in ‘Goldrich’ (S1, S2) when used as a negative control (Fig. 3A). The presence of S8/SC-alleles was indeed confirmed among the tested 18 Turkish cultivars.

Because coding regions of the SC- and S8-RNase alleles are identical, discrimination of SI from SC cultivars could not be achieved in this analysis. The SC- and S8-haplotypes differ only in the SFB gene in that an insertion of 358 bp can be found in the SFBc, 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 SFBc-allele showed an amplification product of ≈500 bp, while genotypes carrying the SFBb-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’ (SSC), was identified. Three cultivars shared the SC-S8-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. S8 was the most frequent S-allele in the tested Turkish germplasm (occurring in 18 cultivars), followed by S8 (14), S8 (12), S2, S13, and S19 (9), S7 (8), SC (7), S3 (6), S11, and S12 (5), while S20-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 Nyéké, 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 S1-S1 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 these 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, Imrahor, 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 SC-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 S11–S14 were only detected in apricots of Armenian origin (Halász...
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 $S_{10}$-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 ($S_9$) 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 Magyarkajszi cultivars are well known in central Europe, and all share the $S_C S_9$ genotype. Among the Turkish cultivars, three have the same $S_C S_9$ 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 Magyarkajszi cultivars. It would be interesting to know whether these cultivars or their ancestors contributed to the formation of the present Hungarian Magyarkajszi 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

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**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                            | *Goldrich, Hargrand, Lambertin-I* | $S_7 S_2$ |
| II                           | Cologlu, Kadioglu, Seftalioglu, *Cegledi őrás, Ligeti őrás* | $S_6 S_9$ |
| III                          | Iri Bittirgen, *Moniqui* | $S_8 S_6$ |
| IV                           | Artvin P.A, *Priana* | $S_2 S_7$ |
| V                            | Aylanak, Ziraat Okulu | $S_2 S_8$ |
| VI                           | Dörtyol 4, Sebbyikí | $S_2 S_9$ |
| VII                          | Sakit 3, Tokaloglu Izmir | $S_6 S_9$ |
| VIII                         | Cataloglu, Ozal, Saganci | $S_6 S_8$ |
| IX                           | No 1 Zerdali, X1 Zerdali | $S_6 S_7$ |
| X                            | Ordubat, X2 Zerdali | $S_6 S_6$ |
| XI                           | Adilcevaz 5, Haci Haliloglu, Kabaasi, Kamelya, No 2 Zerdali | $S_6 S_7$ |
| XII                          | Shalakh (Aprikoz), *Voski* | $S_6 S_6$ |
| XIII                         | Levent, Sakit 1* | $S_6 S_7$ |
| XIV                          | Cekirge 52*, X3 Zerdali | $S_6 S_6$ |
| 0: universal pollen donors   | Canakkale ($S_C S_C$), Ethembey ($S_C S_9$), Karacabey ($S_C S_2$), Mektep ($S_C S_9$), Pasa Mismisi ($S_C S_9$), Sam ($S_C S_2$), Yerli Izmir ($S_C S_2$) | $S_6 S_7$ |
| Adilcevaz 1 ($S_6 S_9$), Adilcevaz 3 ($S_6 S_5$), Agerik ($S_6 S_3$), Akcadag ($S_6 S_6$), Alioglu 49 ($S_8 S_12$), Cigli ($S_6 S_11$), Gec Aprikoz ($S_8 S_11$), Hacikiz ($S_8 S_6$), Hasanbey ($S_6 S_6$), Ismailaga ($S_8 S_13$), Kayseri P.A ($S_8 S_7$), Kurukabuk ($S_8 S_7$), Sekerpare ($S_8 S_6$), Tufandah Izmir ($S_7 S_8$), Yegen ($S_8 S_1$) | $S_6 S_7$ |

*Further analyses are required to confirm self-incompatibility phenotype.*
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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