Determination of self-incompatibility groups of sweet cherry genotypes from Turkey

A. Ipek[1], H. Gulen[1], M.E. Akcay[2], M. Ipek[1], S. Ergin[1] and A. Eris[1]

[1]Horticulture Department, Faculty of Agriculture, Uludag University, Gorukle Bursa, Turkey
[2]Ataturk Central Horticultural Research Institute, Yalova, Turkey

Corresponding author: A. Ipek
E-mail: maipek@uludag.edu.tr

ABSTRACT. Determination of S-allele combinations of sweet cherry genotypes and cultivars has importance for both growers and breeders. We determined S-allele combinations of 40 local Turkish sweet cherry genotypes using a PCR-based method. Ten different S-alleles were detected. Although the most common S-allele was S_3, as also found in Western genotypes and cultivars, there were some differences in the frequencies of some S-alleles between Turkish and Western sweet cherry genotypes. According to their S-allele compositions, 30 local Turkish sweet cherry genotypes were assigned to 10 previously identified incompatibility groups. For the remaining genotypes, whose S-allele combinations did not fit to any previous incompatibility groups, three more incompatibility groups, XLII, XLIII and XLIV, were proposed. Results obtained from this study will help both sweet cherry growers and breeders to better manage these local Turkish sweet cherry genotypes in their orchards.

Key words: Sweet cherry; S-allele; *Prunus avium* L.; S-genotyping; Incompatibility groups

INTRODUCTION

Turkey is a leading sweet cherry (*Prunus avium* L.) producer and exporter country in the world. In 2007, Turkey produced 392,000 metric tons of sweet cherry and exported 57,019 metric tons of this production (FAO, 2007). This significant sweet cherry production has been made using both local and introduced cultivars. Since Anatolia is an important origin and site for domestication of sweet cherry (Watkins, 1976; Gisbert et al., 2008), Turkey has many local sweet cherry cultivars and genotypes whose S-allele combination has not been determined yet. On the other hand, genetic relationships among local Turkish sweet cherry genotypes have been analyzed using DNA markers (Gulen et al., 2010).

During the establishment of new sweet cherry orchards, appropriate pollinators have to be planted to obtain an acceptable fruit set. To do this, cross-(in)compatibility between the major cultivar and pollinator(s) has to be determined prior to establishment of commercial sweet cherry orchards. In addition, determination of cross-(in)compatibility between cultivars is also important for cross breeding of sweet cherry when specific crosses between two cultivars are desired. Therefore, incompatibility groups of western sweet cherry genotypes have been determined with recent studies (Tobutt et al., 2004; Schuster et al., 2007; Gisbert et al., 2008).

Incompatibility in sweet cherry is controlled by multi-allelic S-locus and it is a gametophytic self-incompatibility system (Crane and Lawrence, 1929). In this system, self-incompatibility occurs by arresting pollen tube growth if both the haploid genome of pollen and the diploid genome of style carry the same S-allele. S-alleles in sweet cherry encode for stylar ribonucleases (S-RNase) that inhibit pollen tube growth in style by degrading pollen RNA (Boskovic and Tobutt, 1996; Boskovic et al., 1997).

Recent studies demonstrated that S-RNase genes in *Prunus* are composed of several conserved domains and two introns (Ushijima et al., 1998; Tao et al., 1999). Varying sizes of these two introns interrupting the coding region of S-allele genes have been successfully used to identify S-alleles in sweet cherry genotypes using a polymerase chain reaction (PCR)-based method (Tao et al., 1999; Sonneveld et al., 2001, 2003). Currently, this method has been extensively used for S-genotyping of S-alleles (S1 to S25) in sweet cherry by various studies (Wiersma et al., 2001; Wunsch and Hormaza, 2004; De Cuyper et al., 2005; Schuster et al., 2007; Gisbert et al., 2008). However, S-allele combinations of Turkish sweet cherry genotypes have not been previously determined by S-genotyping to our knowledge. In this study, S-allele combinations of local Turkish sweet cherry genotypes were determined using a PCR-based method for S-genotyping of sweet cherry.

MATERIAL AND METHODS

Plant materials and DNA extraction

A total of 40 local Turkish sweet cherry genotypes were analyzed in the study (Table 1). The young leaf samples of these genotypes were collected from a sweet cherry genetic resource orchard at the Atatürk Central Horticultural Research Institute in Yalova, Turkey, where Turkish sweet cherry germplasm is maintained. Genetic relationships among these lo-

cal Turkish sweet cherry genotypes were determined using AFLP (amplified fragment length polymorphisms) and SSR (simple-sequence repeats) markers (Gulen et al., 2008, 2010). DNA samples were extracted from 150 mg lyophilized and powdered young leaves of these genotypes using the modified CTAB protocol described by Futterer et al. (1995). The DNA concentration of leaf samples was measured by using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and adjusted to 20 ng/µL. DNA samples were stored at -20°C in TE buffer until use.

PCR procedure

Consensus primer pairs for amplification of introns I and II developed by Sonneveld et al. (2003) were used to amplify introns I and II of the S-RNase gene in local Turkish sweet cherry genotypes. Alleles of the S-RNase gene were also amplified using allele specific primer pairs for detection of S1-S16 alleles in local Turkish sweet cherry genotypes (Sonneveld et al., 2001, 2003). Each 25 µL PCR for amplification of S-alleles consisted of 1 U DNA polymerase (Bioron, Ludwigshafen, Germany) with the reaction buffer supplied at 1X concentration, 0.2 µM of each primer, 2 mM $MgCl_2$, dNTPs at 0.25 mM each, and 50 ng template DNA. Thermal cycling conditions were 2 min at 94°C, 35 cycles of 45 s at 94°C, 1 min at the appropriate annealing temperature for each specific primer pair (Sonneveld et al., 2001, 2003) and 2 min (4 min for amplification of intron II) at 72°C and a final extension step of 5 min at 72°C. For these reactions, an Applied Biosystems Thermal Cycler was used. PCR products of intron II and S-alleles (S_1-S_{16}) were separated on a 1.5% agarose gel (Sigma, St. Louis, MO, USA) in 0.5X Tris-borate (TBE) buffer, but the PCR product of intron I was separated on 2% agarose gel. Gels were stained with ethidium bromide (0.5 mg/mL) (Sigma) and photographed.

RESULTS AND DISCUSSION

In order to determine the S-allele composition of local Turkish sweet cherry genotypes, parts of the S-RNase gene spanning introns I and II were amplified with PaConsI and PaConsII consensus primer pairs, respectively (Sonneveld et al., 2003). The amplification of the S-RNase gene with the consensus primers flanking intron I enabled us to determine the sweet cherry genotypes carrying S_3S_{10}, but the amplification of intron I was not enough to discriminate S_4 from S_6, S_1 from S_5 or S_2, S_7, S_9, and S_{12} from each other (Table 1; Figure 1). On the other hand, amplification of intron II allowed us to identify the sweet cherry genotypes carrying S_2, S_3, S_4, S_5, S_6, S_7, S_9 but discrimination of S_1 from S_{13} and S_{10} from S_{14} required additional analysis (Table 1). With PaConsII consensus primer pair, S_1 and S_{13} alleles had the same size of DNA fragment amplified and the sizes of amplified DNA fragments for S_{10} and S_{14} alleles were too close to discriminate them on an agarose gel (Sonneveld et al., 2001, 2003). In order to verify S-allele combinations of local Turkish sweet cherry genotypes determined by consensus primers, the S-RNase gene of each local sweet cherry genotype was amplified using S-allele (S_1-S_{16})-specific primer pairs developed by Sonneveld et al. (2001, 2003) (Figure 1). The S-allele combination of each local Turkish sweet cherry genotype was concluded after combining the results of consensus and S-allele-specific primer pairs (Table 1).

Table 1. S-allele compositions and incompatibility groups of local Turkish sweet cherry genotypes.

Genotypes	S-genotyping with consensus primers		S-allele composition based on allele specific primers													S-allele composition	Incompatibility groups
	First intron	Second intron	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_9	S_{10}	S_{12}	S_{13}	S_{14}	S_{16}		
0843	$S_3/S_4,S_5$	S_3/S_4			+	+		-								S_3S_4	Group III
0848	$S_3/S_4,S_6$	S_3/S_4			+	+		-								S_3S_4	Group III
0894	$S_3/S_5,S_6$	S_3/S_4			+	+		-								S_3S_4	Group III
Cemal	$S_3/S_4,S_6$	S_3/S_4			+	+		-								S_3S_4	Group III
Elifti	$S_3/S_4,S_6$	S_3/S_4			+	+		-								S_3S_4	Group III
Sultan Hisar	$S_3/S_4,S_6$	S_3/S_4			+	+		-								S_3S_4	Group III
Acı Kara 0922	$S_3/S_3,S_6,S_5,S_{12}$	S_2/S_3		+	+				-	-		-				S_2S_3	Group IV
Aydın Siyahı	$S_3/S_2,S_5,S_9,S_{12}$	S_2/S_3		+	+				-	-		-				S_2S_3	Group IV
Edirne	$S_3/S_3,S_5,S_6,S_{12}$	S_2/S_3		+	+				-	-		-				S_2S_3	Group IV
Kara 0908	$S_3/S_3,S_5,S_6,S_{12}$	S_2/S_3		+	+				-	-		-				S_2S_3	Group IV
Sarı	$S_3/S_3,S_5,S_6,S_{12}$	S_2/S_3		+	+				-	-		-				S_2S_3	Group IV
Zeytitali 0906	$S_3/S_3,S_5,S_9,S_{12}$	S_2/S_3		+	+				-	-		-				S_2S_3	Group IV
0889 Yabani	$S_3/S_4,S_6$	-			+	-		+	-	+		-				S_3S_6	Group VI
Artvin 4	$S_3/S_5,S_6$	S_3/S_6			+	-		+	-	+		-				S_3S_6	Group VI
Abdullah	$S_4,S_6/S_2,S_7,S_3,S_9,S_{12}$	S_6/S_9		-		-		+	-	+		-				S_6S_9	Group X
Kadı 0878	$S_6/S_2,S_7,S_9,S_6,S_{12}$	S_6/S_9		-		-		+	-	+		-				S_6S_9	Group X
Turfanda	$S_6/S_6,S_9$	S_6/S_9		-		-		+	-	+		-				S_6S_9	Group X
Yalancı Napolyon	$S_1S_2/S_1,S_5$	$S_1,S_{13}/S_5$	+				+						-			S_1S_5	Group XIV
Şekerpare	S_1/S_5	$S_1,S_{13}/S_6$	+				-	+		+						S_1S_6	Group XVI
Halil Efendi	$S_1,S_2/S_3,S_6$	$S_1,S_{13}/S_6$		-	+			+		+						S_1S_6	Group XX
0900 Ziraat	$S_3/S_3,S_5,S_6,S_{12}$	S_3/S_{12}		-	+	-			-			+				S_3S_{12}	Group XXII
Akşehir Napolyonu	$S_3/S_2,S_5,S_6,S_{12}$	S_3/S_{12}		-	+	-			-			+				S_3S_{12}	Group XXII
Allahdiyen	$S_3/S_3,S_5,S_6,S_{12}$	S_3/S_{12}		-	+	-			-			+				S_3S_{12}	Group XXII
Karakiraz	$S_3/S_3,S_5,S_6,S_{12}$	S_3/S_{12}		-	+	-			-			+				S_3S_{12}	Group XXII
Kazancıoğlu	$S_3/S_3,S_5,S_6,S_{12}$	S_3/S_{12}		-	+	-			-			+				S_3S_{12}	Group XXII
Uluborlu	$S_3/S_3,S_5,S_6,S_{12}$	S_3/S_{12}		-	+				-			+				S_3S_{12}	Group XXII
Kaman Çayırı 0895	$S_2,S_5,S_9,S_{12}/S_4,S_6$	S_2/S_6		+		-		+	-			-				S_2S_6	Group XXV
Kırdar	$S_2,S_5,S_9,S_{12}/S_4,S_6$	S_2/S_6		+		+		+	-			-				S_2S_6	Group XXV
Niğde	$S_4,S_6/S_2,S_7,S_9,S_6,S_{12}$	S_4/S_7		-		+		-	+			-				S_4S_7	Group XXIX
Sultan	$S_4,S_6/S_4,S_7$	S_4/S_7		-		+		-	+			-		-		S_4S_7	Group XXIX
Bademli 0898	$S_2,S_5,S_9,S_{12}/S_{10}$	$S_2/S_{10,14}$		+							+			-		S_2S_{10}	Group XLII†
Tabanlı	$S_2,S_5,S_9,S_{12}/S_{10}$	$S_2/S_{10,14}$		+							+			-		S_2S_{10}	Group XLII
Kara Turani 0888	$S_2,S_7,S_9,S_{12}/S_2,S_7,S_9,S_{12}$	S_2/S_9		+	+	-	+	+	-	+		-				S_2S_9	Group XLIII†
Ferprime*	-	-														S_2S_9	Group XLIII
Primulat**	-	-														S_2S_9	Group XLIII
Acı Bursa	$S_3/S_2,S_3,S_9,S_{12}$	S_3/S_7	-		+				+							S_3S_7	Group XLIV†
Augustkirsche*	-	-			+				+							S_3S_7	Group XLIV
Er. Amasya	$S_4,S_6/--$	$S_4/--$				+		-								S_4-	-
Turfanda Kara	$S_4,S_6/--$	$S_4/--$				+		-								S_4-	-
0885 Erice	$S_3,S_6,S_4,S_7/S_{10}$	$S_3/S_6/S_{10,14}$			+	-		+		+	+					$S_2S_6S_{10}$	-
57	S_2,S_6,S_5,S_9,S_{12}	$S_2/S_5,S_9,S_{14}$			+	-		+	-	+	+					$S_2S_6S_9$	-
0918	S_3,S_6,S_9,S_{10}	$S_3/S_5,S_{10,14}$		+	+	+		+		+	+			-		$S_3S_5S_{10}$	-
Kara Gevrek	$S_3/S_5,S_9,S_6$	$S_3/S_5,S_6$	-	+	+	+	+	+								$S_3S_5S_6$	-

*S-allele combination determined by Schuster et al. (2007). **S-allele combination determined by Gisbert et al. (2008). †Proposed new incompatibility groups.
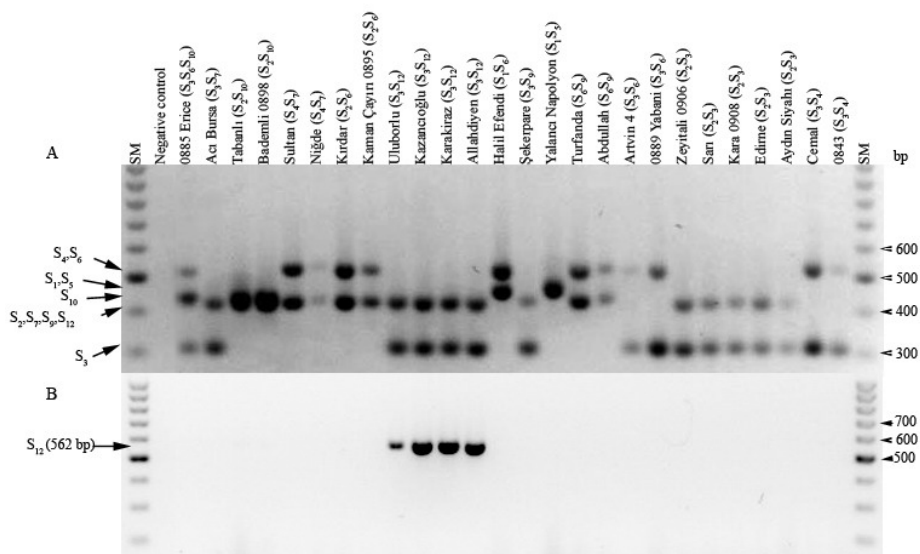
Figure 1. Polymerase chain reaction (PCR) amplification of alleles of S-RNAse in 26 local Turkish sweet cherry genotypes. **A.** With consensus primer pair for intron I. **B.** With an S-allele specific primer for the S_{12} allele. SM = DNA molecular weight markers (100-bp ladder). Water was added to PCR for negative control.

S-genotyping of 40 local Turkish sweet cherry genotypes allowed detection of 10 different S-alleles; S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_9, S_{10}, and S_{12} (Table 1; Figure 1). Although these genotypes were also screened with primer pairs developed for detecting S_{13}, S_{14} and S_{16} alleles, the presence of these alleles was not observed. There were two alleles amplified in 36 local Turkish sweet cherry genotypes. However, in four genotypes, '57', '0918', 'Kara Gevrek', and '0885 Erice', three alleles were amplified suggesting that these genotypes were triploid or S-locus was duplicated (Table 1; Figure 1). In another study, De Cuyper et al. (2005) detected the presence of a triploid genotype in Belgian wild cherry genotypes.

A 381-bp DNA fragment was amplified with consensus primers for intron I in 'Er. Amasya 0849' and 'Turfanda Kara' (data not shown). After cloning and sequencing the 381-bp DNA fragment, the nucleotide sequence of this DNA fragment was blasted against the sequences in GenBank using the BLASTN program at NCBI (Zhang et al., 2000) and matched with the sequences of the S_4 allele of sweet cherry with over 90% identity (data not shown). There were deletions and insertions and single-nucleotide polymorphisms in the coding region of this allele compared to the S4 allele. However, we were unable to amplify this new allele with consensus primer pair for the amplification of intron II. This new allele of S-RNase has to be further characterized before it can be added as a new sweet cherry S-allele to the literature.

Tobutt et al. (2004) compiled the S-genotypes of some 222 self-incompatible and 25 self-compatible cultivars of cherry using data from previous study on S-genotyping. The allele frequencies of S_1, S_2, S_3, S_4, S_5, S_6, and S_9 were 21, 13, 29, 15, 7, 11, and 6%, respectively (Lacis et al., 2008). In our study, the frequencies of S_2 (14.8%), S_3 (29.6%), S_4 (13.6%), S_6 (11.1%), and S_9 (7.5%) were similar to those published by Tobutt et al. (2004) although occurrences of S_1 (2.5%) and S_5 (4.9%) were lower and frequencies of S_7 (3.7%) and S_{10} (4.9%) were higher in local Turkish sweet cherry genotypes (Figure 2).
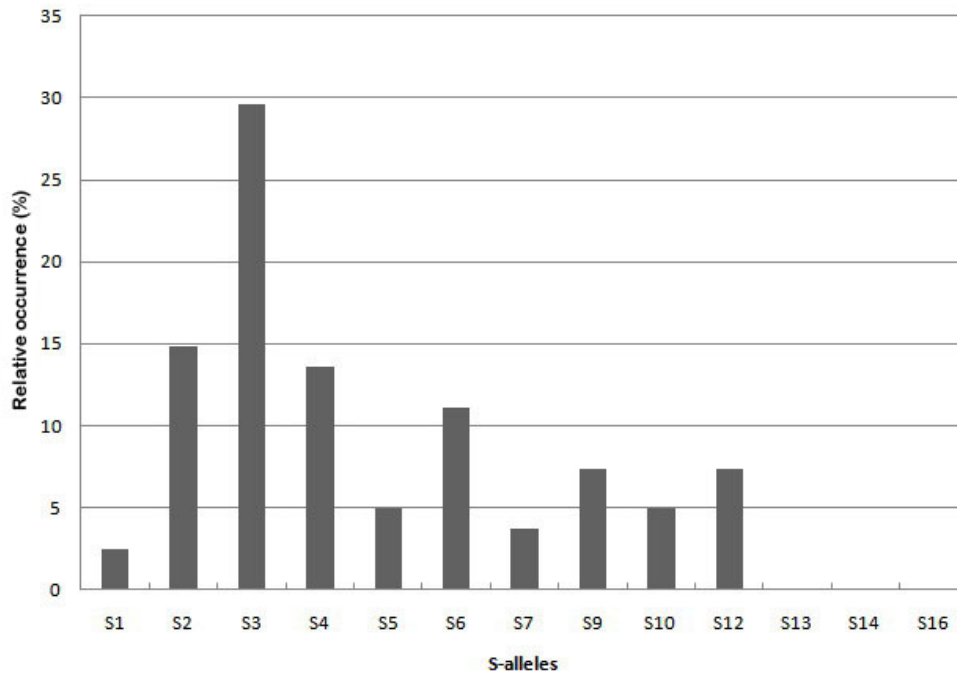
Figure 2. Frequency of S-alleles in local Turkish sweet cherry genotypes.

The S_{12} allele was quite common in Turkish genotypes, with 7.4%, although it was a very rare allele in the western sweet cherry genotypes (Tobutt et al., 2004). '0900 Ziraat', the most common cultivated Turkish sweet cherry cultivar, has an S-allele composition of S_3S_{12}; therefore, introduced (foreign origin) sweet cherry cultivars have been successfully used as pollinators for '0900 Ziraat' in sweet cherry orchards in Turkey. Lynn E. Long, an Oregon State University Extension Horticulturist, compared '0900 Ziraat' and 'Schneiders', and suggested that '0900 Ziraat' and 'Schneiders' are the same genotypes since both cultivars have similar morphological characteristics and carry the rare S-allele S_{12} (Long, 2006). The author indicated that the S_{12} allele was present in only two cherries out of over 200 western sweet cherry genotypes examined. In our study, however, 6 of 40 Turkish cultivars had allelic combination of S_3S_{12}, suggesting that S_{12} is not a very rare allele in Turkish sweet cherry genotypes. Therefore, we suggest using DNA molecular markers, such as SSR or AFLP, for better estimation of the genetic relationship between '0900 Ziraat' and 'Schneiders'.

After determination of S-allele compositions, 30 local Turkish sweet cherry genotypes were assigned to 10 previously determined incompatibility groups (Table 1). Most common incompatibility groups were Group III, Group IV and Group XXII with 6 genotypes in each. There was no previously assigned incompatibility group for the S-allele combination of remaining genotypes. A new incompatibility group, XLII, was assigned for 'Bademli 0898' and 'Tabanlı' containing S_2S_{10} alleles. The remaining four cultivars, 'Kara Turani 0888' (S_2S_9) and 'Acı Bursa' (S_3S_7), have unique S-allele combinations and there were no previously determined incompatibility groups. Previous studies reported that 'Ferprime' and 'Primulat' have the same S-alleles as 'Kara Turani 0888' (S_2S_9) (Schuster et al., 2007; Gisbert et al., 2008). Similarly, 'Acı Bursa'

and 'Augustkirsche' (Schuster et al., 2007) have the same allelic combination of S_3S_7. Therefore, incompatibility group XLIII was allocated for 'Kara Turani 0888', 'Ferprime', and 'Primulat'. Another incompatibility group, XLIV, was assigned for 'Acı Bursa' and 'Augustkirsche'. Genotypes carrying a new S-allele or triploid S-alleles were not assigned to incompatibility groups.

S-allele combinations of western sweet cherry germplasm and sweet cherry genotypes from the breeding programs in Europe, Canada and the U.S. have previously been determined (Wiersma et al., 2001; Sonneveld et al., 2001, 2003; Tobutt et al., 2001, 2004; De Cuyper et al., 2005; Schuster et al., 2007; Marchese et al., 2007; Gisbert et al., 2008; Lacis et al., 2008; Stanys et al., 2008). These studies on the S-genotyping of sweet cherry assigned the sweet cherry genotypes to various incompatibility groups, which is useful for both sweet cherry growers and breeders. Tobutt et al. (2004) compiled the cherry S-genotyping data and determined 26 incompatibility groups. Schuster et al. (2007) extended it to 35 after S-genotyping sweet cherry accessions and cultivars in the German Fruit Gene Bank and private collections. With recent studies on the S-genotyping of sweet cherry genotypes by Marchese et al. (2007), Gisbert et al. (2008) and Stanys et al. (2008), the number of incompatibility groups has reached 41. Our results from the S-genotyping of 35 Turkish sweet cherry genotypes suggested additional three incompatibility groups and we proposed incompatibility groups XLII, XLIII and XLIV. These results indicated that the number of incompatibility groups will increase in the future with the S-genotyping of more sweet cherry genotypes from eastern origin and with the discovery of new S-alleles. This will make following incompatibility groups more complicated. On the other hand, establishment of a public data base containing information on the incompatibility groups of sweet cherry genotypes examined can serve the sweet cherry community by finding incompatibility groups of sweet cherry genotypes and cultivars of interest.

Information obtained from this study on S-allele combinations of local Turkish sweet cherry genotypes will be useful during the establishment of new commercial sweet cherry orchards. In order to maximize the fruit set and consequently yield, choosing the correct pollinator(s) for the main cultivar(s) of an orchard is very important and this information will help in making the correct pollinator choice in Turkey. For example, '0900 Ziraat' and 'Aksehir Napolyonu' are two common cultivars grown in Turkey. We found that these two cultivars are in the same incompatibility group, since both possess S_3S_{12} alleles. Sweet cherry orchards established using these two cultivars will not have satisfactory fruit set due to the lack of an appropriate pollinator. Similarly, Sutyemez and Eti (1999) cross-pollinated '0900 Ziraat' and 'Aksehir Napolyonu' by hand but satisfactory fruit set was not observed, confirming our study on S-allele genotyping. In addition, this information will also be valuable for sweet cherry breeders during the planning of specific crosses in their breeding programs.

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