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ARTICLE

Self-incompatibility alleles in important genotypes for apple breeding in Brazil

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Abstract: *The objective of this study was to identify self-incompatibility (S) alleles of advanced breeding selections of apple (Malus × domestica Borkh.). The S-alleles of 42 apple genotypes were analyzed by markers using allele-specific PCR amplification and amplicons digested with restriction endonucleases. Among the screened genotypes were cultivars, advanced selections, and accessions of the Apple Germplasm Bank of Epagri (Caçador, Santa Catarina, Brazil). Two S-alleles were identified in 36 genotypes, and only one S-allele was determined in the other six genotypes. In all, eleven S-alleles were identified among all the genotypes evaluated. The S_3 and S_5 alleles were most frequent (30.2% and 18.6%, respectively). The identification of S-alleles using molecular markers in important apple tree genotypes is useful for determination of compatible parents for breeding programs.*

Keywords: *Malus × domestica Borkh., artificial hybridization, S-RNases, S-alleles.*

INTRODUCTION

The development of new apple cultivars (*Malus* × *domestica* Borkh.) by classical breeding methods requires from 13 to 17 years of research (Sedov 2014). The process begins with the choice of parents that have traits of interest, which are then crossed to select new cultivars and their pollinizers (Denardi et al. 2019a). The possible parental combinations are restricted by the gametophytic self-incompatibility (GSI) system present in *Malus* (Pereira-Lorenzo et al. 2018). The GSI of a fertile plant is its inability to produce zygotes after self-pollination or pollination among individuals that have *S*-alleles in common (Muñoz-Sanz et al. 2020). The *S*-locus is responsible for determining self-incompatibility and is positioned on chromosome 17 of the apple genome (Maliepaard et al. 1998). Crosses between genetically compatible plants (even between species) are required to generate as many plants as possible with the greatest genetic variability (De Franceschi et al. 2016). The *S*-alleles of several apple cultivars and genotypes have not been genotyped, making it difficult to choose compatible parents for planned crosses.

Traditionally, the presence of *S*-alleles was determined indirectly by pollination and pollen tube growth tests (Bošković and Tobutt 1999), but this methodology is strongly influenced by the environment and requires replications in different growing seasons to ensure the reliability of this identification (Breen et al. 2016). The use of genetic markers to identify *S*-alleles, such as allele-specific

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primers, provides information on their distribution among apple genotypes (Long et al. 2010, Akbari et al. 2016, Larsen et al. 2016, Kasajima et al. 2017) and allows breeders to plan crosses between compatible genotypes (Morita et al. 2009, Breen et al. 2016).

In the GSI mechanism, if the pollen has the same *S*-allele as the pistil, the developed pollen-tubes are recognized and rejected by a pistil-specific ribonuclease (*S-RNase*) encoded by the *S*-locus. The *S-RNase* is always expressed in the pistil, but when the *S*-allele of the pollen is not the same as either of the two *S*-alleles expressed in the pistil, the *S-RNases* are inactivated by at least two genes specifically expressed in pollen - *S-locus F-box Brother* genes (Sassa et al. 2007). To date, 57 *S*-alleles of the *Malus S*-locus encoding a different *S-RNase* have been identified (Kim et al. 2016).

The only active public apple breeding program in Brazil is at the Agricultural Research and Rural Extension Company of Santa Catarina (Epagri), located at the Experimental Stations of Caçador and São Joaquim, SC. For breeding crosses, the Epagri Apple Breeding Program uses selections and cultivars resulting from their own crosses and/or those developed in other countries as parents. It is crucial for this breeding program to choose fully compatible parents for the planned crosses. The objective of the study was to use genetic markers to identify the *S*-alleles of 42 apple genotypes/cultivars used as parents in the Epagri Apple Breeding Program.

MATERIAL AND METHODS

Among the apple genotypes used in the Epagri Apple Breeding Program, a total of 42 were tested (Table 1). Of these, six are cultivars developed by Epagri, 20 are advanced selections developed by the Epagri Apple Breeding Program, and 16 are accessions from the Epagri Apple Germplasm Bank. These genotypes were grown in experimental orchards and on the premises of the Apple Germplasm Bank at the Epagri Experimental Station in the municipality of Caçador in the Midwestern region of the state of Santa Catarina (lat 26° 49' 5'' S, long 50° 59' 12'' W, alt 940 m asl).

Young and healthy leaves were collected from the 42 apple genotypes and deep frozen at -20 ºC in plastic bags until DNA extraction, which was performed according to the protocol proposed by Lefort and Douglas (1999) with modifications (Revers et al. 2005), using 0.1 g of ground plant tissue.

Table 1. The apple cultivars and the apple selections analyzed in this study and their parents

Cultivar/ selection	Parent 1 (♀)	Parent 2 (♂)	Cultivar/ Selection	Parent 1 (♀)	Parent 2 (♂)
21-300-13[1]	Unknown	Unknown	M-10/09[2]	Imperatriz [S3S5]	Cripps Pink [S2S23]
21-300-21[1]	Unknown	Unknown	M-11/00[2]	Fred Hough [S5S19]	Imperatriz [S3S5]
21-361-75[1]	Unknown	Unknown	M-11/01[2]	Fred Hough [S5S19]	Imperatriz [S3S5]
21-373-58[1]	Unknown	Unknown	M-11/92[2]	M-41	Gala [S2S5]
21-379-64[1]	Unknown	Unknown	M-12/00[2]	Fred Hough [S5S19]	Imperatriz [S3S5]
21-502-1[1]	Unknown	Unknown	M-13/00[2]	Fred Hough [S5S19]	Imperatriz [S3S5]
21-555-13[1]	Unknown	Unknown	M-13/91[2]	Princesa [S3S5]	Mollie's Delicious [S3S7]
141/38[2]	Baronesa [S3S9]	O.p.	M-15/01[2]	Fred Hough [S5S19]	Imperatriz [S3S5]
Co-op 8[3]	PRI 558-1	Mollie's Delicious [S3S7]	M-21/08[2]	M-47/94	Princesa [S3S5]
Co-op 14[3]	PRI 10-147	Mollie's Delicious [S3S7]	M-23/07[2]	M-46/94	M-13/91
Co-op 16[3]	PRI 764	PRI 672	M-3/02[2]	Fred Hough [S5S19]	Imperatriz [S3S5]
Co-op 24[3]	NJ. 125355	Prima [S2S10]	M-4/09[2]	Imperatriz [S3S5]	Catarina [S1S19]
Castel Gala[4]	Sport mutation of Gala		M-44/08[2]	Imperatriz [S3S5]	Catarina [S1S19]
D1R102T116[5]	Unknown	Unknown	M-53/08[2]	Imperatriz [S3S5]	Catarina [S1S19]
D1R103T245[5]	Unknown	Unknown	M-58/07[2]	Imperatriz [S3S5]	Baronesa [S3S9]
Galaxy[6]	Sport mutation of Gala		M-8/01[2]	Fred Hough [S5S19]	Imperatriz [S3S5]
Macfree[7]	McIntosh [S10S25]	48-177	M-9/07[2]	M-46/94	Imperatriz [S3S5]
SCS417 Monalisa[4]	Gala [S2S5]	Malus 4	Scifresh[6]	Braeburn [S9S24]	Gala [S2S5]
SCS426 Venice[4]	Imperatriz [S3S5]	Baronesa [S3S9]	SCS427 Elenise[4]	Imperatriz [S3S5]	Cripps Pink [S2S23]
M-1/02[2]	Fred Hough [S5S19]	Imperatriz [S3S5]	SCS416 Kinkas[4]	Fuji [S1S9]	PWR37T133
M-1/07[2]	M-47/94	Princesa [S3S5]	SCS425 Luiza[4]	Imperatriz [S3S5]	Cripps Pink [S2S23]

O.p: open pollinated. [1] Selections from Argentina. [2] Selections from the Epagri Apple Breeding Program. [3] Selections from the PRI disease-resistant apple breeding program. [4] Cultivar developed by Epagri. [5] Selections from USA. [6] Cultivar from New Zealand. [7] Cultivar from Ottawa Research Station breeding program.

Each polymerase chain reaction (PCR) contained 1 U of *Taq* DNA polymerase, 1x enzyme buffer, 2.00 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM of each primer (forward and reverse), and 50 ng of genomic DNA, with a final volume of 15 µL. Primers for the identification of 16 *S*-alleles of apple trees were used: S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_9, S_{10}, S_{16}, S_{19}, S_{20}, S_{22}, S_{23}, S_{24}, and S_{26} (Table 2).

The PCRs were performed in a T100™ thermocycler (BioRad®, California, USA) programmed for 3 min at 94 °C, followed by 30 cycles of 94 °C for 1 min, annealing at 54-62 °C (depending on the primer characteristics; see Table 2) for 1 min, and 72 °C for 1 min, followed by a final extension step (72 °C for 7 min).

Table 2. *S*-alleles tested for apple, respective sequences of each primer, and specific PCR conditions. Values in parentheses indicate the fragment size generated by digestion with the respective restriction enzymes

***S*-allele**	**Primers**	**Sequences (5' -> 3')**	**Annealing temperature ºC, enzyme**	**Amplified fragment** (bp)
S_1	FTC168	ATATTGTAAGGCACCGCCATATCAT	60	530
	FTC169	GGTTCTGTATTGGGGAAGACGCACAA		
S_2	OWB122	GTTCAAACGTGACTTATGCG	60	449
	OWB123	GGTTTGGTTCCTTACCATGG		
S_3	FTC177	CAAACGATAACAAATCTTAC	55	500
	FTC226	TATATGGAAATCACCATTCG		
S_4	FCT5	TCCCACAATACAGAACGAGA	60, *Taq*I	274 (194+77)
	OWB249	CAATCTATGAAATGTGCTCTG		
S_5	FTC10	CAAACATGGCACCTGTGGGTCTCC	59	346
	FTC11	TAATAATGGATATCATTGGTAGG		
S_6	FTC141	ATCAGCCGGCTGTCTGCCACTC	$58^{E\,45}$	850
	FTC142	AGCCGTGCTCTTAATACTGAATAC		
S_7	FTC143	ACTCGAATGGACATGACCCAGT	60	302
	FTC144	TGTCGTTCATTATTGTGGGATGTC		
S_9	OWB154	CAGCCGGCTGTCTGCCACTT	62	343
	OWB155	CGGTTCGATCGAGTACGTTG		
S_{10}	FTC12	CCAAACGTACTCAATCGAAG	60	209
	FTC228	ATGTCGTCCCGTGTCCTGAATC		
S_{10} modified[I]		AACAAATCTTAAAGCCCAGC	60, *Nar*I	-
		GGTTTCTTATAGTCGATACTTTG		
$S_3/S_5/S_{10}$ [II]		CAATTTACGCAGCARTATCAG	58	-
		TGTTTTGAATYGAAAATTARTTAGGAGT		
S_{16}	FTC5	TCCCACAATACAGAACGAGA	60, *Taq*I	274 (243+41)
	OWB249	CAATCTATGAAATGTGCTCTG		
S_{19}	FTC229	TCTGGGAAAGAGAGTGGCTC	60	304
	FTC230	TTTATGAACTTCGTTAAGTCTC		
S_{20}	FTC141	ATCAGCCGGCTGTCTGCCACTC	$60^{E\,45}$, *Nar*I	920 (800+120)
	FTC142	AGCCGTGCTCTTAATACTGAATAC		
S_{22}	FTC5	TCCCACAATACAGAACGAGA	60, *Taq*I	274 (199+44+31)
	OWB249	CAATCTATGAAATGTGCTCTG		
S_{23}	FTC222	CAATCGAACCAATCATTTGGT	60	237
	FTC224	GGTGTCATATTGTTGGTACTAATG		
S_{24}	FTC231	AAATATTGCAACGCACAGCA	60	580
	FTC232	TTGAGAGGATTTCAGAGATG		
S_{26}	FCT14	GAAGATGCCATACGCAATGG	54	194
	FTC9	TTTAATACCGAATATTGGCG		

$^{E\,45}$ = Extension of 45 sec. [I] Primer proposed by Kitahara & Matsumoto (2002). Reaction conditions: 3 min at 94 °C, followed by 30 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72°C for 1 min, with a final extension step at 72 °C for 10 min, set at 4 °C after concluding amplification. Amplified fragment of 282 bp for the *S*-alleles and after treatment with enzyme *Nar*I generates two fragments: 185 and 97 bp. [II] Primer proposed by Larsen et al. (2016) for the identification of alleles S_3, S_5, and S_{10}. Reaction conditions: 2 min at 94 °C, followed by 33 cycles at 94 °C for 20 sec, 58 °C for 20 sec, and 72 °C for 2 min, with a final step of 72 °C for 5 min, maintaining a fixed temperature of 4 °C after concluding amplification. Alleles with respective amplified fragment size before and after the treatment with enzyme *Taq*I: S_3 = 423 bp, 264 bp; S_5 = 399 bp, 273 bp; S_{10} = 382 bp, not fragmented by the restriction enzyme. FTC and OWB primers were developed by Broothaerts (2003).

For discrimination of the S_4, S_{16}, and S_{22} alleles, part of the PCR product (10 µL) was digested by the restriction enzyme *Taq*I (for 1 h in a 65 °C water bath). Likewise, for identification of the S_{20} allele, 10 µL of the PCR product was digested by the restriction enzyme *Nar*I (for 4 h in a 37 °C water bath). For the S_{10} modified and $S_3/S_5/S_{10}$ primers, PCR programming and restriction enzyme digestion are described in Table 2.

As a positive control for the presence of each *S*-allele, cultivars previously characterized for the respective *S*-allele were used (Table 3). The only exception was the S_{16} allele since no genotype is maintained by Epagri with this pre-identified allele. In addition, the same cultivars were used for primer optimization.

After PCR and respective digestions with restriction enzymes, if necessary, the amplification products were analyzed by 3% agarose gel electrophoresis using the 50 bp DNA marker to help identify the size of the PCR product. The gels were stained with GelRed® (Biotium, California, USA) and then observed and photographed with Kodak Gel Logic 212 Pro (Carestream, New York, USA), for registration and interpretation. The samples with bands that coincided with the size of the respective *S*-allele amplifications (Table 2) were considered to be present.

RESULTS AND DISCUSSION

At least one *S*-allele was identified in each genotype characterized (Table 4). The genotypes 'Castel Gala' (Epagri Apple Breeding cultivar) and 'Galaxy' were identified as S_2S_5. They are sport mutations of 'Gala' for early budding and skin color, respectively (Hawerroth et al. 2018, Denardi et al. 2019a), and as originally expected, had the same genotype as the original cultivar (Matsumoto et al. 1999).

Among the genotypes tested, the genotypes 21-300-21, 21-361-75, Co-op 24, M-11/92, 'MacFree', and 'SCS416 Kinkas' manifested only one of the *S*-alleles identified with the primer set used in this study: $S_9S_?$, $S_9S_?$, $S_2S_?$, $S_2S_?$, $S_{20}S_?$,

Table 3. Apple cultivars used as positive controls for the presence of each *S*-allele, their *S*-alleles reported in the literature, and respective references

Allele	Cultivar	Cultivar *S* genotype	Reference
S_1	Catarina	S_1S_{19}	Albuquerque Junior et al. (2011)
	Fuji	S_1S_9	Matsumoto et al. (1999)
S_2	Cripps Pink	S_2S_{23}	Broothaerts et al. (2004)
	Golden Delicious	S_2S_3	Matsumoto et al. (1999)
S_3	Golden Delicious	S_2S_3	Matsumoto et al. (1999)
	Imperatriz	S_3S_5	Albuquerque Junior et al. (2011)
S_4	Gloster	S_4S_{19}	Dreesen et al. (2010)
S_5	Gala	S_2S_5	Matsumoto et al. (1999)
	Joaquina	S_5S_{19}	Albuquerque Junior et al. (2011)
S_6	Marubakaido	S_6S_{26}	Agapito-Tenfen et al. (2015)
S_7	Akane	S_7S_{24}	Kitahara et al. (2000)
	Idared	S_3S_7	Janssens et al. (1995)
S_9	Baronesa	S_3S_9	Albuquerque Junior et al. (2011)
	Fuji	S_1S_9	Matsumoto et al. (1999)
S_{10}	Liberty	$S_3S_5S_{10}$	Broothaerts et al. (2004)
	McIntosh	$S_{10}S_{25}$	Kitahara and Matsumoto (2002)
S_{19}	Delicious	S_9S_{19}	Dreesen et al. (2010)
	Fred Hough	S_5S_{19}	Albuquerque Junior et al. (2011)
S_{20}	Mutsu	$S_2S_3S_{20}$	Dreesen et al. (2010)
S_{22}	Alkmene	S_5S_{22}	Dreesen et al. (2010)
S_{23}	Cripps Pink	S_2S_{23}	Broothaerts et al. (2004)
	Granny Smith	S_3S_{23}	Dreesen et al. (2010)
S_{24}	Braeburn	S_9S_{24}	Dreesen et al. (2010)
	Primícia	$S_{24}S_?$	Albuquerque Junior et al. (2011)
S_{26}	Marubakaido	S_6S_{26}	Agapito-Tenfen et al. (2015)

$S_?$: another unidentified *S*-allele.

Table 4. *S*-alleles identified in relevant apple genotypes for the Epagri Apple Breeding Program

Cultivar/selection	*S*-alleles	Cultivar/selection	*S*-alleles
21-300-13	S_1S_3	M-9/07	S_3S_{19}
21-300-21	$S_9S_?$	M-10/09	S_5S_{23}
21-361-75	$S_9S_?$	M-11/00	S_3S_{19}
21-373-58	$S_3S_5S_9$	M-11/01	S_3S_5
21-379-64	S_3S_{19}	M-11/92	$S_2S_?$
21-502-1	S_1S_{24}	M-12/00	S_3S_5
21-555-13	S_3S_{19}	M-13/00	S_3S_{19}
141/38	S_2S_9	M-13/91	S_3S_5
Co-op 8	$S_3S_7S_{19}$	M-15/01	S_3S_{19}
Co-op 14	S_3S_5	M-21/08	S_3S_5
Co-op 16	S_3S_5	M-23/07	S_3S_5
Co-op 24	$S_2S_?$	M-44/08	S_1S_5
Castel Gala	S_2S_5	M-53/08	S_3S_{19}
D1R102T116	S_3S_{24}	M-58/07	S_5S_9
D1R103T245	S_3S_{24}	Macfree	$S_{20}S_?$
Galaxy	S_2S_5	Scifresh	S_2S_{24}
M-1/02	S_3S_{19}	SCS427 Elenise	S_3S_{23}
M-1/07	S_3S_5	SCS416 Kinkas	$S_9S_?$
M-3/02	S_3S_5	SCS425 Luiza	S_5S_9
M-4/09	S_1S_3	SCS417 Monalisa	S_2S_{10}
M-8/01	S_3S_{19}	SCS426 Venice	S_3S_9

$S_?$: another unidentified *S*-allele.

and $S_9S_?$, respectively. New markers are required for identification of the second *S*-allele of these genotypes, for example, through use of the markers developed by Larsen et al. (2016), which identify *S*-alleles found at a lower frequency among apple cultivars. It is noteworthy that these low-frequency *S*-alleles were not evaluated initially in the present study because they are not commonly found in genotypes developed in Brazil (Albuquerque Junior et al. 2011). The cultivar 'SCS416 Kinkas' ($S_9S_?$) is the result of the cross between 'Fuji' (S_1S_9) and PWR37T133 (*S*-alleles unknown), while M-11/92 ($S_2S_?$) is a descendant of the cross between M-41 [Anna ♀ (S_3S_{29}) × NJ-56 ♂ (*S*-alleles unknown)] and Gala (S_2S_5). Both genotypes have unknown *S*-alleles in their genealogy and were able to exhibit *S*-alleles other than those we attempted to genotype in this study.

The genotype S_3S_5 was determined for the selections M-3/02, M-11/01, and M-12/00, while S_3S_{19} was identified for 'M-1/02', 'M-8/01', 'M-11/00', 'M-13/00', and 'M-15/01'. These selections have the same semi-compatible parents, 'Fred Hough' (S_5S_{19} - ♀) and 'Imperatriz' (S_3S_5 - ♂). All the genotypes had the expected segregation of this cross. As reported by De Franceschi et al. (2016), the semi-compatibility between parents causes the abortion of pollen carrying the common *S*-allele when coming in contact with the pistil of a semi-compatible plant.

For the selection M-13/91, a S_3S_5 genotype was detected, which is different from the S_5S_{10} previously reported by Albuquerque Junior et al. (2011). This confirms the pedigree of 'M-13/91' ['Mollie's Delicious' (S_3S_7 - ♀) × 'Princesa' (S_3S_5 - ♂)], whose parents do not carry the S_{10} allele. Using the FTC12 and FTC228 primers, an allele size corresponding to the S_{10} allele (209 bp) occurred in the selection M-13/91. However, when using the 'S_{10} modified' marker recommended by Kitahara and Matsumoto (2002), the amplification of the expected region in 'M-13/91' was not confirmed. In contrast, for 'SCS417 Monalisa' (S_2S_{10}), the amplification product generated by the 'S_{10} modified' marker treated with restriction enzyme *Nar*I generated specific fragments (185 and 97 bp), indicating the presence of the S_{10} allele. Likewise, when using the '$S_3/S_5/S_{10}$' marker for genotyping of the selection M-13/91, fragments characteristic of S_3 (423 bp and 264 bp) and S_5 (399 bp and 273 bp) alleles were amplified, but not of S_{10} (382 bp). In addition, the final amplified product was 382 bp for the cultivar 'SCS417 Monalisa' when using the '$S_3/S_5/S_{10}$' marker, a size characteristic of the S_{10} allele (Larsen et al. 2016). Based on the sequences of the available *S*-alleles (Benson et al. 2013), alleles S_3 (GenBank code: U12200.1) and S_{10} (GenBank code: AB052683.1) have a percentage of identity of 96%. The S_3 and S_5 alleles (GenBank

code: U19791.1) have a sequence identity of 77%, and the S_5 and S_{10} alleles, 90%. Therefore, the data suggest that the primers FTC12 and FTC228 match homologous sequences at the three alleles (S_3, S_5, and S_{10}), impairing the use of these primers for identification of the S_{10} allele, thus explaining the discrepancy in genotyping compared to results of Albuquerque Junior et al. (2011).

According to the official pedigree, the cultivar 'SCS425 Luiza' is a descendant of 'Imperatriz' (S_3S_5 - ♀) and 'Cripps Pink' (S_2S_{23} - ♂) (Denardi et al. 2019b), and is a sibling of 'SCS427 Elenise' and 'M-10/09' (Table 1). The *S*-allele genotypes were S_3S_{23} and S_5S_{23} for the cultivar 'SCS427 Elenise' and selection M-10/09, respectively. However, 'SCS425 Luiza' exhibited the genotype S_5S_9, which was not expected, based on its genealogy. So, the presence of the S_9 allele in 'SCS425 Luiza' indicates that there may have been cross contamination during the development of the cultivar (pollen contamination, seed exchange between crosses at sowing, or hybrid exchange at planting) or that this cultivar is not the result of the cross 'Imperatriz' × 'Cripps Pink'. Consequently, the true pedigree of 'SCS425 Luiza' must be determined. Similar results have been reported in the literature. Sakurai et al. (2000) admitted the possibility of cross contamination during the development of the cultivar 'Kent' (S_3S_9). In this case, this cultivar was theoretically a descendant from 'Cox's Orange Pippin' (S_5S_9 - ♀) × 'Jonathan' (S_7S_9 - ♂). For that reason, these authors suggested that 'Jonathan' is not the true pollen donor of the cultivar 'Kent'. In this sense, the genotyping of the *S*-alleles in the present study generated information that show possible errors in the genealogy previously registered by the Epagri Apple Breeding Program. Thus, *S*-allele

Table 5. Level of compatibility among the genotypes evaluated in this study based on their *S*-alleles

Genotype	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	21-373-58**	21-502-1	141/38	Co-op 8**	Macfree	Monalisa	Venice	M-10/09	M-44/08	Scifresh	Elenise
Group 1 (S_3S_5) Co-op 14, Co-op 16, M-1/07, M-11/01, M-12/00, M-13/91, M-21/08, M-23/07, M-3/02	-																		
Group 2 (S_3S_{19}) 21-379-64, 21-555-13, M-1/02, M-11/00, M-13/00, M-15/01, M-53/08, M-8/01, M-9/07	SC	-																	
Group 3 ($S_9S_?$) 21-300-21, 21-361-75, SCS416 Kinkas	C	C	-																
Group 4 ($S_2S_?$) Co-op 24, M-11/92	C	C	SC*	-															
Group 5 (S_3S_{24}) D1R102T116, D1R103T245	SC	SC	C	C	-														
Group 6 (S_1S_3) 21-300-13, M-4/09	SC	SC	C	C	SC	-													
Group 7 (S_5S_9) M-58/07, SCS425 Luiza	SC	C	SC	C	C	C	-												
Group 8 (S_2S_5) Castel Gala, Galaxy	SC	C	C	SC	C	C	SC	-											
21-373-58**	I	SC	SC	C	SC	SC	SC	SC	-										
21-502-1	C	C	C	C	SC	SC	C	C	C	-									
141/38	C	C	SC	SC	C	C	SC	SC	SC	C	-								
Co-op 8	SC	SC	C	C	SC	SC	C	C	-	C	C	-							
Macfree	C	C	SC*	SC*	C	C	C	C	C	C	C	C	-						
SCS417 Monalisa	C	C	C	SC	C	C	C	SC	C	C	SC	C	C	-					
SCS426 Venice	SC	SC	SC	C	SC	SC	SC	C	I	C	SC	SC	C	C	-				
M-10/09	SC	C	C	C	C	C	SC	SC	SC	C	C	C	C	C	C	-			
M-44/08	SC	C	C	C	C	SC	SC	SC	SC	SC	C	C	C	C	C	SC	-		
Scifresh	C	C	C	SC	SC	C	C	SC	C	SC	SC	C	C	SC	C	C	C	-	
SCS427 Elenise	SC	SC	C	C	SC	SC	C	C	SC	C	C	SC	C	C	SC	SC	C	C	-

Genotypes in the groups are incompatible – they have the same *S*-alleles. *At least semi-compatible genotypes. **Using triploid genotypes as female parent.

genotyping by markers can be used as an auxiliary tool in the characterization of the descendants of apple crosses, taking the expected segregation of *S*-alleles into account.

The Epagri apple selections and the apple cultivar 'SCS426 Venice' were characterized by the presence of two *S*-alleles (Table 4), and this presence is consistent with the possibility of those *S*-alleles having been inherited from their parents, Imperatriz (♀) and Baronesa (♂) (Denardi et al. 2019c). Two *S*-alleles were also found for the selections 141/38 (S_2S_9 – the S_9 is from the parental 'Baronesa' and the other *S*-allele is from open pollination), M-1/07 (S_3S_5), M-21/08 (S_3S_5), M-23/07 (S_3S_5), M-4/09 (S_1S_3), M-44/08 (S_1S_5), M-53/08 (S_3S_{19}), M-58/07 (S_5S_9), M-9/07 (S_3S_{19}), and the cultivar SCS426 Venice (S_3S_9). Three *S*-alleles were identified for the cultivars 21-373-58 ($S_3S_5S_9$) and Co-op 8 ($S_3S_7S_{19}$). Three *S*-alleles were also identified in other genotypes previously known as triploid (Broothaerts et al. 2004, Dreesen et al. 2010, Agapito-Tenfen et al. 2015), and there is no more molecular information in the literature about these two cultivars that could confirm triploidy. The others were identified as diploid genotypes: 21-300-13 (S_1S_3), 21-379-64 (S_3S_{19}), 21-502-1 (S_1S_{24}), 21-555-13 (S_3S_{19}), Co-op 14 (S_3S_5), Co-op 16 (S_3S_5), D1R102T116 (S_3S_{24}), D1R103T245 (S_3S_{24}), and Scifresh (S_2S_{24}) (Table 4).

There are 11 cultivars from the Epagri Apple Breeding Program that had already been genotyped (Albuquerque Junior et al. 2011): 'Baronesa' (S_3S_9), 'Duquesa' (S_2S_3), 'Fred Hough' (S_5S_{19}), 'Catarina' (S_1S_{19}), 'Fuji Suprema' (S_1S_9), 'Condessa' ($S_2S_?$), 'Imperatriz' (S_3S_5), 'Daiane' (S_3S_5), 'Joaquina' (S_5S_{19}), 'Lisgala' (S_2S_5), 'Primícia' ($S_{24}S_?$), and 'Princesa' (S_3S_5). Other international apple cultivars are likewise important for the Epagri Apple Breeding Program and have been used in several planned crosses. The following cultivars have been genotyped for their *S*-alleles: 'Akane' (S_7S_{24}) (Kitahara et al. 2000), 'Cripps Pink' (S_2S_{23}) (Broothaerts et al. 2004), 'Florina' (S_2S_3) (Long et al. 2010), 'Liberty' ($S_3S_5S_{10}$) (Broothaerts et al. 2004), *Malus floribunda* ($S_{26}S_?$) (Broothaerts 2003), 'Priscila' (S_9S_{20}) (Morita et al. 2009), 'Red Free' (S_3S_7) (Morita et al. 2009), and 'Sansa' (S_5S_7) (Kitahara and Matsumoto 2002).

The compatibility levels between the genotypes based on identification of the *S*-alleles are presented in Table 5. Crosses using the genotypes within groups (Table 5) are impossible because they are incompatible. In the crosses between groups, there is at least semi-compatibility between the groups and the other genotypes, except for triploid genotypes. Pollen from triploid plants is sterile because the chromosomes are unequally divided during meiosis (Sedov et al. 2017). For that reason, triploid plants can only be used as a female parent.

Eleven different *S*-alleles were identified in the 42 genotypes evaluated (Figure 1). The S_3 and S_5 alleles were most frequently identified (30.2% and 18.6%, respectively). One of the reasons for the higher frequency of these alleles is that 26 of the 42 genotypes tested were direct or indirect descendants from the cultivars 'Imperatriz' (S_3S_5) (Albuquerque

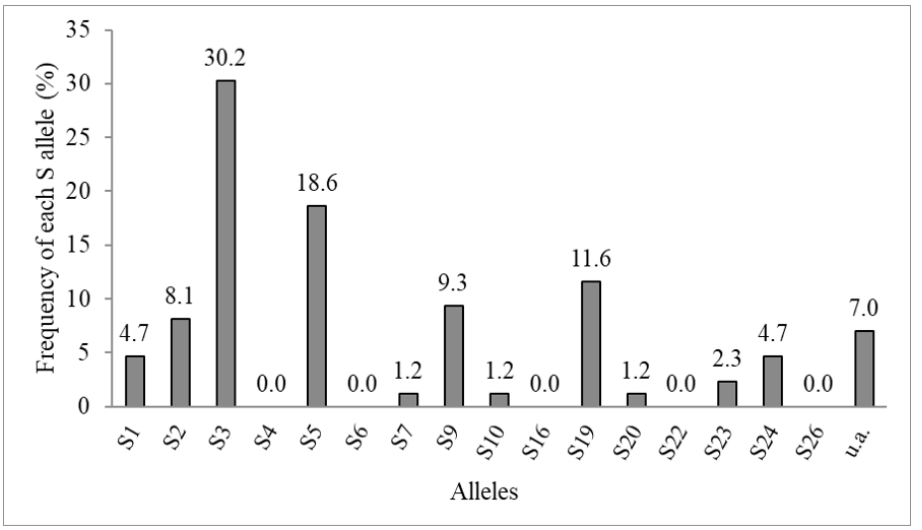

Figure 1. Frequency of occurrence of each *S*-allele in 42 apple genotypes: six cultivars developed by Epagri; 20 selections of the Elite Germplasm Collection of the Epagri Apple Breeding Program; and 16 accessions from the Apple Germplasm Bank of Epagri, located in Caçador, SC. u.a.: unidentified alleles.

Junior et al. 2011), 'Golden Delicious' (S_2S_3) (Matsumoto et al. 1999), and/or 'Gala' (S_2S_5) (Matsumoto et al. 1999). For a long time, these three genotypes served as the basis for the crosses of the Epagri Apple Breeding Program. A consequence of selection through breeding is that the bottleneck in genetic variability is indirectly reflected in the higher frequency of a few *S*-alleles, such as S_3 and S_5 in this situation. Larsen et al. (2016) showed a higher frequency of S_3 alleles (28%) among 432 genotypes of the genus *Malus*. In European apple cultivars, Dreesen et al. (2010) identified S_2, S_3, S_5, and S_9 as the most common *S*-alleles. Meanwhile, Hegedűs (2006) reported that the S_2, S_3, S_5, S_7, S_9, and S_{10} alleles were the most frequent among the commercial apple cultivars, due to the extensive use of the genotypes 'Golden Delicious', 'Delicious', 'Jonathan', 'McIntosh', and 'Cox's Orange Pippin' in apple breeding programs around the world.

According to Halász et al. (2011) and De Franceschi et al. (2016), the presence of the S_2, S_3, and S_5 alleles is associated with resistance to apple scab (*Venturia inaequalis*). Apparently, none of these *S*-alleles are linked to the gene of vertical resistance against apple scab (*Rvi6*), but somehow they are linked to different resistance levels, close to genes of minor effect on horizontal resistance (Halász et al. 2011). Indirect selection for these alleles can be performed by breeders, and most of the parents used for generating scab-resistant plants have at least one of these alleles (S_2, S_3, and S_5), explaining their higher frequency in elite genotypes of the Epagri Breeding Program.

The identification of the *S*-alleles in the genotypes evaluated allows breeders to plan crosses. Furthermore, it provides important information for other breeding programs, which can use the genotypes evaluated in this study as a genetic source in their research.

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