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 S3 and S5 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<sup>1</sup> | Unknown      | Unknown      | M-10/09<sup>2</sup> | Imperatriz [S355] | Cripps Pink [S2523] |
| 21-300-21<sup>1</sup> | Unknown      | Unknown      | M-11/00<sup>2</sup> | Fred Hough [S5519] | Imperatriz [S355] |
| 21-361-75<sup>1</sup> | Unknown      | Unknown      | M-11/01<sup>2</sup> | Fred Hough [S5519] | Imperatriz [S355] |
| 21-373-58<sup>1</sup> | Unknown      | Unknown      | M-11/92<sup>2</sup> | M-41 | Gala [S255] |
| 21-379-64<sup>1</sup> | Unknown      | Unknown      | M-12/00<sup>2</sup> | Fred Hough [S5519] | Imperatriz [S355] |
| 21-502-1<sup>1</sup> | Unknown      | Unknown      | M-13/00<sup>2</sup> | Fred Hough [S5519] | Imperatriz [S355] |
| 21-555-13<sup>1</sup> | Unknown      | Unknown      | M-13/91<sup>2</sup> | Princesa [S355] | Mollie’s Delicious [S357] |
| 141/38<sup>2</sup> | Baronesa [S359] | O.p.         | M-15/01<sup>2</sup> | Fred Hough [S5519] | Imperatriz [S355] |
| Co-op 8<sup>3</sup> | PRI 558-1 | Mollie’s Delicious [S357] | M-21/08<sup>2</sup> | M-47/94 | Princesa [S355] |
| Co-op 14<sup>3</sup> | PRI 10-147 | Mollie’s Delicious [S357] | M-23/07<sup>2</sup> | M-46/94 | M-13/91 |
| Co-op 16<sup>3</sup> | PRI 764 | PRI 672 | M-3/02<sup>2</sup> | Fred Hough [S5519] | Imperatriz [S355] |
| Co-op 24<sup>3</sup> | NJ. 125355 | Prima [S2510] | M-4/09<sup>4</sup> | Imperatriz [S355] | Catarina [S1519] |
| Castel Gala<sup>4</sup> | Sport mutation of Gala | M-44/08<sup>4</sup> | Imperatriz [S355] | Catarina [S1519] |
| D1R102T116<sup>5</sup> | Unknown | Unknown | M-53/08<sup>5</sup> | Imperatriz [S355] | Catarina [S1519] |
| D1R103T245<sup>5</sup> | Unknown | Unknown | M-58/07<sup>5</sup> | Imperatriz [S355] | Baronesa [S359] |
| Galaxy<sup>6</sup> | Sport mutation of Gala | M-8/01<sup>2</sup> | Fred Hough [S5519] | Imperatriz [S355] |
| Macfree<sup>7</sup> | McIntosh [S10525] | 48-177 | M-9/07<sup>2</sup> | M-46/94 | Imperatriz [S355] |
| SCS417 Monalis<sup>4</sup> | Gala [S255] | Malus 4 | SciFresh<sup>4</sup> | Braeburn [S9524] | Gala [S255] |
| SCS426 Venice<sup>7</sup> | Imperatriz [S355] | Baronesa [S359] | SCS427 Elenise<sup>7</sup> | Imperatriz [S355] | Cripps Pink [S2523] |
| M-1/02<sup>7</sup> | Fred Hough [S5519] | Imperatriz [S355] | SCS416 Kinkas<sup>4</sup> | Fuji [S159] | PWR377T133 |
| M-1/07<sup>7</sup> | M-47/94 | Princesa [S355] | SCS425 Luiza<sup>4</sup> | Imperatriz [S355] | Cripps Pink [S2523] |

O.p. open pollinated. <sup>1</sup>Selections from Argentina. <sup>2</sup>Selections from the Epagri Apple Breeding Program. <sup>3</sup>Selections from the PRI disease-resistant apple breeding program. <sup>4</sup>Cultivar developed by Epagri. <sup>5</sup>Selections from USA. <sup>6</sup>Cultivar from New Zealand. <sup>7</sup>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 °C, enzyme | Amplified fragment (bp) |
|-----------|---------|----------------------|-----------------------------|-----------------------|
| $S_1$     | FTC168  | ATATTGTAAGGACCGGCACATCAT                                      | 60                        | 530                   |
|           | FTC169  | GGTCTGTATTGGGGAAGGCCA                                        |                           |                       |
| $S_2$     | OWB122  | GTTCAAGGTGCTATATGGG                                          | 60                        | 449                   |
|           | OWB123  | GGTGGTGGCTCTACTCATG                                          |                           |                       |
| $S_3$     | FTC177  | CAAAGGATACAAACTTCTAC                                        | 55                        | 500                   |
|           | FTC226  | TATAGGAAATACCCATTCCG                                         |                           |                       |
| $S_4$     | FTC5    | TCCCAACATACAGAACAGA                                          | 60, TaqI                   | 274 (194+77)          |
|           | OWB249  | CAATCTAGAAGTGGCTCTG                                          |                           |                       |
| $S_5$     | FTC10   | CAAACATGGCGCTGGTGGG                                          | 59                        | 346                   |
|           | FTC11   | TAAATGGATCATCATGGAG                                          |                           |                       |
| $S_6$     | FTC141  | ATAGGGACGGCTCTGACACT                                          | 58 $^{1, 45}$             | 850                   |
|           | FTC142  | AGCCGTGCTCTTAATGGAAT                                             |                           |                       |
| $S_7$     | FTC143  | ACTCGAATGGGACATGCCAGT                                         | 60                        | 302                   |
|           | FTC144  | TGTGCTTATATGGGGATGTC                                          |                           |                       |
| $S_8$     | OWB154  | CGCGCCGCTCTGCTGCA                                            | 62                        | 343                   |
|           | OWB155  | CCGGGCGACTGAGGATGTC                                          |                           |                       |
| $S_{10}$  | FTC12   | CCAAAGCGTACCATCGAGA                                          | 60                        | 209                   |
|           | FTC228  | ATGTCGTCGGTCTGCAAT                                             |                           |                       |
| $S_{10}$ modified $^{1}$ | FTC35FTC14 | ACAAATTTAAAGCCGCGCACGTTGTATAGATGGAGT | 60, NarI | - |
| $S_3$/$S_5$/S$_{10}$ modified $^{1}$ | FTC5/FTC168/FTC177 | CAAATTTACCGACGCTATACGGGTTCGAATGGAGGATTGTTTGAAYGAAAAATATTAGGAGT | 58 $^{1}$ | - |
| $S_{16}$  | FTC5    | TCCCAACATACAGAACAGA                                          | 60, TaqI                   | 274 (243+41)          |
|           | OWB249  | CAATCTAGAAGTGGCTCTG                                          |                           |                       |
| $S_{19}$  | FTC229  | TCTGGAAGAGAGTGCGCTCC                                          | 60                        | 304                   |
|           | FTC230  | TTATGAACCTTGATGTCGTC                                          |                           |                       |
| $S_{20}$  | FTC141  | ATAGGGACGGCTCTGACACT                                          | 60 $^{1, 45}$             | 920 (800+120)         |
|           | FTC142  | AGCCGTGCTCTTAATGGAAT                                             |                           |                       |
| $S_{22}$  | FTC5    | TCCCAACATACAGAACAGA                                          | 60, TaqI                   | 274 (199+44+31)       |
|           | OWB249  | CAATCTAGAAGTGGCTCTG                                          |                           |                       |
| $S_{23}$  | FTC222  | CAAAGGATACAAACTTCTAC                                        | 60                        | 237                   |
|           | FTC224  | GGTGTCATATGGGTGATCTAG                                          |                           |                       |
| $S_{24}$  | FTC231  | AAATATTGCAAGCAGACAGA                                         | 60                        | 580                   |
|           | FTC232  | TGGAGAGGATTTCAGAGATG                                          |                           |                       |
| $S_{26}$  | FTC14   | GAAGATGTCATACAGAACAGA                                         | 54                        | 194                   |

$^{1, 45}$ = Extension of 45 sec. $^{1}$ 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 NarI generates two fragments: 185 and 97 bp. $^{1}$ 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 TaqI: $S_3 = 423$ 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 TaqI (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 NarI (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: $S_9S_?$, $S_9S_?$, $S_2S_?$, $S_2S_?$, $S_{20}S_?$, $S_?$.

### 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  | $S_5S_{19}$           | Albuquerque Junior et al. (2011) |
|        | Fuji      | $S_5S_9$              | Matsumoto et al. (1999)         |
| $S_2$  | Cripps Pink | $S_2S_{23}$       | Broothaerts et al. (2004)       |
|        | Golden Delicious | $S_2S_3$       | Matsumoto et al. (1999)         |
|        | Golden Delicious | $S_2S_4$       | Matsumoto et al. (1999)         |
| $S_3$  | Imperatriz | $S_3S_5$             | Albuquerque Junior et al. (2011) |
| $S_4$  | Gloster   | $S_4S_{13}$           | Deessen et al. (2010)           |
|        | Gala      | $S_4S_5$              | Matsumoto et al. (1999)         |
| $S_5$  | Joaquina  | $S_5S_{19}$           | Albuquerque Junior et al. (2011) |
| $S_6$  | Marubakaido | $S_6S_{26}$       | Agapito-Tenfen et al. (2015)    |
|        | Akane     | $S_6S_{24}$           | Kitahara et al. (2000)          |
|        | Idared    | $S_6S_9$              | Janssens et al. (1995)          |
| $S_7$  | Baronesa  | $S_7S_9$              | Albuquerque Junior et al. (2011) |
|        | Fuji      | $S_7S_9$              | Matsumoto et al. (1999)         |
|        | Liberty   | $S_7S_{19}$           | Broothaerts et al. (2004)       |
| $S_{20}$ | McIntosh | $S_{20}S_{25}$        | Kitahara and Matsumoto (2002)   |
|        | Delicious | $S_{20}S_{19}$        | Deessen et al. (2010)           |
| $S_{10}$ | Fred Hough | $S_{10}S_{19}$      | Albuquerque Junior et al. (2011) |
|        | Mutsu     | $S_{10}S_{20}$        | Deessen et al. (2010)           |
| $S_{20}$ | Alkmene  | $S_{20}S_{20}$        | Deessen et al. (2010)           |
| $S_{27}$ | Cripps Pink | $S_{27}S_{23}$      | Broothaerts et al. (2004)       |
| $S_{22}$ | Granny Smith | $S_{22}S_{23}$    | Deessen et al. (2010)           |
|        | Braeburn  | $S_{22}S_{24}$        | Deessen et al. (2010)           |
| $S_{24}$ | Primicia | $S_{24}S_{26}$        | Albuquerque Junior et al. (2011) |
| $S_{26}$ | Marubakaido | $S_{26}S_{26}$    | Agapito-Tenfen et al. (2015)    |

$S_?$: another unidentified $S$-allele.
and $S_5S_9$, 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’ ($S_5S_9$) is the result of the cross between ‘Fuji’ ($S_5S_9$) and PWR37T133 ($S$-alleles unknown), while M-11/92 ($S_5S_9$) is a descendant of the cross between M-41 [$Anna \♀ (S_5S_9 \times NJ-56 \♂)$ ($S$-alleles unknown)] and Gala ($S_5S_9$). 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 $S_5S_9$ was determined for the selections M-3/02, M-11/01, and M-12/00, while $S_9S_9$ 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’ ($S_5S_9 - \♀$) and ‘Imperatriz’ ($S_5S_9 - \♂$). 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 $S_5S_9$ 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’ ($S_5S_9 - \♀$) × ‘Princesa’ ($S_5S_9 - \♂$)], whose parents do not carry the $S_9$ 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’ ($S_5S_{10}$), the amplification product generated by the ‘$S_{10}$ modified’ marker treated with restriction enzyme $NarI$ generated specific fragments (185 and 97 bp), indicating the presence of the $S_{10}$ allele. Likewise, when using the ‘$S_9/S_9/S_{10}$’ marker for genotyping of the selection M-13/91, fragments characteristic of $S_9$ (423 bp and 264 bp) and $S_9$ (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 ‘$S_{10}$/$S_9/S_{10}$’ 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 $S_9$ (GenBank code: U12200.1) and $S_{10}$ (GenBank code: AB052683.1) have a percentage of identity of 96%. The $S_9$ and $S_{10}$ alleles (GenBank

| Cultivar/selection | $S$-alleles | Cultivar/selection | $S$-alleles |
|--------------------|-------------|--------------------|-------------|
| 21-300-13          | $S_5S_9$    | M-9/07             | $S_7S_9$    |
| 21-300-21          | $S_5S_9$    | M-10/09            | $S_7S_9$    |
| 21-361-75          | $S_5S_9$    | M-11/00            | $S_7S_9$    |
| 21-373-58          | $S_5S_9$    | M-11/01            | $S_7S_9$    |
| 21-379-64          | $S_5S_{19}$ | M-11/92            | $S_7S_9$    |
| 21-502-1           | $S_5S_{19}$ | M-12/00            | $S_7S_9$    |
| 21-555-13          | $S_5S_{19}$ | M-13/00            | $S_7S_9$    |
| 141/38             | $S_5S_9$    | M-13/91            | $S_7S_9$    |
| Co-op 8            | $S_5S_{19}$ | M-15/01            | $S_7S_9$    |
| Co-op 14           | $S_5S_9$    | M-21/08            | $S_7S_9$    |
| Co-op 16           | $S_5S_9$    | M-23/07            | $S_7S_9$    |
| Co-op 24           | $S_5S_9$    | M-44/08            | $S_7S_9$    |
| Castel Gala        | $S_5S_9$    | M-53/08            | $S_7S_9$    |
| D1R102T116         | $S_5S_{24}$ | M-58/07            | $S_7S_9$    |
| D1R103T245         | $S_5S_{24}$ | Macfree            | $S_{10}S_{19}$ |
| Galaxy             | $S_5S_9$    | SCS427 Elenise     | $S_7S_{23}$ |
| M-1/02             | $S_5S_{19}$ | SCS416 Kinkas      | $S_7S_{23}$ |
| M-1/07             | $S_5S_9$    | SCS425 Luiza       | $S_7S_9$    |
| M-3/02             | $S_5S_9$    | SCS417 Monalisa    | $S_7S_{10}$ |
| M-4/09             | $S_5S_9$    | SCS426 Venice      | $S_7S_9$    |
| M-8/01             | $S_5S_{19}$ | SCS427 Elenise     | $S_7S_{23}$ |

$S_9$: another unidentified $S$-allele.
According to the official pedigree, the cultivar ‘SCS425 Luiza’ is a descendant of ‘Imperatriz’ (S, S, ♂) and ‘Cripps Pink’ (S, S, ♀) (Denardi et al. 2019b), and is a sibling of ‘SCS427 Elenise’ and ‘M-10/09’ (Table 1). The S-allele genotypes were S, S, S, for the cultivar ‘SCS427 Elenise’ and selection M-10/09, respectively. However, ‘SCS425 Luiza’ exhibited the genotype S, which was not expected, based on its genealogy. So, the presence of the S 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, S, ♂). In this case, this cultivar was theoretically a descendant from ‘Cox’s Orange Pippin’ (S, S, ♀) × ‘Jonathan’ (S, S, ♂). 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, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Co-op 14, Co-op 16, M-1/07, M-11/01, M-12/00, M-21/08, M-3/02 | -       |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Group 2 (S, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| 21-379-64, 21-555-13, M-1/02, M-11/00, M-13/00, M-15/01, M-8/01, M-9/00, M-53/08, M-3/02 | SC       |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Group 3 (S, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| 21-300-21, 21-361-75, SCS416 Kinkas | C        | C       |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Group 4 (S, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Co-op 24, M-11/92 | C       | C       | SC*     |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Group 5 (S, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| D1R102T116, D1R103T245 | SC       | SC      | C       | C       |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Group 6 (S, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| 21-300-13, M-4/09 | SC       | SC      | C       | C       | SC       |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Group 7 (S, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| M-58/07, SCS425 Luiza | SC       | C       | SC      | C       | C       | C       |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Group 8 (S, S)   |         |         |         |         |         |         |         |         |           |           |        |         |         |         |        |         |         |          |         |
| Castel Gaia, Galaxy | SC       | C       | C       | SC      | C       | C       | SC       |         |           |           |        |         |         |         |        |         |         |          |         |
| 21-373-58**     | SC       | SC      | C       | C       | SC      | SC      | SC      | SC      |           |           |        |         |         |         |        |         |         |          |         |
| 21-502-1        |          | SC      | C       | C       | SC      | SC      | SC      | C       |           |           |        |         |         |         |        |         |         |          |         |
| 141/38          | SC       | C       | SC      | SC      | C       | SC      | SC      | C       |           |           |        |         |         |         |        |         |         |          |         |
| Co-op 8         | SC       | SC      | C       | C       | SC      | SC      | C       | C       |           |           |        |         |         |         |        |         |         |          |         |
| Macfree         | SC       | C       | SC*     | SC*     | C       | C       | C       | C       |           |           |        |         |         |         |        |         |         |          |         |
| SCS417 Monalisa | SC       | SC      | SC      | C       | C       | SC      | C       | C       |           |           |        |         |         |         |        |         |         |          |         |
| SCS426 Venice   | SC       | SC      | SC      | C       | SC      | SC      | SC      | C       |           |           |        |         |         |         |        |         |         |          |         |
| M-10/09         |         | SC      | C       | C       | C       | SC      | SC      | C       |           |           |        |         |         |         |        |         |         |          |         |
| M-44/08         | SC       | C       | C       | C       | SC      | SC      | SC      | C       |           |           |        |         |         |         |        |         |         |          |         |
| Scifresh        | SC       | C       | SC      | SC      | C       | C       | SC      | C       |           |           |        |         |         |         |        |         |         |          |         |
| SCS427 Elenise  | SC       | 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.
Self-incompatibility alleles in important genotypes for apple breeding in Brazil
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\_♀ S\_♂ – the S\_♀ is from the parental ‘Baronesa’ and the other S-allele is from open pollination), M-1/07 (S\_♀ S\_♂), M-21/08 (S\_♀ S\_♂), M-23/07 (S\_♀ S\_♂), M-4/09 (S\_♀ S\_♂), M-44/08 (S\_♀ S\_♂), M-53/08 (S\_♀ S\_♂), M-58/07 (S\_♀ S\_♂), M-9/07 (S\_♀ S\_♂), and the cultivar SCS426 Venice (S\_♀ S\_♂). Three S-alleles were identified for the cultivars 21-373-58 (S\_♀ S\_♂) and Co-op 8 (S\_♀ S\_♂). 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\_♀ S\_♂), 21-379-64 (S\_♀ S\_♂), 21-502-1 (S\_♀ S\_♂), 21-555-13 (S\_♀ S\_♂), Co-op 14 (S\_♀ S\_♂), D1R102T116 (S\_♀ S\_♂), D1R103T245 (S\_♀ S\_♂), and Scifresh (S\_♀ S\_♂) (Table 4).

There are 11 cultivars from the Epagri Apple Breeding Program that had already been genotyped (Albuquerque Junior et al. 2011): ‘Baronesa’ (S\_♀ S\_♂), ‘Duquesa’ (S\_♀ S\_♂), ‘Fred Hough’ (S\_♀ S\_♂), ‘Catarina’ (S\_♀ S\_♂), ‘Fuji Suprema’ (S\_♀ S\_♂), ‘Condessa’ (S\_♀ S\_♂), ‘Imperatriz’ (S\_♀ S\_♂), ‘Daiane’ (S\_♀ S\_♂), ‘Joaquina’ (S\_♀ S\_♂), ‘Lisgala’ (S\_♀ S\_♂), ‘Primícia’ (S\_♀ S\_♂), and ‘Princesa’ (S\_♀ S\_♂). 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\_♀ S\_♂) (Kitahara et al. 2000), ‘Cripps Pink’ (S\_♀ S\_♂) (Broothaerts et al. 2004), ‘Florina’ (S\_♀ S\_♂) (Long et al. 2010), ‘Liberty’ (S\_♀ S\_♂) (Broothaerts et al. 2004), Malus floribunda (S\_♀ S\_♂) (Broothaerts 2003), ‘Priscila’ (S\_♀ S\_♂) (Morita et al. 2009), ‘Red Free’ (S\_♀ S\_♂) (Morita et al. 2009), and ‘Sansa’ (S\_♀ S\_♂) (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\_♀ and S\_♂ 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\_♀ S\_♂) (Albuquerque

![Figure 1](https://example.com/figure1.png)

**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.
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Junior et al. (2011), ‘Golden Delicious’ (S₂S₃) (Matsumoto et al. 1999), and/or ‘Gala’ (S₅S₅) (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 S₂ and S₃ in this situation. Larsen et al. (2016) showed a higher frequency of S₂ alleles (28%) among 432 genotypes of the genus Malus. In European apple cultivars, Dreesen et al. (2010) identified S₂, S₃ and S₅ as the most common S-alleles. Meanwhile, Hegedűs (2006) reported that the S₂, S₃, S₅, S₇, S₉ and S₁₀ 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 S₂, S₅, and S₇ 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 (S₂, S₅, and S₇), 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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