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BASIC AREAS
Article

Identification of pollinizers for apple 'SCS426 Venice'

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ABSTRACT: Apple orchards require the presence of a different genotype to pollinate the fruit producing cultivar. This is due to the process of gametophytic self-incompatibility present in most species and cultivars of the genus *Malus*. The fruit producing cultivar and the pollinizer must be genetically compatible to ensure fruit set and symmetrical and adequate fruit formation. The aim of this work was to evaluate five potential pollinizers for the new apple cultivar SCS426 Venice by genotyping the self-incompatibility locus (*S*-locus) and by controlled pollination in the field. The *S*-locus was screened using molecular markers and the fertilization capacity was evaluated by monitoring the fruit set after artificial pollination. Three genotypes were identified as semi-compatible (selection 135/140, cultivar SCS433 Felix 3 and SCS425 Luiza) and two as fully compatible ('SCS431 Felix 1' and 'SCS434 Felix 4') with 'SCS426 Venice'. Regardless of the level of compatibility, all genotypes tested are efficient for the fertilization of 'SCS426 Venice' flowers and can be used as pollinizers in commercial orchards of this cultivar.

Key words: *Malus* × *domestica* Borkh., *S*-alleles, gametophytic self-incompatibility, pollination.

INTRODUCTION

Although presenting hermaphrodite flowers, apples (*Malus* × *domestica* Borkh.) depend on cross-pollination for the fertilization of the flowers and consequent fruit set. This necessity is due to the gametophytic self-incompatibility mechanism presented in most species of the genus *Malus* and in several genera of the Rosaceae family (Fujii et al. 2016). Most apple cultivars do not produce fruit by self-pollination or by crosses between genotypes that have the same alleles in the *S*-locus, responsible for gametophytic self-incompatibility control (Ramírez and Davenport 2013; Kasajima 2017).

For this reason, most commercial apple orchards are composed of fruit-producing cultivars (scion cultivars) and at least another genotype used as a pollinizer. Pollinizers must coincide at flowering time with the scion cultivar (Matsumoto 2014; Albuquerque Junior et al. 2011). In addition, scion cultivars and pollinizers need to be genetically compatible (Orcheski and Brown 2012), since the pollen tube does not grow when the *S*-allele in the haploid cell of the pollen grain is the same to one of the two *S*-alleles expressed in the pistil (Matsumoto et al. 1999; Sassa et al. 2007).

The gametophytic self-incompatibility is a barrier to identify and use compatible plants for directed crosses or pollination in the field that enables fruit set (de Nettancourt 1977). Likewise, fruit growers cannot produce fruit optimally when incompatible genotypes are grown in the orchards (Orcheski and Brown 2012). In order to ensure the formation of symmetrical fruit with normal shape development, at least one seed must be formed in four of the five fruit carpels (Denardi and Stuker 2008; Sheffield 2014). The normal apple shape formation is induced through hormones released by the developing embryos, so the ovule fertilization and the consequent seed formation in the carpels are necessary to obtain fruit with appropriate shape (Matsumoto et al. 2012).

The number of fruit formed and the number of seeds in each carpel are some of the parameters that have been used in the evaluation of genetic compatibility between apple trees, but the results range from season to season due to the environmental factors (Matsumoto 2014; Heo et al. 2012). Nowadays, the use of molecular markers linked to the *S*-locus has been shown to be an efficient tool for determining the compatibility between genotypes.

The apple cultivar SCS426 Venice was developed and recently released by the Agricultural Research and Rural Extension Company of Santa Catarina State (Epagri), as a result from the cross between 'Imperatriz' (♀) and 'Baronesa' (♂) (Denardi et al. 2019 a). The *S*-locus of 'SCS426 Venice' is S_3S_9 (Brancher et al. 2020). However, 'SCS426 Venice' does not have the pollinizers characterized yet. Therefore, the aim of this work was to evaluate five potential pollinizers for the new apple cultivar SCS426 Venice by genotyping the self-incompatibility locus (*S*-locus) and by controlled pollination in the field.

MATERIAL AND METHODS

Five apple genotypes were tested as pollinizers for 'SCS426 Venice': the selection 135/140 and the cultivars SCS431 Felix 1, SCS433 Felix 3, SCS434 Felix 4 and SCS425 Luiza. The selection 135/140, 'SCS431 Felix 1', 'SCS433 Felix 3' and 'SCS434 Felix 4' were selected by the Epagri Apple Breeding Program from an open pollination progeny and do not produce fruits with commercial value (the fruits are very small and with a bitter flavor). 'SCS425 Luiza' is a commercial cultivar with good fruit quality and promising market value (Denardi et al. 2019 b).

Field compatibility tests

The experiment was conducted at Caçador municipality, in the Midwestern region of the state of Santa Catarina (26°49'5"S; 50°59'12"W; average altitude of 940 m above sea level), Brazil, during the crop seasons 2014/2015 and 2017/2018. In the season 2015/2016, the experiment was compromised due to late frost in the orchard and in 2016/2017 the experimental area was affected by the drift of thinning product sprayed from marginal orchards that harmed the fruit set of artificial pollinations. Therefore, only the 2014/2015 and 2017/2018 seasons were considered for the experiment.

Forty replicated trees of 'SCS426 Venice' were planted in 2010 and the experiment was conducted in a randomized block design with four replications. Each replication consisted of 25 inflorescences with two flowers in the pink balloon stage (stage E2; Fleckinger (1965)), distributed in the 40 trees. The flowers were emasculated by removing the petals, sepals and anthers and were artificially pollinated with the five pollinizers tested soon after. Then, the inflorescences were protected with brown kraft paper bag for at least 72 h to avoid possible contamination by pollen from other sources. To identify possible exogenous pollen contamination, 25 inflorescences in each block were selected, emasculated and just after protected without perform artificial pollination.

The pollen used in the artificial pollination was collected in the seasons prior to the experiment. The flowers were collected from the pollinizer plants in the pink balloon stage (Stage E2) (Fleckinger (1965). The anthers from each pollinizer flowers were removed and dried for 48 h at 25°C. Thereafter, they were stored in a glass vial on silica at -22 °C for up to five days before use. The vials were placed at 4 °C for 24 h and then the pollen germination test was performed according to Kvitschal et al. (2013). The pollen with more than 50% of the germinated grains was used in the artificial pollination.

The evaluation of the pollination efficiency was carried out by measuring the number of fruits formed per inflorescence, the number of seeds per fruit and the number of true seeds per fruit in both seasons. Only seeds that presented a completely formed tegument and endosperm were considered as true seeds. Additionally, for the 2017/2018 season, the number of seeds per carpel were counted.

The data was analyzed by ANOVA on Genes Software (Cruz 2013), considering the effects of season, genotype and their interaction on the characters number of fruits formed per inflorescence, number of seeds per fruit and number of true seeds per fruit. The effect of the pollinizers was analyzed for the number of seeds per carpel for the 2017/2018 season. When necessary, Tukey's test was performed.

Determination of genetic compatibility

The *S*-alleles of the pollinizers 'SCS431 Felix 1', 'SCS433 Felix 3', 'SCS434 Felix 4' and selection 135/140 were identified by allele-specific PCR. To confirm that the fruit formed was the result of each five tested crosses, ten random plants originated from seeds of each crossing were genotyped.

DNA extraction was performed with the FastDNA Spin kit (MP Biomedicals, California, USA) using 150 mg of young leaves, according to manufacturer's guidelines. The quality and concentration of the DNA samples were quantified using spectrophotometry (Libra S50, Biochron, Cambridge, UK) and treated with 2 µg of RNase A.

The reactions were carried out based on Broothaerts (2003) methodology. In each *S*-allele reaction, standard cultivars (Table 1) were used as positive controls for each allele to confirm the effectiveness of the reaction.

Each 15 µL reaction was composed by 1 U of *Taq* DNA polymerase, 1 × enzyme buffer, 2 mmol·L^{-1} of $MgCl_2$, 0.2 mmol·L^{-1} of each dNTP, 1 µmol·L^{-1} of each primer (forward and reverse) and 50 ηg of genomic DNA. The amplification of each *S*-allele was performed on a programmable thermal cycler (Bio Rad T100, California-USA) with 3 min at 94 °C, followed by 30 cycles composed of 94 °C for 1 min, annealing for 1 min from 54 to 62 °C, depending on the primer's characteristics, and 1 min at 72 °C, followed by a final extension step of 72 °C for 7 min, except for the allele S_{10}, as indicated in Table 2. For the discrimination of the S_4, S_{10}, S_{16} and S_{22}, half of the amplified product was digested by the restriction enzyme *Taq*I (1 h at 65 °C) and for S_{20} by the restriction enzyme *Nar*I (4 h at 37 °C). The *S*-alleles were chosen because they are the 16 more commonly *S*-alleles reported in apple cultivars developed in Brazil (Albuquerque Junior et al. 2011).

After the PCR and digestion, the products were analyzed by 3% agarose gel electrophoresis using the 50 bp molecular weight marker to assist the identification of each *S*-allele. The samples were stained with the GelRed intercalating fluorophore (Biotium, California, USA). Scoring of the amplified fragments was performed by image analysis captured in photodocumentary Kodak Gel Logic 212 Pro (Carestream, New York, USA).

The *S*-alleles were considered as present when the fragments of known size occurred, as indicated in the literature, and coincide with the amplified fragments in the cultivars used as positive control for each *S*-allele.

Table 1. Standard apple (*Malus x domestica* Borkh.) cultivars used as positive control for each *S*-allele tested and respective GeneBank number.

Standard cultivar	*S*-allele	Gene Bank No.
Fuji (S_1S_9)	S_1	D50837
Golden Delicious (S_2S_3)	S_2	U12199
Golden Delicious (S_2S_3)	S_3	U12200
Gloster (S_4S_{19})	S_4	AF327223
Gala (S_2S_5)	S_5	U19791
Marubakaido (S_6S_{26})	S_6	*
Idared (S_3S_7)	S_7	AB032246
Fuji (S_1S_9)	S_9	D50836
McIntosh ($S_{10}S_{22}$)	S_{10}	AB052683
Baskatong ($S_{16}S_{26}$)	S_{16}	AF016919
Alkmene (S_5S_{22})	S_{22}	AF327222
Delicious (S_9S_{19})	S_{19}	AB035273
Mutsu ($S_2S_3S_{20}$)	S_{20}	AB019184
Granny Smith (S_3S_{23})	S_{23}	AF239809
Braeburn (S_9S_{24})	S_{24}	AF016920
Marubakaido (S_6S_{26})	S_{26}	*

* *S*-locus genotype identified by Agapito-Tenfen et al. (2015) for Marubakaido apple rootstock.

Table 2. Primer sequences and temperature conditions for allele-specific PCR to identify the *S*-alleles of apple tree (*Malus x domestica* Borkh.) and restriction enzyme digestion.

S- allele	Primers	Sequence (5' → 3')	Annealing temperature (°C) / restriction enzymes	Amplified size (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	FTC5	TCCCACAATACAGAACGAGA	60 / *Taq*I	274 (194+77)
	OWB249	CAATCTATGAAATGTGCTCTG		
S_5	FTC10	CAAACATGGCACCTGTGGGTCTCC	59	346
	FTC11	TAATAATGGATATCATTGGTAGG		
S_6	FTC141	ATCAGCCGGCTGTCTGCCACTC	58 [1]	850
	FTC142	AGCCGTGCTCTTAATACTGAATAC		
S_7	FTC143	ACTCGAATGGACATGACCCAGT	60	302
	FTC144	TGTCGTTCATTATTGTGGGATGTC		
S_9	OWB154	CAGCCGGCTGTCTGCCACTT	62	343
	OWB155	CGGTTCGATCGAGTACGTTG		
S_{10}	[2]	AACAAATCTTAAAGCCCAGC	60	-
		GGTTTCTTATAGTCGATACTTTG		
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 [1] / *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}	FTC14	GAAGATGCCATACGCAATGG	54	194
	FTC9	TTTAATACCGAATATTGGCG		

Values in parentheses refer to the fragment size generated after digestion with the respective restriction enzymes. [1]Cycle extension time of 45 sec. [2]Primers proposed by Kitahara and 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 allele S_{10} and after treatment with enzyme *Nar*I generates two fragments: 185 and 97 bp.

RESULTS AND DISCUSSION

The pollinizers were proposed considering the flowering phenological historic data from the Epagri Apple Breeding Program (Fig. 1), prioritizing the flowering period coincidence with 'SCS426 Venice', associated to the historical production and germination of the pollen grains (viability) determined under laboratory conditions (data not shown).

In Fig. 1, a sample of historical data of each cultivar collected from experimental orchards previously installed are presented and it is possible to access their coincidence of flowering period. The artificial pollinations were made using stored pollen

to pollinate plants of 'SCS426 Venice' in an experimental orchard, where not all pollinizers evaluated were planted. So, the verification of phenology of all genotypes in the same conditions as the crosses were made (season and place) was not possible.

All pollinizers were efficient to pollinate the 'SCS426 Venice' based on the number of fruits formed per inflorescence, number of seeds per fruit and number of true seeds per fruit. In the artificial pollination, no significant effect was identified for the interaction between pollinizers and season, as well as for the major effect of pollinizer (Table 3). However, the

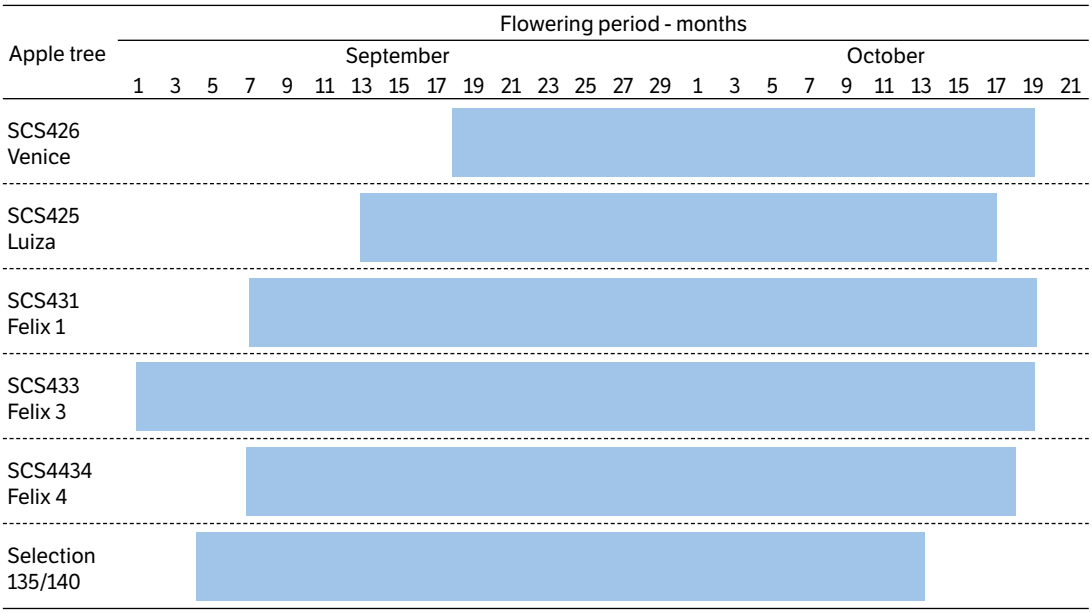

Figure 1. Flowering period of apple tree (*Malus x domestica* Borkh.) cultivars and selection used in this work along six seasons of evaluation (2007/2008 to 2013/2014).

Table 3. Summary of variance analysis considering the effects of the apple (*Malus* spp.) pollinizers and seasons for number of fruits formed per inflorescence, number of seeds per fruit and the number of true seeds per fruit in during the seasons 2014/2015 and 2017/2018 and variance analysis for number of seeds per carpel in the 2017/2018 season.

Source of variation	Df	Mean square - Seasons 2014/2015 and 2017/2018		
		Number of fruits formed per inflorescence	Number of seeds per fruit	Number of true seeds per fruit
Pollinizer	4	0.058^{ns}	0.464^{ns}	0.334^{ns}
Season	1	0.700**	8.770**	10.000**
Pollinizer x Season	4	0.028^{ns}	0.243^{ns}	0.271^{ns}
Block	3	0.276	0.237	0.133
Error	27	0.066	0.417	0.431
Mean		1.204	7.838	7.347
CV (%)		21.376	8.243	8.934

Number of true seeds per carpel in season 2017/2018		
Source of variation	Df	Mean square
Pollinizer	4	0.011^{ns}
Block	3	0.007
Error	12	0.007
Mean		1.670
CV (%)		4.883

Df: Degrees of freedom. CV: Coefficient of variation.

season showed a significant effect ($p < 0.01$) on the number of fruits formed per inflorescence, number of seeds per fruit and number of true seeds per fruit (Table 3). The field pollination tests are highly influenced by environmental conditions and cultural management adopted in the orchards. The number of fruits produced and number of seeds formed may be different from one year to the next (Yamane and Tao 2009).

In the 2017/2018 season, a higher number of fruits formed per inflorescence was observed compared to the 2014/2015 season (Fig. 2a). Nevertheless, the number of fruits formed per inflorescence was greater than one for both seasons (> 50%), which is considered sufficient for an apple commercial crop (Breen et al. 2016). According to Racskó et al. (2007), it is necessary that 15-20% of fruit set be observed to ensure adequate levels of commercial production in apple orchards, indicating that all the pollinizer plants tested presented satisfactory effect for fruit set of 'SCS426 Venice'.

Despite the number of seeds per fruit and number of true seeds per fruit being higher in 2014/2015 (Fig. 2b, c), all pollinizers had satisfactory performance in both seasons, as more than five seeds per fruit were observed. Similarly, although there was no significant difference in the number of seeds formed by carpel (Table 3), on average more than one seed was formed (1.67). The development of seeds, especially of one true seed per carpel, is important to allow the development of a symmetrical fruit (Denardi and Stuker 2008; Sheffield 2014). Therefore, the results of the present work suggest that 'SCS426 Venice' can produce fruit with acceptable shape and size when pollinated by all five pollinizers tested. Even if there are cases of semi-compatibility, there were no differences in the number of fruits per inflorescence, number of seeds per fruit, number of true seeds per fruit and number of true seeds per carpel among the pollinizers. This can be explained by the amount of pollen deposited on the stigmas by manual pollination. A large amount of pollen available can compensate the semi-compatibility between the genotypes, which may explain why it is indistinguishable based on the number of seeds (Hoebee et al. 2011). The production of fruit with good commercial value can be achieved when there is a high density of pollinizer plants in the orchards (translating into high pollen density), even in cases of semi-compatibility.

No quality apple fruit characters were considered in this work because they are not influenced by any different pollinizers. The apple quality is determined by the mother plant (the apple tree where the fruit subsequently forms) and the environmental action (EPAGRI 2006). Therefore, the pollinizer is responsible just for the donation of pollen grains capable of fertilizing the flowers of the mother plant.

As the number of seeds formed between the crosses was satisfactory, this suggests good compatibility between pollen and stigma among 'SCS426 Venice' and all the pollinizers tested (Galletta 1983). However, were identified compatible and semi-compatible crosses among the evaluated pollinizers based on the genotyping of the *S*-alleles (Table 4).

The *S*-locus of 'SCS426 Venice' were identified as S_3S_9 (Brancher et al. 2020). The pollinizers 'SCS431 Felix 1' and 'SCS434 Felix 4' were characterized as fully-compatible with 'SCS426 Venice', both being genotyped as S_4S_5. Considering the possible *S*-locus genotypes of the offspring of these crosses, the seedlings were identified with three of the four possible genotypes

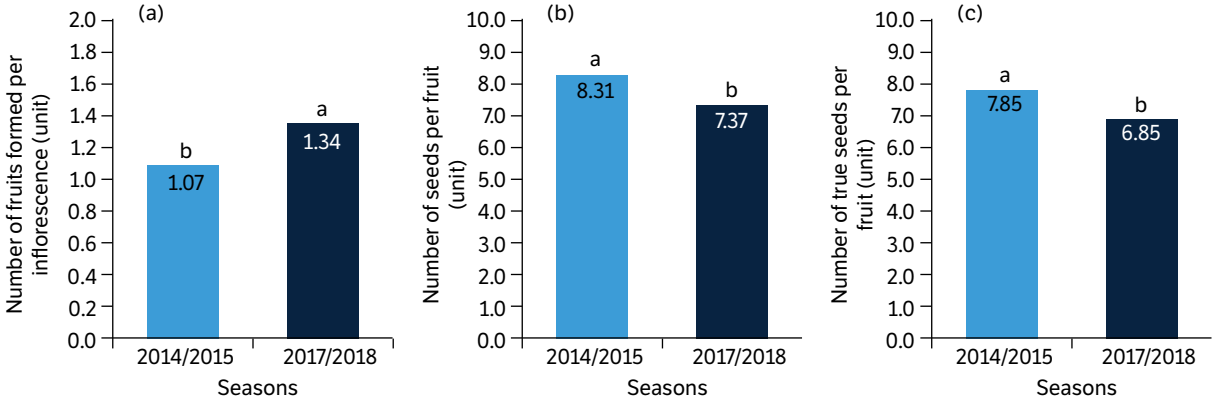

Figure 2. Performance of 'SCS426 Venice' for number of fruits formed per inflorescence (a), number of seeds per fruit (b) and number of true seeds per fruit (c) in both seasons of evaluation, considering the mean obtained with all the pollinizers tested. The letters above the columns indicate the significant differences between the seasons.

Table 4. *S*-alleles genotype of pollinizers, compatibility with 'SCS426 Venice' (S_3S_9), possible segregant genotypes from the cross with 'SCS426 Venice' and number of seedlings that have each *S*-locus genotyped among the 10 individuals random chosen per crossing.

Pollinizer	*S*-locus	Compatibility with 'Venice' (S_3S_9)	Possible seedlings genotype when crossed with 'Venice'	Number of seedlings
SCS425 Luiza	S_5S_9	Semi-incompatible	S_3S_5	2
			S_5S_9	8
SCS431 Felix 1	S_4S_5	Compatible	S_3S_4	2
			S_3S_5	5
			S_5S_9	3
			S_4S_9	0
SCS433 Felix 3	$S_3S?$	Semi-incompatible	$S_3S?$	3
			$S_9S?$	7
SCS434 Felix 4	S_4S_5	Compatible	S_3S_4	4
			S_3S_5	0
			S_5S_9	4
			S_4S_9	2
Selection 135/140	$S_3S?$	Semi-incompatible	$S_3S?$	7
			$S_9S?$	3

S? is an unidentified allele, being considered different from any of the evaluated ones.

for the *S*-alleles (Table 4). Among the 10 seedlings randomly tested from the 'SCS426 Venice' × 'SCS434 Felix 4', four, four and two were S_3S_4, S_5S_9 and S_4S_9, respectively. In the 'SCS426 Venice' × 'SCS431 Felix 1' cross, five, three and two seedlings were identified as S_3S_5, S_5S_9 and S_3S_4, respectively.

The other three pollinizers were identified as semi-compatible with 'SCS426 Venice' (Table 4). 'SCS425 Luiza' was genotyped as S_5S_9 (Brancher et al. 2020) and, both selection 135/140 and 'SCS433 Felix 3', were genotyped as $S_3S?$. The markers used for genotyping are allele-specific and did not enable the characterization of the missing *S*-allele for selection 135/140 and 'SCS433 Felix 3'. However, the uncharacterized *S*-alleles are different from SCS426 Venice's alleles and were labeled as '*S?*' (Table 4). Selection 135/140 and 'SCS433 Felix 3' were selected from open pollinated plants (male parent unknown), so the unidentified *S*-alleles could be originated from any apple plant grown in the orchard where they were bred. Since none of the 16 *S*-alleles tested were identified in these plants, it is believed that the unidentified *S*-allele might be one or two of the other nine *S*-alleles identified worldwide in apple trees (Larsen et al. 2016).

The 'SCS426 Venice' × selection 135/140 and 'SCS426 Venice' × 'SCS433 Felix 3' crosses are expected to be semi-compatible, where two genotypes ($S_3S?$ and $S_9S?$) should be observed in the offspring (Ramalho et al. 2012). Indeed, in the cross 'SCS426 Venice' × selection 135/140, seven seedlings were identified as $S_3S?$ and three as $S_9S?$. In the cross 'SCS426 Venice' × 'SCS433 Felix 3' seven seedlings were $S_9S?$ and three were $S_3S?$. Among the ten random seedlings genotyped from the crossing 'SCS426 Venice' × 'SCS425 Luiza', eight had the S_5S_9 and two had the S_3S_5 alleles. Thus, the identification of the *S*-alleles of the 10 seedlings randomly genotyped confirmed that the fruits were originated from the respective crosses and were not the result of contamination by other exogenous pollen.

The gametophytic self-incompatibility is known to be a determining factor for pollination success and apple fruit set in most of apple cultivars, including fruit and seeds development (Orcheski and Brown 2012; Ramírez and Davenport 2013; Matsumoto 2014). Under ideal conditions, compatible plants may have all seeds formed (normally around ten in *Malus* spp.) while incompatible plants do not grow any seeds (if these plants do not have parthenocarpy, they will not form fruits either) (Matsumoto et al. 2012).

Matsumoto et al. (2012) concluded that a partial pollination (when not all stigmas are pollinated and, consequently, the ovules are not fertilized) increases considerably the development of lopsided-shaped fruits, which reduces their commercial value. Although the semi-compatibility, no fruit deformation was observed in 'SCS426 Venice' when using 'SCS425 Luiza'

as pollinizer. In the crosses between 'SCS426 Venice' and selection 135/140 or 'SCS433 Felix 3', very few deformed fruits were identified. It was found an average of 7.84 seeds per fruit, with 1.67 seeds per carpel, which would be expected as a result of a fully-compatible crosses (Sheffield 2014). Apple tree's flowers present 10 ovules, the more ovules are fertilized, larger are the fruit sizes (Vizzotto et al. 2018). The theoretically expected in semi-compatible crosses was the presence of large numbers of deformed fruits by the abortion of common *S*-allele pollen to both parents (semi-compatible). However, the formation of symmetrical fruits again indicates that the amount of pollen available compensates the semi-compatibility of the pollinizers.

For the apple tree commercial production, both 'SCS426 Venice' and 'SCS425 Luiza' are cultivars with excellent fruit quality (Betinelli et al. 2017; Magrin et al. 2017). Furthermore, both present great pollen production and suitable release to cross-fertilization, even though they are semi-compatible. Alternate rows planting schemes for commercial orchard formation using 'SCS426 Venice' and 'SCS425 Luiza' can be a suitable commercial option, as it is done for 'Gala' and 'Fuji' orchards in Brazil. Both cultivars need similar management in the orchard and it would be possible to produce fruit of commercial value in all planted area without losing the 20% of pollinizer plants usually recommended for commercial orchards, without income. An alternative for increasing the pollen available is to increase the density of beehives distributed in apple orchards. The increase of pollinizer plants promotes a higher frequency of bee visits to the flowers, greater fruit set and allows adequate pollination of the plants with greater amount of pollen.

CONCLUSION

The pollinizers 'SCS431 Felix 1' (S_4S_5) and 'SCS434 Felix 4' (S_4S_5) are genetically compatible with 'SCS426 Venice (S_3S_9)'. The 135/140 (S_3S?), 'SCS433 Felix 3' (S_3S?) and 'SCS425 Luiza' (S_5S_9) are semi-compatible with 'SCS426 Venice'.

Regardless of the levels of compatibility, all genotypes tested are effective to fertilize flowers of the 'SCS426 Venice' and can be used efficiently as pollinizers in commercial orchards of this cultivar.

AUTHOR'S CONTRIBUTION

Conceptualization: Brancher T. L., Hawerroth M. C. and Kvitschal M. V.; Methodology: Brancher T. L. and Hawerroth M. C.; Investigation: Brancher T. L., Carlesso C., Hawerroth M. C. and Kvitschal M. V.; Writing – Original Draft: Brancher T. L.; Writing – Review and Editing: Brancher, T. L., Couto M., Denardi F., Guidolin A. F., Hawerroth M. C. and Kvitschal M. V.; Funding Acquisition: Hawerroth M. C.; Resources: Hawerroth M. C. and Kvitschal M. V.; Supervision: Hawerroth M. C. and Kvitschal M. V.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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