CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Pedigree testing for the SCS425 Luiza apple cultivar

Thyana Lays Brancher[1*], Marcus Vinicius Kvitschal[2], Luane Bosetto[3], Flavia Anan Saiki[3], Maraisa Crestani Hawerroth[4] and Altamir Frederico Guidolin[3]

Crop Breeding and Applied Biotechnology
23(4): e45232343, 2023
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332023v23n4a38

Abstract: *According to literature, the apple cultivar SCS425 Luiza is a hybrid of Imperatriz♀ and Cripps Pink♂; however, molecular analysis of gametophytic self-incompatibility alleles showed the presence of an unexpected allele in SCS425 Luiza. This raised doubts about the fidelity of the pedigree. Thus, this study aimed to investigate the real genealogy of the SCS425 Luiza cultivar via fingerprint analysis. A total of 19 pairs of SSR primers covering 12 of the 17 chromosomes were used. 'Imperatriz' was tested as the female parent, and either 'Cripps Pink' or 'Baronesa' was tested as the presumed male parents. The results excluded 'Cripps Pink' as the male parent because most of the markers showed alleles of exclusion with Likelihood ratio (LR) value equal to zero. In contrast, when Baronesa was tested as the male parent, all alleles followed the expected segregation with very high LR values (>10.000). Therefore, it is concluded that the correct genealogy of the SCS425 Luiza cultivar is Imperatriz♀ and Baronesa♂.*

Keywords: Malus x domestica *Borkh., paternity test, molecular fingerprint, SSR markers*

INTRODUCTION

Apple (*Malus x domestica* Borkh.) is the third most produced fruit in the world, and its production has been rising in recent years (FAO 2022). It is a deciduous fruit species of temperate climate, belonging to the family Rosaceae, subfamily Pomoideae. Apple trees usually need the accumulation of a minimum amount of chilling hours (below 7.2 °C) to overcome the dormancy of their buds (endodormancy) and start a new reproductive cycle, but how much chilling accumulation is needed varies according to the cultivar (Petri 2002). The basic number of chromosomes for the genus Malus is x = 17, possibly originating from hybridization between two wild species from Prunoideae (x = 8) and Spiraeoideae (x = 9) subfamilies, suggesting an allopolyploid origin (Phipps et al. 1991). Most apple trees are diploid (2n = 34), but triploid individuals can also occur naturally (Brown 2012). Plants of the genus Malus are allogamous due to the mechanism of gametophytic self-incompatibility, even with hermaphrodite flowers (Batlle et al. 1995, Matsumoto 2014, De Franceschi et al. 2016).

SCS425 Luiza cultivar is an agronomically important cultivar released by the Apple Breeding Program of the Agricultural Research and Rural Extension Company of Santa Catarina – Epagri, Brazil (Denardi et al. 2019a). The cultivar is characterized by having a medium chilling requirement, being resistant to

***Corresponding author:**
E-mail: thyanabrancher@gmail.com
ORCID: 0000-0003-3337-6314

Received: 04 August 2023
Accepted: 30 August 2023
Published: 10 October 2023

[1] Universidade Federal de Lavras, Trevo Rotatório Professor Edmir Sá Santos, Universidade Federal de Lavras, 37203-202, Lavras, MG, Brazil
[2] Epagri, Rua Abílio Franco, 1500, Bom Sucesso, 89501-032, Caçador, SC, Brazil
[3] UDESC - Centro de Ciências Agroveterinárias, Avenida Luiz de Camões, 2090, Conta Dinheiro, 88520-000, Lages, SC, Brazil
[4] InnoveAgro Pesquisa e Consultoria Agronômica, Avenida Militar, 107, Santa Terezinha, 95201-000, Vacaria, RS, Brazil

glomerella leaf spot (*Colletotrichum* spp.) and having high fruit quality. According to Denardi et al. (2019a), this cultivar is the result of hybridization between cultivars Imperatriz (♀) and Cripps Pink (♂), which are characterized by having genotype S_3S_5 and S_2S_{23}, respectively, at self-incompatibility locus (Albuquerque Junior et al. 2011, Brancher et al. 2020). However, molecular analysis of this locus in the SCS425 Luiza cultivar identified an S_5 allele and an unexpected S_9 allele (Brancher et al. 2020). This raised suspicions about the paternal genealogy information described in the records of Epagri's Apple Breeding Program about the original population, from which the SCS425 Luiza cultivar was selected.

Thus, the present study aimed to investigate, through SSR marker fingerprinting, whether the Cripps Pink cultivar is the male parent, and if not, to identify which was the pollen donor plant that pollinated the Imperatriz cultivar to generate the hybrid population, from which the cultivar SCS425 Luiza was selected.

MATERIAL AND METHODS

Plant material

In the 2017/2018 growing season, leaves were collected from apple trees in the initial development phase of the Imperatriz, Baronesa, Cripps Pink, Luiza, Elenise, Venice and Isadora cultivars, which were used in the directed crosses of the 2000/2001 cycle of Epagri's Apple Breeding Program. The leaves were taken from the orchard at the Epagri's Experimental Station of Caçador (EECd), Santa Catarina State, Brazil. Young leaves not fully expanded from the respective cultivars were placed in plastic bags and stored at -20 °C until the moment of DNA extraction. The cultivars classified as progeny are Luiza, Venice, Elenise and Isadora, resulting from two directed crosses (Imperatriz ♀ x Cripps Pink ♂; and Imperatriz ♀ x Baronesa ♂) made in the spring of 2000 season.

DNA extraction

For DNA extraction, the 2% CTAB (CetylTrimethylAmmonium Bromide) protocol, developed by Doyle and Doyle (1990), was used with adaptations for the species and laboratory conditions. Approximately 200 mg of plant tissue were placed in a high-resistance tube, with five metallic spheres inside, and then 1.0 mL of extraction buffer (2% CTAB; 1.4 M NaCl; 20 mM EDTA; 100 mM Tris-HCl pH 8.0; 2% PVP-40; 0.5% β-mercaptoethanol) heated to 60 °C. The maceration was performed in the Precellys® equipment at 14,000 xg 8,000 rpm for 20 seconds, and the procedure was repeated six times, with intervals of 10 seconds. The other extraction steps followed the protocol developed by Doyle and Doyle (1990). After extraction, the DNA concentration (ng μL^{-1}) and the A260/A280 and A260/A230 quality parameters were measured using a NanodropOne® spectrophotometer (Thermo Scientific, Waltham, Massachusetts, U.S.). DNA samples were then diluted to the standard concentration of 10 ng μL^{-1}.

Polymerase chain reaction (PCR) conditions

The reactions were carried out in a multiplex scheme, according to the color of the fluorophore, the size of the amplified fragment and the PCR conditions, using a total of 19 pairs of SSR (*Simple Sequence Repeats*) primers developed exclusively for *Malus x domestica* Borkh. (Table 1). The choice of SSR primers was based on the literature data (Klabunde et al. 2016, Hawerroth et al. 2018) and sampling genomic regions from 12 of the 17 chromosomes of apple species. The PCR reactions were made using multiplex panels, as detailed in Table 1. For each PCR reaction, 1.2 ng of genomic DNA was used, plus 1x buffer, 0.2 µM of each primer, 0.2 µM of dNTPs, 1.5 mM of $MgCl_2$, 1 U of Platinum® Taq DNA Polymerase (Invitrogen, Waltham, Massachusetts, U.S.), and ultrapure water to complete the final volume of 12.5 µL. The reactions were performed in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Waltham, Massachusetts, U.S.), with the following cycling conditions: (I) initial denaturation of chain: 94 °C for 5 min; (II) denaturation: 94 °C for 45 sec; (III) annealing: 60 °C for 60 sec; (IV) extension of chain: 72 °C for 60 sec; and (V) final extension: 72 °C for 7 min. Steps II, III and IV were performed for 28 cycles for multiplex panels A, B, C, D, F and G; 30 cycles for panel E; and 31 cycles for panel H (Table 1).

Capillary electrophoresis

The AB 3130 Genetic Analyzer (Applied Biosystems) was used for analyzing the PCR products. Each sample mix contained 1.0 µL of PCR products, 4.85 µL of HI-DI™ formamide (Applied Biosystems) and 0.15 µL of GS600 LIZ® (Applied

Table 1. List of 19 pairs of SSR primers used to genotype apple (*Malus x domestica* Borkh.) cultivars and their respective oligonucleotide sequences, units of repetition (UR), fluorophore (Dye), multiplex panel (M) and size range of amplified PCR fragments expected (bp)

M	SSR	*Forward* sequence *(5' → 3')*	*Reverse* sequence *(5' → 3')*	Repetition Unit	Dye	Range (bp)
A	CH04d02 [a]	CGTACGCTGCTTCTTTTGCT	CTATCCACCACCCGTCAACT	$(TC)_{19}$	6-FAM	118-146
	CH03a08 [a]	TTGGTTTGCTAGGAAAAGAAGG	AAGTTTATCGGGCCTACACG	$(CT)_{19}$	HEX	146-218
	CH01f02 [a]	ACCACATTAGAGCAGTTGAGG	CTGGTTTGTTTTCCTCCAGC	$(AG)_{22}$	NED	174-206
B	CH01g12 [a]	CCCACCAATCAAAAATCACC	TGAAGTATGGTGGTGCGTTC	$(AG)_{22}$	6-FAM	174-206
	CH01h01 [a]	GAAAGACTTGCAGTGGGAGC	GGAGTGGGTTTGAGAAGGTT	$(TC)_{25}$	HEX	114-134
	CH02c11 [a]	TGAAGGCAATCACTCTGTGC	TTCCGAGAATCCTCTTCGAC	$(CT)_{15}CC(CT)_{8}$	NED	219-239
C	Hi03g06 [b]	TGCCAATACTCCCTCATTTACC	GTTTAAACAGAACTGCACCACATCC	$(TC)_{7}$	6-FAM	182-204
	CH03b06 [a]	GCATCCTTGAATGAGGTTCACT	CCAATCACCAAATCAATGTCAC	$(CT)_{20}$	HEX	111-131
	CH02d08 [a]	TCCAAAATGGCGTACCTCTC	GCAGACACTCACTCACTATCTCTC	$(GA)_{20}$	VIC	210-254
D	CH05d11 [a]	CACAACCTGATATCCGGGAC	GAGAAGGTCGTACATTCCTCAA	$(AG)_{22}$	6-FAM	171-211
	CH02b03b [a]	ATAAGGATACAAAAACCCTACACAG	GACATGTTTGGTTGAAAACTTG	$(CT)_{22}$	HEX	77-109
	NZ02b1 [c]	CCGTGATGACAAAGTGCATGA	ATGAGTTTGATGCCCTTGGA	$(GA)_{14}$	HEX	212-238
E	CH01f09 [a]	ATGTACATCAAAGTGTGGATTG	GGCGCTTTCCAACACATC	$(AG)_{22}$	HEX	125-160
F	CH02b12 [a]	GGCAGGCTTTACGATTATGC	CCCACTAAAAGTTCACAGGC	$(CT)_{21}TT(TC)_{5}$	6-FAM	101-143
	CH03c02 [a]	TCACTATTTACGGGATCAAGCA	GTGCAGAGTCTTTGACAAGGC	$(CT)_{22}$	HEX	116-136
G	CH04c07 [a]	GGCCTTCCATGTCTCAGAAG	CCTCATGCCCTCCACTAACA	$(AG)_{23}$	6-FAM	98-135
	CH05c06 [a]	ATTGGAACTCTCCGTATTGTGC	ATCAACAGTAGTGGTAGCCGGT	$(CT)_{6}CA(CT)_{20}$	HEX	104-126
H	CH02b10 [a]	CAAGGAAATCATCAAAGATTCAAG	CAAGTGGCTTCGGATAGTTG	$(CT)_{19}$	6-FAM	121-159
	CH05a05 [a]	TGTATCAGTGGTTTGCATGAAC	GCAACTCCCAACTCTTCTTTCT	$(GA)_{21}$	HEX	198-230

[a] Liebhard et al. (2002); [b] Silfverberg-Dilworth et al. (2006); [c] Guilford et al. (1997).

Biosystems, Waltham, Massachusetts, U.S.). Peak interpretation, allele size calling and genotyping were performed using the GeneMapper® Id-X software, v. 1.2.

Data set analysis

The allele frequencies used in the present work were calculated based on the previous genotyping data of the SCS425 Luiza cultivar and 27 other apple tree cultivars used as parental germplasm in the hybridization routine of the Epagri's Apple Breeding Program, namely: Imperatriz, Baronesa, Cripps Pink, Venice, Elenise, Isadora, Fuji, Fuji Suprema, Fuji Precoce, Royal Gala, Lisgala, Castel Gala, Gala Gui, NJ-76, Coop-14, Joaquina, Fred Hough, Kinkas, D1R103T245, Primícia, Princesa, Condessa, Duquesa, Daiane, Monalisa, Serrana and Granny Smith. Pedigree analysis was performed using the Familias 3 software (Kling et al. 2014). 'Imperatriz' was fixed as female parent in all tests. The cultivars 'Baronesa' and 'Cripps Pink' were tested as possible male parent. To be considered as a male parent, the cultivar must show a total *Likelihood ratio* (LR) value greater than or equal to 10,000. In addition, exclusion markers were also analyzed to support the interpretation of exclusion of a possible male parent based on LR values. Furthermore, based on the alleles of each genotype, a dissimilarity matrix was made using the Jaccard method, which was used for grouping genotypes in a UPGMA dendrogram (Unweighted Pair Group Method with Arithmetic Mean). The UPGMA grouping adjustment to the dissimilarity matrix was determined by the cophenetic correlation coefficient based on the average dissimilarity to separate the groups (Sokal and Rohlf 1962). The significance of the cophenetic correlation was calculated using t and Mantel tests. The grouping and cophenetic correlation analysis were performed using the computer program GENES (Cruz 2013).

RESULTS AND DISCUSSION

The approach for pedigree checking for SCS425 Luiza cultivar in the present work followed the same methods used in human and animal paternity tests. Although this approach is rarely used for plants (Meagher 1986), there was a need to elucidate the correct genealogy of the cultivar SCS425 Luiza. The main factor that led to doubt in 'Luiza' genealogy was the identification of an unexpected self-incompatibility *S* allele for the cross of 'Imperatriz' x 'Cripps Pink' (Brancher et al. 2020). The original information was that the cultivar SCS425 Luiza is a result of cross between Imperatriz (♀) and

Cripps Pink (♂) (Denardi et al. 2019a), but after genotyping the alleles of the *S* allelic series, responsible for the control of gametophytic self-incompatibility between apple trees, the S_5S_9 genotype was identified for 'Luiza' (Brancher et al. 2020). As the cultivar Imperatriz has the genotype S_3S_5 and 'Cripps Pink' has the genotype S_2S_{23} (Albuquerque Junior

Table 2. New apple cultivars (self-incompatibility genotype) released by Epagri's Apple Breeding Program, recorded pedigree, *S* alleles and *likelihood ratio* (LR) value based on their respective parents (female ♀ and male ♂)

Cultivar	♀	♂	LR value
Luiza[1] (S_5S_9)	Imperatriz (S_3S_5)	Cripps Pink (S_2S_{23})	0
Venice[2] (S_3S_9)	Imperatriz (S_3S_5)	Baronesa (S_3S_9)	1.996.340,6
Elenise[3] (S_3S_{23})	Imperatriz (S_3S_5)	Cripps Pink (S_2S_{23})	311.786,6
Isadora[4] (S_5S_{23})	Imperatriz (S_3S_5)	Cripps Pink (S_2S_{23})	40.621.290,3

[1] Denardi et al. (2019a); [2] Denardi et al. (2020); [3] Denardi et al. (2019b); [4] Denardi et al. (2023).

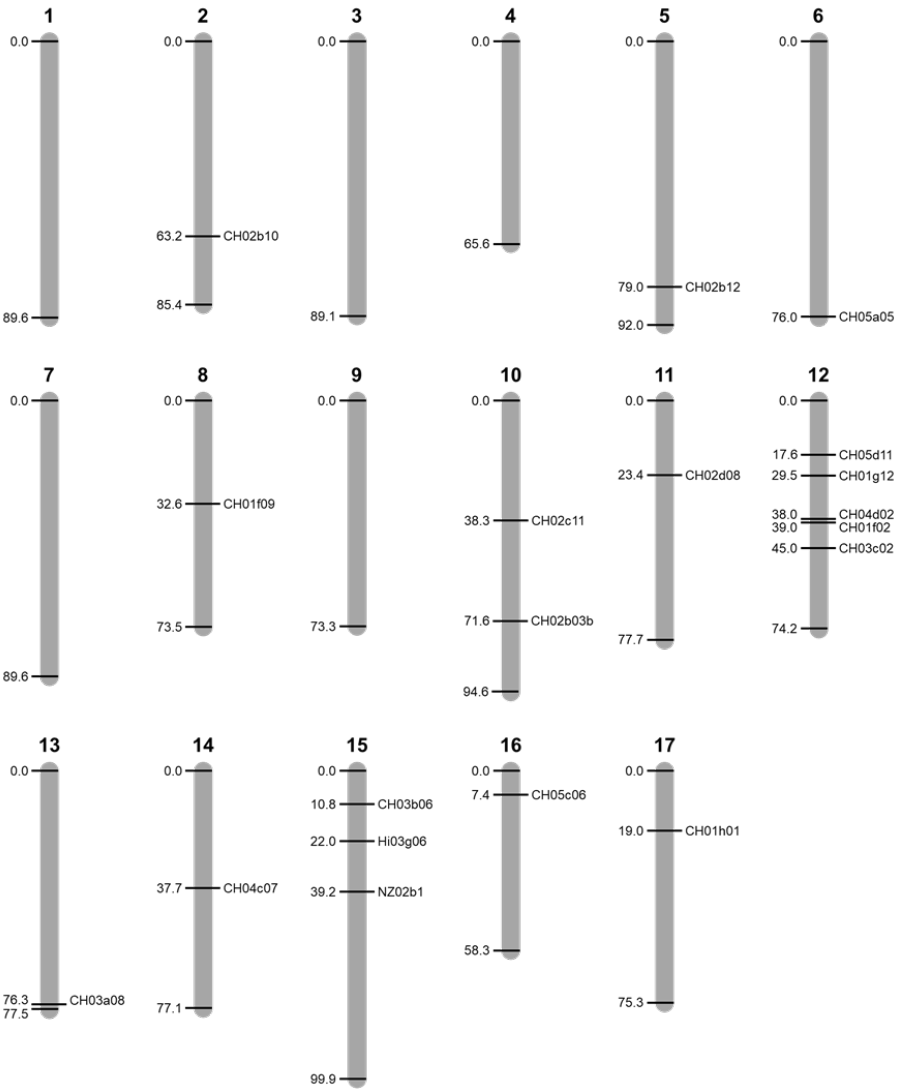

Figure 1. A diagram of genetic map of apple (*Malus x domestica* Borkh.), indicating the targeted positions of the 19 pairs of SSR primers on 12 of the 17 chromosomes (cM). The diagram was built from the database available at http://www.rosaceae.org/search/markers.

et al. 2011), it is unlikely that 'SCS425 Luiza' is a cross progeny between these two cultivars, because none of them has the S_9 allele. However, this occurrence is not unique among the apple tree breeding programs around the world. Divergences in the genealogy of hybrid apple cultivars have also been identified via genotyping of *S* alleles in the Kent cultivar (Sakurai et al. 2000).

In an attempt to identify the correct male parent of Luiza cultivar, the three parental cultivars (Imperatriz, Baronesa and Cripps Pink) that were used in the crossing programs of Epagri's Apple Breeding Program in the same year, as well as four sister cultivars originating from the same combinations (Venice, Elenise, Isadora and Luiza) were tested.

Imperatriz was the female parent for 'Venice', 'Elenise', 'Isadora' and 'Luiza'. Baronesa was the male parent for 'Venice', and Cripps Pink was the male parent for 'Elenise' and 'Isadora'. The observed *likelihood ratio* (LR) values were much higher than 10,000 for 'Venice', 'Elenise' and 'Isadora', corroborating the pedigrees previously reported by the breeders for these three cultivars (Table 2). However, the LR value for SCS425 Luiza cultivar testing was equal to zero when the genealogy reported by Denardi et al. (2019b) was checked out. It indicates that 'Cripps Pink' is not the male parent of 'Luiza' (Table 2).

The 19 pairs of SSR primers amplified (Figure 1) a total of 126 alleles in the cultivars set evaluated, the mean number of alleles identified for each genome region was 6.63. Two alleles were observed in each genome region, suggesting diploid genotypes for all cultivars. The alleles identified at each genome region in Luiza, Venice, Elenise, Isadora, Imperatriz, Baronesa and Cripps Pink cultivars are shown in Table 3.

It is observed in Table 3 that the cultivars Venice, Elenise and Isadora follow the expected segregation pattern for all markers amplified in PCR considering the genotypes of male and female parents, respectively, as reported in the literature (Denardi et al. 2019a, 2019b, 2020, Denardi et al. 2023). However, for the cultivar Luiza, of the 19 evaluated genome regions, only 9 regions followed the expected pattern of allele segregation, showing the presence of only one allele common to the Cripps Pink cultivar, previously described as the male parent. However, when carefully observing the result of the genotypic characterization of Luiza cultivar for all 19 SSR markers, it appeared that all alleles followed an expected segregation pattern if the male parent considered was cv. Baronesa (Table 3), which corroborates the results

Table 3. SSR alleles amplified by 19 pairs of SSR primers from the cultivars Imperatriz, Baronesa, Cripps Pink, Luiza, Venice, Elenise and Isadora

SSR marker	Alleles amplified (bp)													
	Imperatriz		Baronesa		Cripps Pink		Luiza		Venice		Elenise		Isadora	
	Female parent		Male parent suggested 1		Male parent suggested 2		Progeny		Progeny		Progeny		Progeny	
CH01f02	182	204	168	182	178	206	**182**	**182**	168	204	204	206	204	206
CH01f09	126	136	**136**	**136**	**126**	**126**	126	136	**136**	**136**	126	136	**126**	**126**
CH01g12	103	143	125	143	99	179	103	143	125	143	99	143	143	179
CH01h01	117	127	113	127	109	115	113	117	117	127	115	127	109	127
CH02b03b	72	90	72	92	72	92	72	92	72	72	72	72	72	92
CH02b10	113	119	127	151	115	119	113	127	119	151	**119**	**119**	**119**	**119**
CH02b12	**135**	**135**	131	135	**135**	**135**	**135**	**135**	131	135	**135**	**135**	**135**	**135**
CH02c11	**229**	**229**	225	231	203	229	225	229	225	229	203	229	203	229
CH02d08	**252**	**252**	208	222	208	220	222	252	222	252	220	252	220	252
CH03a08	156	186	**180**	**180**	180	242	156	180	180	186	156	180	180	186
CH03b06	111	115	**111**	**111**	111	133	111	115	111	115	111	115	111	115
CH03c02	**121**	**121**	101	157	121	123	121	157	101	121	**121**	**121**	**121**	**121**
CH04c07	92	132	104	132	92	104	92	104	92	132	104	132	92	104
CH04d02	115	117	115	117	**117**	**117**	**115**	**115**	**117**	**117**	**117**	**117**	**117**	**117**
CH05a05	204	218	**218**	**218**	216	218	204	218	204	218	204	218	216	218
CH05c06	111	119	97	111	97	121	97	111	**111**	**111**	97	111	97	111
CH05d11	168	172	178	192	164	166	172	192	168	192	164	172	164	168
Hi03g06	190	190	190	194	176	198	190	194	**190**	**190**	176	190	176	190
NZ02b1	213	235	**213**	**213**	213	225	213	235	213	235	213	235	213	235

Alleles highlighted in bold and underlined are in homozygosis.

previously observed. It suggests that the Cripps Pink cultivar is not the true male parent of the cv. Luiza, and that the 'Baronesa' tree is the pollen donor.

In the grouping dendrogram made from the dissimilarity data matrix calculated between cultivars for the 19 SSR markers (Figure 2), a great similarity can be observed between Imperatriz and Luiza cultivars, reinforcing the already known genetic relationship between both as reported by Denardi et al. (2019b). However, it is also observed that Luiza cultivar showed greater genetic similarity to the group of Venice and Baronesa cultivars, and not to the group that includes the cultivar Cripps Pink. This is another indication that the Cripps Pink cultivar is not the male parent used to generate the population from which the Luiza cultivar was selected.

Based on the paternity analysis, the Cripps Pink cultivar was also discarded as a male parent, since the calculated LR (*likelihood ratio*) value was equal to zero. Furthermore, from the 19 SSR markers tested, more than half (10 genomic regions) were characterized as exclusion alleles when considering Cripps Pink cultivar as the male parent of 'Luiza'

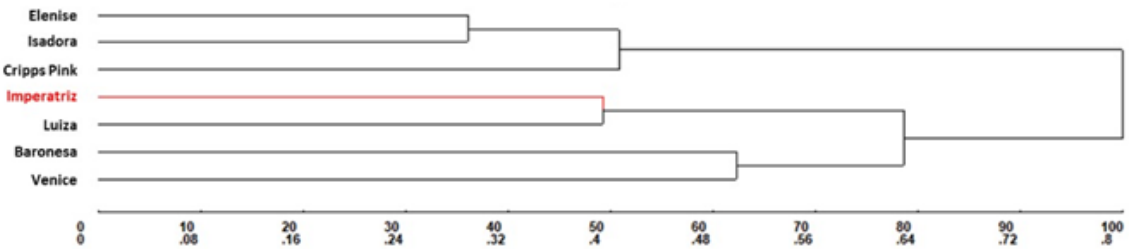

Figure 2. Dendrogram of the cultivars Imperatriz, Baronesa, Cripps Pink, Luiza, Venice, Elenise and Isadora by the UPGMA method based on their SSR allele dissimilarity matrix produced by 19 pairs of SSR primers.

Table 4. *Likelihood ratio* (LR) values and the size of fragments amplified by 19 pairs of SSR primers in paternity analysis of the SCS425 Luiza cultivar

	Hypothesis 1							Hypothesis 2						
SSR	Imperatriz (♀) x Cripps Pink (♂) = Luiza							Imperatriz (♀) x Baronesa (♂) = Luiza						
marker	LR	Imperatriz		Cripps Pink		Luiza		LR	Imperatriz		Baronesa		Luiza	
CH01f02	0.000	182	204	**178**	**206**	182	182	1.558	182	204	168	182	182	182
CH01f09	1.219	126	136	126	126	126	136	1.219	126	136	136	136	126	136
CH01g12	0.000	103	143	**99**	**179**	103	143	0.933	103	143	125	143	103	143
CH01h01	0.000	117	127	**109**	**115**	113	117	2.157	117	127	113	127	113	117
CH02b03b	2.793	72	90	72	92	72	92	2.793	72	90	72	92	72	92
CH02b10	0.000	113	119	**115**	**119**	113	127	4.000	113	119	127	151	113	127
CH02b12	1.557	135	135	135	135	135	135	0.778	135	135	131	135	135	135
CH02c11	0.000	229	229	**203**	**229**	225	229	3.493	229	229	225	231	225	229
CH02d08	0.000	252	252	**208**	**220**	222	252	1.748	252	252	208	222	222	252
CH03a08	1.166	156	186	180	242	156	180	2.331	156	186	180	180	156	180
CH03b06	0.757	111	115	111	133	111	115	1.514	111	115	111	111	111	115
CH03c02	0.000	121	121	**121**	**123**	121	157	4.673	121	121	101	157	121	157
CH04c07	2.334	92	132	92	104	92	104	2.334	92	132	104	132	92	104
CH04d02	0.000	115	117	**117**	**117**	115	115	2.334	115	117	115	117	115	115
CH05a05	0.700	204	218	216	218	204	218	1.401	204	218	218	218	204	218
CH05c06	2.002	111	119	97	121	97	111	2.002	111	119	97	111	97	111
CH05d11	0.000	168	172	**164**	**166**	172	192	4.004	168	172	178	192	172	192
Hi03g06	0.000	190	190	**176**	**198**	190	194	7.042	190	190	190	194	190	194
NZ02b1	0.824	213	235	213	225	213	235	1.647	213	235	213	213	213	235
LR total:	0.000							2.375.957,07						

Alleles highlighted in bold and underlined represent alleles of exclusion, which were not inherited from the parent under test.

(Table 4), since any of the alleles of the supposed parent could inherit into the supposed descendant progeny (cv. Luiza). On the other hand, when considering Imperatriz and Baronesa to be female and male parents, respectively, a high LR (2,375,957.07) was observed (Table 4), which is much higher than that recommended in the literature (≥ 10,000) to accept a given cultivar as a paternal parent in a genealogy under checking analysis.

Therefore, the Cripps Pink cultivar is excluded as the male parent of the SCS425 Luiza cultivar. By analyzing molecular fingerprints of the 19 SSR markers used, it is safe to state that the paternal parent of 'SCS425 Luiza' is the Baronesa cultivar. Thus, the SCS425 Luiza cultivar is derived from the cross between Imperatriz (♀) and Baronesa (♂), but not from the cross between Imperatriz (♀) and Cripps Pink (♂) as reported by Denardi et al. (2019b). During the routine process of breeding and selection of trees in the Epagri's Apple Breeding Program, there must have been some mistake in the manipulation of pollen, hybrid seeds or young trees, or there could also have been an exchange of trees identification tags between different segregating populations performed in that season. So, it shows the high importance of careful work by the Breeding Program employees as a way to avoid mistakes of this nature.

ACKNOWLEDGEMENTS

The authors wish to thank the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC), Universidade do Estado de Santa Catarina (UDESC), and Universidade Federal de Santa Catarina (UFSC) for financial support to this research and for the scholarships granted.

REFERENCES

Albuquerque Junior CL, Denardi F, Dantas ACM, Nodari RO, Albuquerque CL, Denardi F, Mesquita Dantas AC and Nodari RO (2011) The self-incompatible RNase S-alleles of Brazilian apple cultivars. **Euphytica 181**: 277-284.

Batlle I, Alston FH and Evans KM (1995) The use of the isoenzymic marker gene Got-1 in the recognition of incompatibility S alleles in apple. **Theoretical and Applied Genetics 90**: 303-306.

Brancher TL, Hawerroth MC, Kvitschal MV, Manenti DC and Guidolin AF (2020) Self-incompatibility alleles in important genotypes for apple breeding in Brazil. **Crop Breeding and Applied Biotechnology 20**: 1-9.

Brown S (2012) Apple. In **Fruit breeding**. Springer US, Boston, p. 329-367.

Cruz CD (2013) GENES - a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum. Agronomy 35**: 271-276.

De Franceschi P, Cova V, Tartarini S and Dondini L (2016) Characterization of a new apple S-RNase allele and its linkage with the Rvi5 gene for scab resistance. **Molecular Breeding 36**: 1-11.

Denardi F, Kvitschal MV, Argenta LC, Couto M, Araujo L (2023) SCS443 Isadora: late ripening apple cultivar with very high fruit storage ability. **Revista Brasileira de Fruticultura 45**: e-161.

Denardi F, Kvitschal MV, Hawerroth MC and Argenta LC (2019a) 'SCS425 Luiza': new apple cultivar with medium chilling requirement and resistant to glomerella leaf spot (colletotrichum spp.). **Revista Brasileira de Fruticultura 41**: e-109.

Denardi F, Kvitschal MV, Hawerroth MC and Argenta LC (2019b) SCS426 Venice: new apple cultivar with glomerella leaf spot resistance and picking time in march. **Crop Breeding and Applied Biotechnology 19**: 481-486.

Denardi F, Kvitschal MV, Hawerroth MC and Argenta LC (2020) SCS427 Elenise: late-ripening apple variety of good storability and resistance to Glomerella Leaf Spot. **Revista Agropecuária Catarinense 33**: 32-36.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. **Focus 12**: 13-15.

FAO - Food and Agriculture Organization of the United Nations (2022) FAOSTAT. Crop production quantity. Available at <http://www.fao.org/faostat/en/#data/QC>. Accessed on May 4, 2021.

Guilford P, Prakash S, Zhu JM, Rikkerink E, Gardiner S, Bassett H and Forster R (1997) Microsatellites in Malus X domestica (apple): abundance, polymorphism and cultivar identification. **Theoretical and Applied Genetics 94**: 249-254.

Hawerroth MC, Brancher TL and Kvitschal MV (2018) Dissimilaridade entre genótipos elite de macieira da Epagri com base na caracterização fenotípica e molecular. **Agropecuária Catarinense 31**: 67-72.

Klabunde GHF, Junkes CFO, Tenfen SZA, Dantas ACM, Furlan CRC, Mantovani A, Denardi F, Boneti JI and Nodari RO (2016) Genetic diversity and apple leaf spot disease resistance characterization assessed by SSR markers. **Crop Breeding and Applied Biotechnology 16**: 189-196.

Kling D, Tillmar AO and Egeland T (2014) Familias 3 – Extensions and new functionality. **Forensic Science International: Genetics 13**: 121-127.

Liebhard R, Gianfranceschi L, Koller B, Ryder CD, Tarchini R, Van de Weg E and Gessler C (2002) Development and characterisation of 140 new microsatellites in apple (Malus x domestica Borkh.). **Molecular Breeding 10**: 217-241.

Matsumoto S (2014) Apple pollination biology for stable and novel fruit production: Search system for apple cultivar combination showing incompatibility, semicompatibility, and full-compatibility based on the S-RNase allele database. **International Journal of Agronomy 2014**: 1-9.

Meagher TR (1986) Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. **The American Naturalist 128**: 199-215.

Petri JL (2002) Fatores edafoclimáticos. In EPAGRI (ed) **A cultura da macieira**. EPAGRI, Florianópolis, p. 105-112.

Phipps JB, Robertson KR, Rohrer JR and Smith PG (1991) Origins and evolution of subfam. Maloideae (Rosaceae). **Systematic Botany 16**: 303.

Sakurai K, Brown SK and Weeden N (2000) Self-incompatibility alleles of apple cultivars and advanced selections. **Hortscience 35**: 116-119.

Silfverberg-Dilworth E, Matasci CL, Weg WE Van de, Kaauwen MPW Van, Walser M, Kodde LP, Soglio V, Gianfranceschi L, Durel CE, Costa F, Yamamoto T, Koller B, Gessler C and Patocchi A (2006) Microsatellite markers spanning the apple (*Malus x domestica* Borkh.) genome. **Tree Genetics & Genomes 2**: 202-224.

Sokal RR and Rohlf FJ (1962) The comparison of dendrograms by objective methods. **Taxon 11**: 33.