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ARTICLE

Genetic diversity and apple leaf spot disease resistance characterization assessed by SSR markers

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Abstract: *Among the cultivation problems of apple production in Brazil, Apple Leaf Spot (ALS) disease represents one of the main breeding challenges. This study aims at analyzing the genetic diversity among 152 apple scion accessions available at the Apple Gene Bank of EPAGRI, located in Caçador, Santa Catarina/ Brazil. Eleven genomic SSR loci were analyzed to assess genetic diversity of ALS resistant and susceptible accessions. Results revealed high genetic diversity of the studied accessions, being 120 exclusive alleles (67 unique) from scion accessions resistant to ALS, and a mean PIC of 0.823. The locus Probability of Identity (I) ranged from 0.017 to 0.089. The combined I was 4.11 x 10^{-16}, and the Power of Exclusion was 99.99999259%. In addition, the DNA fingerprint patterns will contribute as additional descriptors to select parental for crosses and early identification of apple accessions for breeding purposes, and also for cultivar protection.*

Key words: *Colletotrichum gloeosporioides, DNA fingerprinting, Malus spp, genetic identity, microsatellite.*

INTRODUCTION

Brazil is the tenth largest apple producer country, with a total production of 1.335 million tons in 2012 (FAO 2014). However, the growers still face serious limiting problems, such as poor climatic adaptation of the current varieties due to insufficient chilling during the winter. High rainfall, associated with high temperatures, during the growing season are the main causes of development of many serious diseases problems in Southern Brazil. Breeding programs carried out by researchers and germplasm bank curators in this region focus on identifying promising germplasm to be used as parent for specific traits, such as short juvenility, high yield, fruit quality, disease-resistance rootstocks, and for low to moderate chilling requirement (Furlan et al. 2010). In addition, the development of scion cultivars resistant to Apple Leaf Spot disease (ALS) is one of the major challenges for apple breeders in southern states of Brazil. The disease is caused by the fungus *Colletotrichum gloeosporioides,* which triggers the appearance of several brown patches, followed by intense defoliation of trees, and consequently decrease in fruit production (Weir et al. 2012).

One of the most effective strategies to control ALS is to develop disease-resistant cultivars. In this context, the Apple Gene Bank plays an important role as a source of high genetics (Janick et al. 1996, Hokanson et al. 1997). *Malus* ×

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domestica Borkh. is one of the most important domesticated fruit species, and its distinct varieties has been submitted to countless crossings and selections over the years. However, a limited number of *Malus* species has been used up to present for breeding disease resistance, except few breeding programs, like Purdue Rutgers, Illinois, USA, and some European apple breeding programs (Korban and Tartarini 2009). The majority of the parents used in breeding programs have explored a strait gene pool, involving crosses among widespread commercial cultivars (Kumar et al. 2010). For instance, the varieties Red Delicious, Golden Delicious and Jonathan, have been frequently utilized on apple breeding, producing several of the current cultivars (Noiton and Alspach 1996). In addition, the selection and release of sport mutants of widespread cultivars have also potentialized the trend towards genetic similarity in today's commercial cultivars (Brooks and Olmo 1994).

The accessibility to diverse apple germplasm is essential for pursuing successful breeding, since it increases the number of allele combinations and allows the development of new cultivars with improved and desirable traits. Consequently, broadening the germplasm used in breeding will increase the diversity in cultivation, avoid genetic vulnerability, and preserve their unique genetic characteristics available in this new genetic combinations for future generations.

In plant germplasm characterization, duplicates and mislabeling of accessions might occur, which are unwanted, costly and time consuming (Gustavsson et al. 2008). In these cases, molecular markers are very useful to detect identical, synonymous and homonymous accessions, and also to help breeders to form their core collections (Gross et al. 2012). In addition, proper identification and characterization of accessions will help better protect cultivars under intellectual property rights and identify parents carrying alleles of interest for apple breeding (Goulão et al. 2001).

The EPAGRI´s Apple Gene Bank (EAGB) contains 442 apple accessions introduced from more than 20 countries. Among them, there are several wild apple species, such as *M. aldenhamensis, M. atrosanguinea, M. baccata, M. eley, M. hillieri, M. platicarpa, M. robusta, M. prunifolia, M. pumila, M. floribunda,* and 19 *M. pumila* rootstock accessions. The referred Gene Bank can be then considered a potential source of germplasm for many genetic resistances. There are 33 accessions with vertical resistance to apple scab (*Venturia inaequalis*). Furlan et al. (2010) also identified 187 resistant and 58 susceptible accessions to *Colletotrichum gloeosporioides*.

The use of molecular markers has been valuable for assessing species and cultivars' genetic diversity, determining phylogenetic relationships and identifying alleles of interest (Han and Korban 2010). Among the molecular ones, microsatellite markers (SSR-*simple sequence repeats*) are highly polymorphic, multiallelic, co-dominant, reproducible and are distributed throughout the entire genome, making them ideal for revealing genetic diversity (Morgante et al. 2002). Therefore, the use of molecular markers to access the apple germplasm is a trustworthy tool (Gustavsson et al. 2008, Zhuang et al. 2011, Potts et al. 2012, Reim et al. 2013, Burak et al. 2014). Almost 400 microsatellite markers have already been identified and mapped in *Malus* and other fruit species (Guilford et al. 1997, Liebhard et al. 2002, Silfverberg-Dilworth et al. 2006, Han and Korban 2008). These molecular markers have been successfully used for assessing the gene pool and relatedness among distinct *Malus* germplasm (Pereira-Lorenzo et al. 2008, Gasi et al. 2010); and also for identifying loci associated with target alleles and for map-based cloning, regarding their co-dominant heritage and high polymorphism level (Hokanson et al. 1998, Liebhard et al. 2002, Oraguzie et al. 2005, Naik et al. 2006, Han and Korban 2010, Zhuang et al. 2011).

Thus, the primary goal of this work was to assess genetic diversity and relatedness of a large collection of apple germplasm maintained by EPAGRI´s *Malus* breeding program. The objective was to identify and establish DNA fingerprinting patterns based on SSR markers, and to associate specific alleles and genotypes to ALS resistant and susceptible phenotypic data.

MATERIAL AND METHODS

Plant material and DNA isolation

The 152 apple scion accessions (Table 1) selected for this study represent a wide genetic spectrum containing several target alleles of particular interest to apple breeding in Southern Brazil. The selected apple accessions used in this work are originated from 18 countries and are currently located at EPAGRI – Caçador Experimental Station (lat 26º 49' 07" S, long 50º 59' 07" W, and alt 960 m asl), Caçador, SC, Brazil. Total DNA was extracted from 100 mg of leaf material, based on CTAB method from Doyle and Doyle (1990). DNA quality and quantity were determined by spectrophotometry at 260 nm, using Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Table 1. Names and country of origin of apple accessions genotypes used in this study

	Accessions	Origin		Accessions	Origin		Accessions	Origin
1	2137964	*	52	Greensleeves	UK	103	† Planaltina	Brazil
2	† Akagui	Japan	53	GrothRed	*	104	POME 10	Brazil
3	Angius	*	54	† Hame 6	*	105	POME 12	Brazil
4	Argentina 2	Argentina	55	Harrold Red	USA	106	† POME 13	Brazil
5	Arlet	Switzerland	56	† Hatsuaki	Japan	107	POME 14	Brazil
6	Baronesa	Brazil	57	Hokuto	Japan	108	POME 15	Brazil
7	Bem Davis	Brazil	58	Holland	USA	109	POME 16	Brazil
8	Black Jon	USA	59	Honey Gold	USA	110	POME 17	Brazil
9	Bonita[a]	Brazil	60	† Horey	Japan	111	† POME 19	Brazil
10	Bonita[b]	Brazil	61	Imperatore	Brazil	112	POME 20	Brazil
11	Braebum	New Zealand	62	Imperatriz	Brazil	113	† POME 22	Brazil
12	Carla	Brazil	63	Israel 8-3	Israel	114	POME 28	Brazil
13	Catarina	Brazil	64	† Ivette	N.lands	115	POME 03	Brazil
14	Centenária	Brazil	65	Jersey Mac	USA	116	Porporate	*
15	† Condessa	Brazil	66	† Jona Free	USA	117	Priam	USA
16	Comrade Red	England	67	† Jonagold	USA	118	† PX1032	France
17	Coop 14	USA	68	Jonared	USA	119	PX1033[a]	France
18	Coop 16	USA	69	Liberty	USA	120	PX1033[b]	France
19	Coop 24	USA	70	† Lisgala	Brazil	121	†PX216	France
20	Coop 06	USA	71	M.Aldenhamensis	China	122	† PX565	France
21	† D1R100T209	USA	72	M.Atrosanguinea	UK	123	Quinte	Canada
22	D1R102T116	USA	73	† M. Baccata	China	124	† Rainha	Brazil
23	D1R103T245	USA	74	M.Eleyi	China	125	Red Delicious	USA
24	D1R68T571	USA	75	M.Robusta	Canada	126	Red Free	USA
25	D1R73T94	USA	76	M6039	*	127	Redgold	USA
26	† D1R98T188	USA	77	Maayan	Israel	128	Reinette du Canada	Canada
27	D1R99T15	USA	78	Mac Free	USA	129	Reinette du Mans	France
28	D2R30T30	USA	79	† Marquesa	Brazil	130	René Reinetes	France
29	D2R31T237	USA	80	Mechinoku	Japan	131	Rome Beauty	USA
30	D2R38T126	USA	81	Melrose	USA	132	Sansa	Japan
31	D2R39T243	USA	82	Mere	*	133	† Senshu	Japan
32	D2R40T253[a]	USA	83	Monroe	USA	134	Shellred	USA
33	D2R40T253[b]	USA	84	† Mutsu	Japan	135	SM69-3	Brazil
34	Daiane	Brazil	85	Natsumidori	Japan	136	† Splendor	New Zealand
35	Delcon	USA	86	† Nero 26	USA	137	Stark J. Grimes	USA
36	† Discovery	UK	87	NewtonPippin	USA	138	Starkjonadel	New Zealand
37	Dorsett Golden	Bahamas	88	Niagara	USA	139	Stayman	USA
38	Ein Shemer	Israel	89	NJ36	USA	140	† Summered	Canada
39	† Elstar	Netherlands	90	NJ41	USA	141	Summerland	Canada
40	Erwin	Germany	91	NJ44	USA	142	Suntan	UK
41	Eva	Brazil	92	† NJ45	USA	143	Supper Kidd's	UK
42	Fiesta (Red Pippin)	UK	93	NJ49	USA	144	† Toukou	Japan
43	FR8	Brazil	94	NJ50	USA	145	Tropical Beauty	South Africa
44	Fuji Suprema	Japan	95	† NJ96	USA	146	Wealthy	USA
45	† Gala	New Zealand	96	NJR75	USA	147	Webster	USA
46	Galícia	Brazil	97	Nova Easygro	USA	148	Wemershock	*
47	Gloster 69	Germany	98	Ohrin	Japan	149	† Willie Sharp	Netherlands
48	† Golden Delicious	USA	99	Orankis Tem	*	150	† Wilmuta	Belgium
49	Goldjon	Italy	100	Pachacamac	Peru	151	Winter Gold	*
50	Gorden	*	101	Paulared	USA	152	Yoko	Japan
51	Grangille Red	*	102	Pilot	USA			

* Unknown origin; † Indicates cultivar susceptibility to ALS disease.

SSR genotyping

All accessions were genotyped using 11 perfect dinucleotide microsatellite loci from seven apple linkage groups (Table 2). PCR reactions were carried out in a 13.75 µL volume containing 40 ng of genomic DNA, 1X *Taq* buffer, 1 U *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.25 µM of each dNTP (Fermentas, Vilnius, Lithuania), 1.5 mM magnesium chloride and 0.3 µM of each primer pair. A Mastercycler Gradient (Eppendorf, Hamburg, Germany) thermalcycler was used to amplify the eleven SSR loci with the following cycles: 94 ºC for 5 min; followed by 30 cycles at 95 ºC for 30 s; annealing temperature (according to the related literature of each primer) for 1 min; 72 ºC for 1 min; and a final extension of 5 min at 72 ºC. Each 5´ forward oligo was labeled with a fluorophore HEX or 6-FAM to enable the automated genotyping (Table 2). After amplification reactions, PCR products were diluted 10X in ultrapure water in order to be genotyped by capillary electrophoresis in a MegaBACE 1000 DNA Analysis System (GE Healthcare, Little Chalfont, UK).

Table 2. SSRs names, linkage groups, 5´fluorescent modifications, and sequences of the primers used in this study and the expected size range of the amplified fragments

SSR name	Linkage group*	5´ Labeling	Forward sequence	Reverse sequence	**Size range** (bp) **Original publication**
Ch03c02 [a]	12	Hex	tca cta ttt acg gga tca agc a	gtg cag agt ctt tga caa ggc	116-136
Ch02b10 [a]	02	6-FAM	caa gga aat cat caa aga ttc aag	caa gtg gct tcg gat agt tg	121-159
Ch05a05 [a]	06	Hex	tgt atc agt ggt ttg cat gaa c	gca act ccc aac tct tct ttc t	198-230
Ch05c06 [a]	16	Hex	att gga act ctc cgt att gtg c	atc aac agt agt ggt agc cgg t	104-126
Ch02c11 [a]	10	Hex	tga agg caa tca ctc tgt gc	ttc cga gaa tcc tct tcg ac	219-239
Ch02b03b [a]	10	Hex	ata agg ata caa aaa ccc tac acag	gac atg ttt ggt tga aaa ctt g	77-109
Ch01g12 [a]	12	6-FAM	ccc acc aat caa aaa tca cc	tga agt atg gtg gtg cgt tc	112-186
Nz02b01 [b]	15	Hex	ccg tga tga caa agt gca tga	atg agt ttg atg ccc ttg ga	212-238
Ch02g09 [a]	08	6-FAM	tca gac aga aga gga act gta ttt g	caa aca aac cag tac cgc aa	98-138
Ch03b06 [a]	15	Hex	gca tcc ttg aat gag gtt cac t	cca atc acc aaa tca atg tca c	111-131
Hi03g06 [c]	15	6-FAM	tgc caa tac tcc ctc att tac c	gtt taa aça gaa ctg cac cac atc c	182-204

[a] Liebhard et al. (2002), [b] Guilford et al. (1997), [c] Silfverberg-Dilworth et al. (2006)
*Fiesta x Discovery apple genetic reference linkage map (Liebhard et al. 2003)

Data analysis

Alleles peaks from raw data were scored by comparison with ET 550-R size standard (GE Healthcare) using Fragment Profiler analysis software version 1.2 (GE Healthcare). Observed and Expected heterozygosity (*Ho and He*); number of alleles per locus; allele frequencies; exclusive and unique alleles; Polymorphism Information Content $PIC = 1 - J = 1 - \Sigma(1 = J)p2ij$, where *pij* is the frequency of the *j*th allele for *i*th marker (Anderson et al. 1993); Probability of Identity (1; being1 = $\Sigma pi^1 + \Sigma(2pipi)^2$), where p_i and p_j are the frequencies of the *i*th and *j*th alleles and i ≠ j), and (1 unbiased = $n^3 (2a^2 2 - a_1) - 2n^2 2(a_3 + 2a_2) + n(9a_2 + 2) - 6/(n - 1)(n - 2)(n - 3))$ where *n* is the sample size, a_i equals Σpj^i and p_j is the frequency of the *j*th allele (Paetkau and Strobeck 1994) and Power of Exclusion (Q) (Vandeputte 2012) were calculated using the CERVUS 3.0 software (Kalinowski et al. 2007). Alleles were considered rare when their frequencies were lower than 0.05. A dendrogram was constructed with the use of unweighted pair-group method with arithmetic means - UPGMA (Sneath and Sokal 1973), using the software NTSYSpc version 2.02 (Rohlf 2000), and visualized using FigTree software (tree.bio.ed.ac.uk/*software*/*figtree*/).

RESULTS AND DISCUSSION

The eleven genomic SSR loci amplified a total of 242 alleles in the 152 apple accessions (Table 3), with a mean of 22 alleles per locus. Availability of marker data ranged from 99.98% (Ch02b03), 99.99% (Ch02g09 and Ch03c02) to 100% (remaining markers), with a total of 0.42% missing data. The joint analyses of all plants and all loci showed a proportion of heterozygotes to homozygotes of 3.38. When only ALS resistant plants were analyzed (118 accessions), the proportion of heterozygotes to homozygotes was 3.41. Similar proportion value (3.58) was observed for the 34 susceptible accessions. The number of alleles per locus ranged from 16 (Nz02b01) to 29 (Ch01g12) (Table 2). One hundred and twenty alleles (49.59%) were found to be exclusive to resistant accessions, and 13 alleles (5.37%) were

Table 3. Primers, observed size range (bp), number of alleles (A), Polymorphism information content (PIC), Observed heterozygosity (H*o*), Expected heterozygosity (H*e*), Probability of Identity (I) and Power of Exclusion (Q) for resistant, susceptible and total accessions

Primer	range (bp)	Resistant accession/cultivars (n=118)				Susceptible accessions/cultivars (n=34)				Total (n=152)					
		A (*)	PIC	H*o*	H*e*	A (*)	PIC	H*o*	H*e*	A	PIC	H*o*	H*e*	I	Q
Ch03c02	92-218	21(10)	0.786	0.508	0.813	13(2)	0.798	0.438	0.832	23	0.790	0.493	0.815	0.025	0.3174
Ch02b10	106-182	25(12)	0.907	0.746	0.917	14(1)	0.809	0.824	0.838	26	0.895	0.763	0.905	0.022	0.3105
Ch05a05	160-250	19(11)	0.788	0.805	0.812	11(3)	0.617	0.676	0.669	22	0.759	0.776	0.785	0.017	0.3029
Ch05c06	92-226	17(10)	0.761	0.881	0.792	7	0.553	0.824	0.619	17	0.729	0.868	0.762	0.086	0.3892
Ch02c11	200-250	17(6)	0.857	0.720	0.873	11	0.833	0.559	0.863	17	0.854	0.684	0.870	0.031	0.3243
Ch02b03b	76-124	20(11)	0.887	0.836	0.900	9	0.790	0.909	0.824	20	0.880	0.852	0.893	0.030	0.3244
Ch01g12	96-188	29(17)	0.858	0.856	0.873	12	0.850	0.971	0.877	29	0.868	0.882	0.881	0.058	0.3581
Nz02b01	190-260	14(7)	0.740	0.814	0.771	9(2)	0.631	0.765	0.692	16	0.734	0.803	0.767	0.089	0.3923
Ch02g09	90-212	26(15)	0.861	0.771	0.876	12(1)	0.806	0.875	0.838	27	0.854	0.793	0.869	0.071	0.3764
Ch03b06	90-154	17(9)	0.858	0.856	0.875	12(4)	0.767	0.706	0.807	21	0.847	0.822	0.864	0.048	0.3497
Hi03g06	164-240	24(12)	0.787	0.712	0.807	12	0.780	0.882	0.816	24	0.808	0.750	0.827	0.033	0.3277
Combined														**	***

(*) Exclusive alleles between parenthesis
** Combined probability of identity for the eight analyzed SSR loci = $4{,}11 \times 10^{-16}$
*** Power of exclusion for the eight analyzed SSR loci = 99.99999259%

exclusive to susceptible accessions (Tables 3 and 4). Exclusive alleles for ALS resistant accessions were found in all SSR markers, with a mean of 10.91 alleles per locus (Table 4). On the other hand, exclusive alleles were also found in six loci for susceptible ALS accessions. A large number of alleles (163 alleles, 67.36 %) were classified as rare alleles (frequency < 0.5%), ranging from 10 (Ch02c11) to 23 (Ch01g12). A total of 67 unique alleles (27.69%) were detected, out of which 30 were detected in ALS resistant, and 37 in susceptible accessions.

To measure the informativeness of these markers, the polymorphism information content (PIC) for each SSR locus was calculated. The PIC value varied from 0.740 to 0.907, with mean of 0.823 for resistant accession, and from 0.553 to 0.850 for susceptible accessions, with mean of 0.702. Total PIC values for all markers varied from 0.734 (Nz02b01) to 0.895 (Ch02b10), with mean of 0.820 (Table 3). Although Ch02b10 was the most informative locus, with the highest PIC value, Ch01g12 marker presented the highest number of alleles. Total observed heterozygosity varied from 0.493 (Ch03c02) to 0.868 (Ch05c06), with mean of 0.771. Total expected heterozygosity varied from 0.762 (Ch05c06) to 0.905 (Ch02b10), with mean value of 0.840 (Table 3).

In the present study, it was found 16 to 29 alleles per locus. Higher number of alleles, Ho, PIC (Table 3), and wider size ranges were observed for all SSR markers in this study, when compared to those reported on the original primer note studies (Guilford et al. 1997, Liebhard et al. 2002, Silfverberg-Dilworth et al. 2006). The higher genetic diversity values are mainly related to the great diversity of apple accessions in conjunction with the large sample size (152). Guilford et

Table 4. Alleles (bp) identified in 152 accessions in each of the 11 SSRs used in this work

Primer	Alleles
Ch03c02	92 104 106 108 110 116 118 120 122 124 126 128 130 132 134 136 144 146 154 164 166 172 218
Ch02b10	106 108 110 112 116 118 120 122 124 126 128 130 132 134 136 138 140 144 146 148 152 158 160 164 170 182
Ch05a05	160 162 194 198 200 202 204 206 208 210 212 214 216 218 220 222 226 228 232 234 250 264
Ch05c06	92 102 104 106 108 110 112 114 116 118 120 122 126 128 132 218 226
Ch02c11	200 204 208 210 218 220 222 226 228 230 232 234 236 238 240 244 250
Ch02b03b	76 78 80 82 84 86 88 90 92 94 96 98 100 102 104 106 110 116 122 124
Ch01g12	96 104 106 108 110 112 114 116 118 120 124 128 132 134 136 138 140 142 146 148 152 154 156 158 162 166 180 186 188
Nz02b01	190 198 208 218 220 222 228 232 234 236 240 242 244 246 250 260
Ch02g09	90 92 96 98 100 102 104 106 108 110 112 114 116 118 120 122 126 128 134 136 138 140 142 150 154 168 212
Ch03b06	90 96 100 102 104 106 108 110 112 114 116 118 120 122 124 128 130 138 140 152 154
Hi03g06	164 168 172 174 178 182 184 188 192 194 196 198 200 202 204 206 208 210 214 216 218 228 236 240

Dark gray background indicates exclusive alleles for susceptible accessions. Gray background indicates exclusive alleles for resistant accessions.

al. (1997) found 4.5 alleles per locus in 21 apple cultivars; Gianfranceschini et al. (1998) found 8.2 alleles per locus in 19 cultivars, and Coart et al. (2003) found 19.58 alleles per locus in 119 apple genotypes. Oraguzie et al. (2005) found 9.7 alleles per locus in 66 apple rootstocks cultivars; Hokanson et al. (1998) found 12.1 alleles per locus in 142 accessions of 23 *Malus* species; and Gharghani et al. (2009) found 17 alleles per locus in other 159 apple cultivars and wild species from several geographical origins and species.

High level of genetic diversity was expressed also in terms of total expected heterozygosity (He=0.84). The high level of heterozygosity of the cultivars is in accordance with former studies on *M. sylvestris* and *M. domestica* (Coart et al. 2003, Larsen et al. 2006, Koopman et al. 2007). Allelic frequencies showed wide variations, ranging from 0.017 to 0.828. The most frequent alleles were detected in three SSR loci, i.e., Hi02b07, Hi08g06, and CTG1066091, with frequencies higher than 0.8.

It was taken the most diverse cluster of accessions as one cluster. In fact, the accessions of this cluster are 92% genetically different from the remaining accessions. In addition, they are not used in breeding programs, the reason why they diverge so much from the other accessions. Thus, the dendrogram (Figure 1) based on genetic similarity among the 152 accessions showed two major clusters. Only Winter Gold, *M. eley* and *M. atrosanguinea* accessions form the first major cluster. *M. eley* and *M. atrosanguinea* accessions are wild apples, non-domesticated and share very few alleles with the other major cluster. In addition, these two species have not recently been used for breeding of modern cultivars; however they could be used, since all wild accessions are resistant to ALS. Other wild accessions, such as *M. robusta, M. baccata* and *M. andenhamensis,* clustered near the first major cluster. The second major cluster is sub-clustered in several minor ones, with similarity between pairs of accessions ranging from 17% to 86%. The highest similarity value

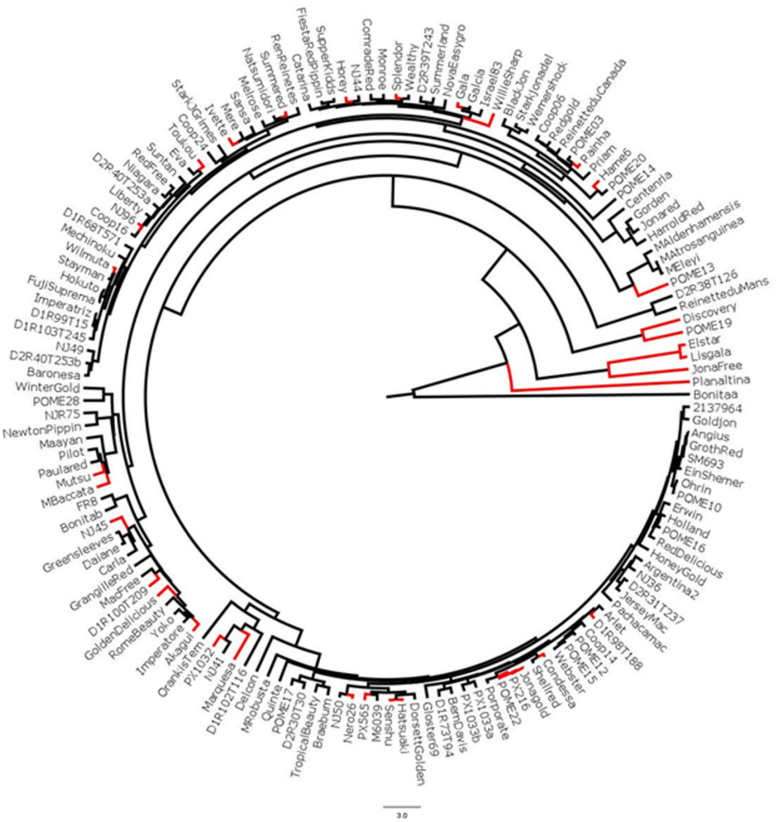

Figure 1. UPGMA dendrogram based on polymorphisms of 11 SSR loci in 152 apple accessions. The red branches indicate susceptibility to Apple Leaf Spot disease (*Colletotrichum gloeosporioides*), being all other accessions resistant to ALS. The scale represents the dissimilarity coefficient between accessions.

of 86% was found in two pairs of accessions; however, identical accessions were not found. The first pair is formed by Fuji Suprema and Hokuto, and the second pair, by Imperatriz and D1R99T15. This high similarity could be the result of a second backcross generation (BC_2). The dendrogram revealed also that the great majority of susceptible accessions were positioned far from wild resistant accessions. Pairs of accessions sharing 82% of alleles were found five times. In three of these cases, the pair is formed by a resistant and a susceptible accession, suggesting that one or more amplified alleles linked to resistance QTLs may be found at the 18% non-shared alleles (Table 4).

The Power of Exclusion (Q) of 99.99999259% from all combined data set provides reliability to the results as discussed above. In addition, the extremely low Probability of Identity (I) of $4.11x10^{-16}$ provides an unambiguous way to discriminate apple accessions (Table 3). These indices allow cultivar identification by analyzing a relatively smaller number of loci than the 11 that were used in the present work.

Brazilian apple breeding programs are in constant need of new resistant scion cultivars which include fruit quality traits. Therefore, combining knowledge of the genetic markers with potential link to ALS resistant phenotype could lead to the faster development of ALS resistant cultivars for use in crossbreeding. Marker assisted selection techniques could make use of such markers associated to ALS resistant phenotypes for further analysis of their genetic linkage and chromosomal location, either for a single locus or quantitative trait loci (QTLs). The development of new ALS resistant cultivars would benefit not only Brazilian and worldwide apple breeders, but also consumers due to the lesser use of pesticides. In addition, the selection of these markers was successful in characterizing a reasonable large number of apple accessions, which could be of great use to other apple germplasm curators worldwide. Nonetheless, further works should be carried out in order to characterize the interaction between resistant accessions and environmental conditions by performing phenotypic analysis in farm conditions.

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