Molecular Characterization of the S-alleles and Compatibility among Hybrid Pear Tree Cultivars for Subtropical Regions

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Abstract. Pear (*Pyrus* spp.) is a temperate-climate fruit species that has gametophytic self-incompatibility. Cross-pollination among intercompatible cultivars can be useful in selecting for breeding programs. The objective of the present study was to evaluate effective fruiting from cross-pollination between hybrid pear cultivars and to characterize pear tree S-alleles. Seven cultivars were evaluated: Cascatense, Centenária, D'água, Primorosa, Seleta, Tenra, and Triunfo. Controlled crosses were carried out in two seasons and consisted of spontaneous self-pollination, parthenocarpy, and cross-pollination between cultivars. During the 2 years of research, the overlap of the entire flowering periods of all cultivars was higher than 50%. Phenology was evaluated from the beginning of pruning, and the time elapsed from pruning to the flowering phenophase was computed. Finally, the flowering-period overlap of the cultivars was analyzed. S-alleles were characterized by polymerase chain reaction (PCR) using primers specific to previously known alleles. Under field conditions, the Primorosa cultivar has high potential as a pollinizer for D'água, Seleta, Tenra, and Triunfo. Pear tree hybrid cultivars have a high frequency of the S1 and S5 alleles. The S5S8 and S1S4 alleles are amplified in the D'água and Seleta cultivars, respectively, conferring compatibility between these cultivars. The S1 and S5 alleles are amplified in 'Primorosa', 'Cascatense', and 'Triunfo', conferring interincompatibility.

The pear tree (*Pyrus* spp.) is a temperate-climate fruit species, and its cultivation in subtropical regions was made possible by hybrid cultivars obtained from the cross *Pyrus communis* × *Pyrus pyrifolia* (Curi et al., 2017). This cross combines the quality of European pear trees (*P. communis*) with the low number of chill hours required by the Asian pear tree (*P. pyrifolia*) (Chagas et al., 2008).

Low fruit set is one of the limiting factors in the expansion of pear tree crops in subtropical regions (Bettiol Neto et al., 2014). The fact that no pollinizer cultivars have been reported for these hybrids in subtropical re-

gions might be the reason for their low effective fruiting.

Most pear tree cultivars have gametophytic self-incompatibility, which causes a plant to reject its own pollen. Therefore, pear trees depend on cross-pollination for fruit production (Franklin-Tong and Franklin, 2003). In general, the use of two to three pear cultivars with a coincident flowering period is recommended for commercial crops. Thus, identifying compatibility between cultivars is of economic importance because partial compatibility may reduce yield (Goldway et al., 1999; Tatari et al., 2017).

Several methods can be applied to the study of the compatibility/incompatibility between cultivars and the determination of suitable pollinizers (Ortega and Dicenta, 2004). Field-controlled pollination allows the performance of several cultivars in the orchard to be estimated and is suggested for determining appropriate pollinizer groups (Mahmoudi et al., 2007). However, the use of this method alone in the determination of

the incompatibility between cultivars may be misleading due to environmental and physiological effects that hinder differentiation between fully compatible and semicompatible crosses. Therefore, the use of another method to increase the reliability of the evaluation is crucial; such a method includes the analysis of genes encoding for S-RNase to identify the S-loci in cultivars, which can define the compatibility level between cultivars, complementing and corroborating the results observed in the field (Jacquemart, 2007).

Self-incompatibility based on S-RNase occurs in Rosaceae species. Compatibility is controlled by the polymorphic S-locus, which encodes at least two genes. S-RNases determine the specificity of pollen rejection in the pistil, and the F-locus and F-box proteins fulfill this function in pollen. S-RNase is believed to function as a specific S cytotoxin and as a recognition protein. Thus, the incompatibility is a consequence of the cytotoxic activity of S-RNase. Conversely, S-RNase cytotoxicity does not occur in compatible pollen tubes (McClure et al., 2011).

Pear breeding programs are carried out in cold regions and seek to develop cultivars adapted to these climatic conditions, with high-yield performance, which fit in the fresh fruit chain. However, pear tree exploitation in subtropical (Bettiol Neto et al., 2014) and tropical regions (Oliveira et al., 2015) has increased. In this sense, breeding programs for cultivars adapted to regions with high temperatures must be intensified.

This study aimed to characterize S-alleles in hybrid pear cultivars (*P. communis* × *P. pyrifolia*) adapted to subtropical climates and evaluate the effective fruiting from the cross between these cultivars.

Materials and Methods

The experiments were conducted in Lavras, MG, Brazil (lat. 21°14'S; long. 45°00'W; altitude 841 m above sea level), in the 2015 and 2016 seasons. The climate of the study region is classified as Cwa (subtropical climate, with cold and dry winter and hot and humid summer) (Souza et al., 2017). This study used seven *P. communis* × *P. pyrifolia* cultivars: Cascatense, Centenária, D'água, Primorosa, Seleta, Tenra, and Triunfo (Table 1).

Pear cultivars were grafted on the *Pyrus calleryana* L. rootstock and transplanted into the field in Oct. 2010, spaced 3 m between rows and 4 m of row distance (density of 833 plants/ha). Pears were conducted in "central leader" system.

Phenological stages and field crosses were evaluated in the Fruiticulture Sector of UFPA in seven different pear cultivars (Table 1). The phenological stages were evaluated in 2015 and 2016, and the crosses were analyzed in the 2015–16 and 2016–17 seasons.

Climatic variables and flowering phenology. The time elapsed between the phenophases of each cultivar was recorded from the beginning of pruning. The beginning, the

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end, and the duration of flowering were verified and the overlap of the flowering periods was analyzed. These data were correlated with climatic variables during the experimental period and used to compare the flowering period between cultivars. Figure 1 shows the climatic data for the period.

Molecular analysis. For the molecular analysis of the cultivars (Table 1), completely expanded, healthy, young leaves from adult plants were collected in 2016 in the field for DNA extraction. Samples were identified and stored in Styrofoam boxes containing ice until storage in an ultrafreezer. The DNA extraction protocol was adapted from Doyle and Doyle (1987). Initially, 2 g of leaf tissue was macerated in liquid nitrogen. Then, 10 mL of cetyl trimethylammonium bromide (CTAB) extraction buffer [2% CTAB, 100 mM TRIS (pH 8.0), 1.4 M NaCl, and 1% polyvinylpyrrolidone] was added to each sample, the suspension was transferred to a 50-mL falcon tube, and the set was incubated in a water bath at 65 °C for 30 min. During this period, the macerated material was homogenized every 10 min. The first DNA extraction was performed by adding chloroform:isoamyl alcohol at a ratio of 24:1 to a volume of 10 mL. The centrifuge tube was gently shaken to homogenize the suspension. At this stage, the organic and aqueous phases were separated by centrifugation at 10,000 rpm for 5 min. The supernatant was collected,

and the remainder was discarded. Afterward, the nucleic acids were precipitated in a mixture of 30 mL of 95% alcohol: 7.5 M ammonium acetate (6:1) for 24 h in a freezer (−20 °C). After precipitation, the nucleic acids were transferred to microcentrifuge tubes and centrifuged again. The supernatant was discarded, and the pellet was placed at room temperature for drying. Then, the nucleic acids were hydrated in TE buffer (1 mM TRIS and 0.1 mM EDTA). In the second extraction, phenol:chloroform:isoamyl alcohol (25:24:1) was added. The organic and aqueous phases were separated by centrifugation at 14,000 rpm for 5 min. The supernatant was transferred to a 2-mL microtube. In a new tube, chloroform:isoamyl alcohol was added to the aqueous phase at a ratio of 24:1, followed by centrifugation. The supernatant was collected and transferred to a new 2-mL microtube and precipitated by adding three volumes of a mixture of 3 M sodium acetate:95% ethanol (1:20) for at least 1 hour in the freezer (−20 °C). After DNA precipitation and drying, the material was hydrated in TE buffer and quantified in a ultraviolet-Vis Nanodrop ND-1000 spectrophotometer (NanoDrop Technology, Rockland, DE). The quantified material was diluted in pure water to a concentration of 10 ng/μL and then used in the PCR.

PCR and gel electrophoresis. PCRs were adapted from Sanzol et al. (2006) using the

primers shown in Table 2. Each reaction was composed of 50 ng genomic DNA, 0.2 mM deoxyribonucleotide triphosphate, 50 mM Tris-HCl, 20 mM KCl, 0.6 μM of each primer, 2 mM MgCl₂, 1 U of Taq polymerase (Invitrogen, Carlsbad, CA), and ultrapure water to complete the 25 μL total volume. The primers MPyC1F and MPyC5R were used to amplify the S-RNase products associated with the S1, S3, S4, and S5 alleles, and the S1 and S2 alleles were amplified using the reverse primer PycomS2R, as reported by Sanzol et al. (2006). The primers PycomC1F and PycomS2R (5' gtaatggttctgtctattattgtgg 3') were used to amplify other alleles, as described by Sanzol and Robbins (2008). The sequences of these primers are shown in Table 2.

Amplifications were performed in a thermocycler (2720; Applied Biosystems, Foster City, CA) programmed for a denaturation cycle of 2 min at 94 °C, followed by 36 cycles of 30 s at 94 °C, 1 min at specific annealing temperature (Table 2), 2 min at 72 °C, and a final extension at 72 °C for 10 min, after this procedure, samples were held at 4 °C. Amplified DNA fragments were subjected to electrophoresis on 2% agarose gel, stained with ethidium bromide (0.5 mg/mL), and immersed in 1×TBE buffer (45 mM Tris-borate, 1 mM EDTA) for 3 h at 80 W. The molecular weight marker was 100 base pairs (bp) DNA Ladder (Ludwig Biotec, Rio Grande Do Sul, Brazil).

Controlled crosses. Controlled crosses were carried out in the 2015–16 and 2016–17 seasons. Because there were different flower stages in the same umbrella, flowers were thinned by selecting only five flowers at the balloon stage per umbrella. Different treatments were performed in each of the seven cultivars before anthesis (Fig. 2):

1. Spontaneous self-pollination, during which five flowers of each umbrella were bagged;
2. Parthenocarpy, during which five flowers of each umbrella were emasculated and bagged;
3. Manual cross-pollination between cultivars, during which flowers were emasculated and pollinated by each of the other cultivars and then bagged.

Each treatment had seven replications with five flowers per experimental plot. Effective fruiting was evaluated 30 d after treatment installation. During the harvest, the number of fruits of each treatment was counted again. Field data cannot distinguish between completely compatible and semi-compatible crosses because, in some cases, parthenocarpy may occur. Therefore, fruits with nonviable seeds were not considered for fruit set.

Results and Discussion

Climatic variables and flowering phenology. The flowering period of the cultivars varied among seasons, resulting in slight differences between

Table 1. List of cultivars with their respective identification number, genealogy, and origin information.

Identification	Cultivar	Genealogy	Origin
1	Cascatense	Packham's Triumph × Le Conte	Embrapa - Brazil
2	Centenária	Hood × Packham's Triumph	IAC - Brazil
3	D'água	Unknown	Unknown
4	Primorosa	Hood Packham's Triumph	Embrapa - Brazil
5	Tenra	Madame Sieboldt × Packham's Triumph	IAC - Brazil
6	Triunfo	Hood × Packham's Triumph	IAC - Brazil
7	Seleta	Hood × Packham's Triumph	IAC - Brazil

IAC = Agronomic Institute of Campinas.

Fig. 1. Climatic data from January 2015 to December 2016. Lavras, Minas Gerais, Brazil.

Table 2. Primers used for the determination of S-alleles in pear tree cultivars (*Pyrus* sp.), with their respective nucleotide sequences.

Primers	Sequences (5'→3')	Direction	Annealing temp (°C)	Reference
MPyC1F	attattwtcaattacgcakcartatcag	Forward	52 °C	Sanzol et al. (2006)
MPyC5R	caaakasyrayctcracyaattcmg	Reverse		
PycomC1F	attttcaattacgcagcaaatatcagc	Forward	54 °C	Sanzol and Robbins (2008)
PycomS2R	gtaatgggtctgtctattattgtgg	Reverse		
MPyC1F	attattwtcaattacgcakcartatcag	Forward	50 °C	Sanzol et al. (2006)
PycomS2R	gtaatgggtctgtctattattgtgg	Reverse		

plants and within each growing season. The beginning of the flowering period was delayed when compared with the previous productive cycle (Fig. 3). This phenomenon may be directly related to environmental factors, as observed in Fig. 1. In 2015, greater rainfall occurred in February and March than in other months; in 2016, a considerable amount of rainfall occurred in June. Moreover, based on the average temperatures of the 2 years, relatively low temperatures, followed by an increase in temperature in 2015, were anticipated in 2014 and 2015 compared with 2016. Together, these factors may have influenced the early flowering in 2015. Castro et al. (2016), in a study on apple trees, also observed that the full bloom period of some cultivars was delayed in years with a low number of chill hours. The same was observed in the present study in 2016.

During the 2 years of research, the overlap between the entire flowering periods of cultivars was higher than 50%. The flowering progression was not uniform between cultivars, and at least two flowering peaks were observed in each cultivar during the flowering period (data not shown). In mild-winter regions, the chill hours and the temperature during the flowering period were the main factors affecting (directly or indirectly) the performance of pollination and fruiting (Soltész, 2003).

Molecular analysis. The combination of the MPyC1F/MPyC5R primers (Fig. 4A) allowed the amplification of three distinct alleles (S1, S4, and S5), and four of the seven cultivars studied had at least one of these three alleles. The PCR amplification products of these primers indicated that the cultivars Seleta, Primorosa, and Cascatense had the 1300-bp fragment associated with the S1 allele, whereas cultivar Seleta also had the 750-bp product associated with the S4 allele. 'D'água' had the S5 allele associated with the 650-bp product. The primer pair MPyC1F/PycomS2R (Fig. 4B) allowed detection of the S1 allele, with a 1150-bp fragment, in four of the evaluated cultivars (Triunfo, Seleta, Primorosa, and Cascatense). The combination of the PycomC1F/PycomS2R primers (Fig. 4C) allowed the amplification of three different S-alleles (S1, S5, and S8). The sizes of these alleles, according to the visualization of the amplification results, ranged from 650 to 1300 bp. The S1 allele, with a 1300-bp fragment, was detected in 'Triunfo', 'Seleta', 'Primorosa', and 'Cascatense'. The S5 allele, with a 650-bp fragment, was found in 'Cascatense', 'Centenária', 'D'água', 'Primorosa', 'Tenra', and 'Triunfo'. The S8 allele, characterized by a 675-bp product, was detected only in cultivar D'água.

Four different S-alleles were detected in the seven evaluated cultivars. Their PCR products ranged from 650 to 1300 bp. In general, when these primers were used, high frequencies of the S5 and S1 alleles were detected; the former was present in six cultivars, and the latter was found in four cultivars.

Fig. 2. Conduction of the experiment at different stages of the pear flower, cultivar Cascatense. (A) Inflorescence with pre-anthesis flowers. (B) Flower at anthesis: wet stigma (red arrow) and anthers (black arrow) (flowers at anthesis were removed). (C) Flower emasculating. (D) Emasculated flowers, only with the pistil. (E) Petri dishes with pollen (black arrow) for pollination. (F) Emasculated and pollinated flowers wrapped in organza. (G) Fruits at the beginning of the development stage. (H) Fruits at the final development stage.

According to Crane and Lewis (1942), in the past, gametophytic incompatibility between pear tree cultivars was unusual, and therefore, the expression of S-alleles was seldom investigated in comparison with other fruit trees. However, Sanzol and Herrero (2002) believe that the limited number of parents used in pear tree breeding programs could have increased the frequency of incompatibility between new cultivars.

Sanzol et al. (2006) also explained the high frequencies of S1 and S5 alleles. The

authors confirmed that, in a breeding program of European pear trees, cultivars Williams (S1S2), Coscia (S3S4), and Doyenne du Comice (S4S5) are predominantly used as parents, and as a result, the S1 and S5 alleles are expected to be represented at higher frequencies.

The presence of the S1 allele in hybrid pear trees can be explained by the genealogy of the cultivars, because all of the cultivars have a common parent, that is, Packham's Triumph. Sanzol and Robbins (2008) ob-

served the expression of S1 and S3 alleles in this cultivar. The S-alleles of the other parents are unknown.

Only cultivars Seleta (S1S4) and D'água (S5S8) showed intercompatibility (Fig. 3). All the other studied hybrids had at least one S-allele in common, conferring partial or total incompatibility to these plants. This phenomenon may hinder the selection of pollinizer cultivars for subtropical regions and compromise the fruit set and yield of these genotypes.

Mota et al. (2010) stated that the analysis of S-alleles, based on the PCR, has a high potential to be used in the identification of groups of self-incompatible cultivars. The knowledge of the S-alleles responsible for gametophytic incompatibility and the identification of an S-allele that can suppress this incompatibility will allow the controlled transfer of this alleles between cultivars with flowering synchrony. This capability will increase the efficiency of breeding programs in obtaining new cultivars and facilitate the reproductive management of orchards, aiming to improve fertilization and fruit set.

Cultivars Cascatense, Primorosa, and Triunfo showed the same PCR products when the three different primers were used. These results suggest that the three cultivars are incompatible. In fact, among the seven cultivars evaluated, Cascatense, Primorosa, and Triunfo were the only ones that showed total incompatibility between each other.

'Centenária' and 'Tenra' showed only the amplification product corresponding to the S5 allele. Yamane et al. (2003), in a study of cherry tree, almond tree, and apricot tree genotypes, also observed variations in the number of alleles between loci, as well as the absence of PCR products in some cultivars. This result, in some cases, can be justified by the occurrence of only one allele or null alleles for a given locus. Null alleles are

Fig. 3. Flowering period of cultivars in 2015 and 2016. *Indicates the first fortnight of the month. **Indicates the second fortnight of the month.

Fig. 4. Polymerase chain reaction amplification of S-alleles obtained from the primers combinations: (A) MPyC1F/MPyC5R; (B) MPyC1F/PycomS2R; and (C) PycomC1F/PycomS2R. M = 100 bp molecular marker, T = Tenra, Tr = Triumph, C = Centenária, Se = Seleta, Pr = Primorosa, Cs = Cascatense, Da = D'água, B = Branco.

Table 3. Compatibility between seven different pear cultivars, based on the S-alleles.

S-allele	Cultivars	Cascataense	Centenária	D'água	Primorosa	Seleta	Tenra	Triunfo
S1×S5	Cascataense	SI	SC	SC	I	I	SC	SC
S5	Centenária	SC	SI	SC	SC	SC	SC	SC
S5×S8	D'água	SC	SC	SI	SC	SC	SC	SC
S1×S5	Primorosa	I	SC	SC	SI	I	SC	SC
S1×S4	Seleta	SC	SC	SC	I	SI	SC	SC
S5	Tenra	SC	SC	SC	SC	SC	SI	SC
S1×S5	Triunfo	I	SC	SC	I	SC	SC	SI

SI = self-incompatible cross; SC = semicompatible cross; I = incompatible cross.

Table 4. Percentage of fruit set obtained from the self-pollination, crosses, and parthenocarpy of seven pear tree cultivars.

Pollinizer	Receptor							
	Cascataense	Centenária	D'água	Primorosa	Seleta	Tenra	Triunfo	Parthenocarpy
Cascataense	0.00	60.00	24.00	8.00	np	np	np	28.00
Centenária	46.67	0.00	42.00	42.67	np	np	np	26.67
D'água	14	14.00	0.00	36.00	np	np	np	1.33
Primorosa	0	18.67	26.67	0.00	np	np	np	2.67
Seleta	57.33	44.00	50.00	60.00	17.33	np	np	14.67
Tenra	44	0.00	38.67	69.33	np	0.00	np	21.33
Triunfo	23.33	56.00	12.00	53.33	np	np	14.00	16.00

np = nonpollinated (no pollination was performed due to the lack of pollinizer cultivars).

Table 5. Data of fruit set percentage and viable seeds percentage from the self-pollination of seven pear tree cultivars.

Self-pollination	Fruit set (%)	Viable seeds (%)
Cascataense	18.00	0.00
Centenária	36.67	0.00
D'água	0.00	0.00
Primorosa	17.33	0.00
Seleta	17.33	16.00
Tenra	10.67	0.00
Triunfo	14.00	8.00

described in several microsatellite systems and are the result of mutations such as substitutions, insertions, and deletions in one or both annealing regions of the primers in a homologous sequence of template DNA (Callen et al., 1993).

According to the amplification patterns of the S-alleles in the studied cultivars, Cascataense, Primorosa, and Triunfo were incompatible with each other. Cultivars D'água and Seleta were compatible. All the other cultivars were semicompatible with each other (Table 3).

Controlled crosses. Based on the field pollination results (Table 4), cultivars Cascataense, Centenária, D'água, Primorosa, and Tenra showed self-incompatibility. All the fruits harvested had unviable seeds. These fruits were considered parthenocarpic and were therefore not counted as self-pollinated fruits. Conversely, the self-pollinated cultivars Seleta and Triunfo showed fruits with viable seeds, with fruit set percentages of 17.33% and 14.0%, respectively (Table 5). This is an interesting characteristic for crops that have self-incompatibility. No pollinizers are required when cultivars with self-incompatibility breakdown are grown. This self-incompatibility may be related to environmental factors or mutations; however, when more than 30% of all flowers are fertilized, the cultivar is considered self-compatible (Ishimizu et al., 1998).

The fruit set resulting from the self-pollination of Seleta and Triunfo may be related to the concentrations of S-RNases in their flower style. The high total concentration of S-RNases in the style controlled by the two S-alleles in each cultivar prevents pollen tube growth and effective fruiting, justifying the occurrence of cultivars with different self-incompatibility levels (Hiratsuka and Zhang, 2002; Zhang and Hiratsuka, 1999).

In addition, high (20 to 25 °C) or low (15 to 20 °C) temperatures during the flowering stage may induce self-fertility by the deactivation or inhibition of the S-locus, which causes insufficient production of RNases (Nyéki, 1996). This phenomenon possibly occurs due to the insertion of several CH3s in the region that promotes the L receptor, consequently blocking the transcription of the S gene (Pancaldi, 1995). Furthermore, this phenomenon allows self-pollination between genotypes that have two identical S-alleles of gametophytic incompatibility, with the consequent formation of few viable seeds and low fruit set (1% to 5%). High (20 to 25 °C) or low (15 to 20 °C) temperatures also can increase the percentage of parthenocarpic fruits (i.e., fruits without seeds) (Lombard, 1990). Thus, it may be unclear which of these two processes occurs if the formation or nonformation of viable seeds is not observed, because, in most of the Brazilian regions where pear trees are cultivated, average temperatures higher than 16 °C are commonly recorded during the flowering stage.

According to Sassa (2016), S-locus products are not sufficient to determine self-incompatibility in Rosaceae species, and other factors not linked to S-loci can also influence self-incompatibility. Some of these factors have already been identified in other species, such as the mutation of part of the pollen in sweet cherry (Cachi and Wünsch, 2011). Therefore, further studies are required

to elucidate self-compatibility in Rosaceae species.

When flowers are self-pollinated and produce seedless fruits, the process is designated as stimulative parthenocarpy (Moriya et al., 2005). According to Crane and Lewis (1942), sterility and self-incompatibility, which prevent self-pollination of pear flowers, may be hindered by stimulative parthenocarpy.

Regarding the fruit set data (Table 5), cultivar Centenária, despite being self-incompatible (0% of viable seeds), had a fruit set higher than 30%. Kozma et al. (2003) argue that the application of sterile pollen stimulates stigmatic secretion, which triggers reactions in the ovary, stimulating fruit development and preventing the formation of abscission tissue at the base of the fruit peduncle, which will grow without developing viable seeds. Jackson (2005) found that pollination, even without fertilization, stimulates the cytoplasmic and biochemical activity of the pistil and the development of the ovary and thus increases the viability of the embryo sac. Tomimoto et al. (1996) reported an example of stimulative parthenocarpy, in which the cross between different cultivars with the same allelic series generated high fruit set due to the induction of stimulative parthenocarpy, mainly in cultivars with high potential to express this trait.

When the parthenocarpy method was evaluated, 'Cascataense', 'Centenária', and 'Tenra' showed a fruit set higher than 20%; 'Seleta' and 'Triunfo' had a fruit set higher than 10%; and 'D'água' and 'Primorosa' had a fruit set lower than 5%. According to Jalili Marandi (2002), when 5% to 8% of pear tree flowers are converted into fruits, the yield is considered economically acceptable. Parthenocarpy is the production of fruits without seeds and ovule fertilization. This process is desirable in several fruit trees that show incompatibility because it may reduce many problems associated with pollination, such as the need for manual pollination or pollinator insects. However, physical or chemical stimuli are usually required for fruit development and formation (Nishitani et al., 2012).

The crosses 'Tenra' × 'Primorosa' (69.3%), 'Seleta' × 'Primorosa' (60%), 'Cascataense' × 'Centenária' (60%), 'Seleta' × 'Cascataense' (57.3%), 'Triunfo' × 'Centenária' (56%), 'Triunfo' × 'Primorosa' (53.3%), and 'Seleta' × 'D'água' (50%) had fruit set percentages equal to or higher than 50%. The cross 'Tenra' × 'Primorosa' stood out for showing the highest fruit set.

Cultivars Seleta and D'água were compatible in relation to the S-allele series; these cultivars also showed high fruit set in the crosses carried out in the field. In addition, semicompatible cultivars showed high fruit set. The fact that fully compatible cultivars did not have a fruit set of 100% can be explained by other aspects involved in this process, such as the availability and viability of the pollen grains of the pollinizer cultivar.

'Primorosa', when used as the receptor, showed low fruit set (lower than 30%) regardless of the pollinizer. However, when

used as a pollinizer for 'Seleta', 'Tenra', and 'Triunfo', cultivar Primorosa showed the best fruit set performance in the evaluated crosses, despite being semicompatible, according to the S-allele results (Table 3).

'Cascataense', when pollinated by 'Primorosa', showed a fruit set of only 8%. The reciprocal cross showed a 0% fruit set and was considered incompatible. This result corroborates the PCR results, because both analyses showed the S1 and S5 alleles.

'Triunfo', when pollinated by 'Cascataense', showed 23% fruit set and was considered incompatible. When pollinated by 'Primorosa', cultivar Triunfo had high fruit set (53%), mainly because these cultivars showed incompatibility when the S-alleles were molecularly identified as S1 and S5.

Sanzol et al. (2006) stated that physiological and environmental factors might influence cultivar compatibility. Wojciechowski and Antkowiak (2009), working with pear cultivars in different environments, observed that some crosses were incompatible in the laboratory and greenhouse but compatible in the field. Hiratsuka and Tomita (1989) observed reduced expression of self-incompatibility in pear trees under high-temperature conditions and at different flower development stages. The present results suggest that the high fruit set of the 'Triunfo' × 'Primorosa' cross occurred due to the relatively high temperatures at the experimental site.

Future experiments with crosses of 'Triunfo' under controlled environments are necessary to detect whether the incompatibility breakdown is related to extrinsic or intrinsic factors because even the self-incompatibility was broken down. If this phenomenon is related to high temperatures, 'Triunfo' is a promising cultivar for mild-winter regions. However, if the breakdown is due to intrinsic factors, such as S-allele mutations, the cultivar is advantageous for pear tree breeding programs that seek self-compatible cultivars. Although self-compatibility is beneficial to genetic diversity, it is unfavorable to agriculture. Thus, self-compatible mutants, particularly self-incompatible commercial fruit species, have been exploited in agricultural production (Mase et al., 2014).

Centenária showed a fruit set ranging from 42% to 46%, and the fruit set percentage of this cultivar did not significantly vary in response to different pollinizers. 'D'água' showed low fruit set when pollinated by 'Cascataense' and 'Centenária' (14%) and a fruit set of 36% when pollinated by 'Primorosa'. Cultivar Tenra showed very high fruit set when pollinated by Primorosa, despite being semicompatible.

In general, when Seleta was used as a receptor, the cultivar showed high fruit set percentages varying from 44% to 60% in the crosses. This result shows that a high fruit set percentage dramatically influences the mother plant. In addition to the gametophytic incompatibility between cultivars, other factors inherent to pear plants also influence fruit set. The flowering period is one of the main

events during the productive cycle of fruit tree species because fruiting is defined at this stage. During this period, environmental and physiological factors interact with each other, which defines the next fruiting and production stages.

The choice of pollinizer plants is based mainly on the coincidence of flowering, which does not always result in satisfactory yields due to incompatibility between cultivars. In addition to the physiological aspects evaluated, this strategy, which is associated with molecular characterization data of S-alleles, facilitates the understanding of the reproductive system and the choice of more compatible genotypes, both for use in breeding programs and in the establishment of orchards of this species (Bandeira et al., 2011).

Conclusions

Pear tree hybrid cultivars have high frequency of the S1 and S5 alleles.

Under field conditions, the cultivar Primorosa has high potential as a pollinizer for the cultivars D'água, Seleta, Tenra, and Triunfo.

Cultivars with relatively high fruit set are compatible and semicompatible.

Cultivar D'água amplifies the S5S8 alleles and Seleta amplifies the S1S4 alleles, conferring full compatibility to these plants.

Cultivars Primorosa, Cascataense, and Triunfo amplify the S1 and S5 alleles, conferring interincompatibility to these plants.

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