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Determination of Potential Pollinizer Grafted Pear Hybrids (*Pyrus communis* × *Pyrus pyrifolia*) for Subtropical Regions in Brazil

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Abstract

The pear tree is originally a temperate-climate fruit tree. The cross *Pyrus communis* × *Pyrus pyrifolia* generated hybrid cultivars adapted to subtropical regions. However, the low effective fruiting is a limiting factor for the expansion of pear tree crops in subtropical regions and no pollinizer plants have been identified for these cultivars in subtropical regions. This work aimed to identify possible pollinizer pear tree cultivars in subtropical regions by evaluating the stigmatic receptivity, the germination percentage, and the number of pollen grains. Seven hybrid pear cultivars were used. Flowers of each cultivar at each floral stage were collected (E, E2, F, F2, and G). The stigmatic receptivity was evaluated by immersing the material in 3% hydrogen peroxide. Flowers were collected for the quantification of anthers, pollen grains per anther and per flower, and pollen grain germination. Stigmas were 100% receptive for all cultivars at the F2 and G stages. Cultivar Cascatense showed a large number of anthers and pollen grains per anther and per flower. The highest pollen grain germination percentage was detected for Cascatense (72.75%). Cultivars Seleta and Triunfo have high stigmatic receptivity for a longer period during the flowering stage, proven to be a potential female parent. Cascatense has a higher germination percentage and a larger number of anthers per flower, pollen grains per anther, and pollen grains per flower. Cascatense has a high potential to be used as a pollinizer, both in pear tree breeding programs and commercial orchards.

Keywords: stigmatic receptivity, pollination, plant breeding

1. Introduction

Despite being a temperate-climate fruit tree, some pear tree hybrid cultivars obtained from the cross *Pyrus communis* × *P. pyrifolia* are adapted to subtropical regions, where the climate is characterized by mild winter and higher temperatures in the summer, compared to temperate regions (Curi et al., 2017). The cultivation of these hybrid cultivars in subtropical regions was made possible by the combination of the quality of the European pear (*P. communis*) with the low chill hours required by the Asian pear (*P. pyrifolia*) (Chagas et al., 2008).

Most pear tree cultivars have gametophytic self-incompatibility, causing the plant to reject its own pollen. Therefore, they depend on cross-pollination for fruit production (Franklin-Tong & Franklin 2003). In general, the use of two to three pear cultivars with a coincident flowering period is recommended for commercial crops. The low effective fruiting due to lack of pollinizer plants, is a limiting factor for the expansion of pear tree crops in subtropical regions (Bettiol Neto et al., 2014).

The absence of the ovule fertilization in pollinated flowers, in both temperate and subtropical regions, results in a small number of fruits set per tree, resulting in reduced fruit yield. Fewer seeds were formed under insufficient pollination condition due to limited supply of phytohormones for ovary and seed development (Tavares et al., 2002).

To overcome the gametophytic incompatibility and provide adequate fertilization for increasing the effective fruiting in pear orchards, pollen grains of genotypes compatible with favorable "S" allele series must be transferred by pollinator insects (Sezerino & Orth, 2015). Due to the self-incompatibility in pear cultivars,

pollinizer plants are used; however, they must be compatible and provide a large number of pollen grains to the main fruiting cultivar (Nakasu & Faoro, 2003). The flowering time and flowering stages of the pollinizer cultivar and the recipient cultivar must be synchronized. Moreover, the pollinizer tree must produce viable pollen in adequate quantity and quality to guarantee fruit production (Bettiol Neto et al., 2014).

The receptive stigmatic surface and the duration of its availability is fundamental to determine the best period of pollen deposition on the flower. For successful pear fertilization, the pollen grain must present high viability and germination rate (Brito et al., 2010).

The in vitro pollen germination test is essential for breeding programs as they allow verifying pollen viability (Ramos et al., 2008). Pollen grain viability and germination are crucial factors to determine fruit set, and they influence fruit yield (Irenaeus & Mitra, 2014).

There is a wide variation among the cultivars within the same species in terms of pollen viability, germination and the specific requirements for the reproductive process to occur (Irenaeus & Mitra, 2014).

Therefore, this study aimed to identify possible pear tree pollinizer pear cultivars in subtropical regions by evaluating the stigmatic receptivity, the pollen germination percentage, and the number of pollen grains.

2. Material and Methods

The plant material was collected during 2015 and 2016 in Lavras, Brazil located at latitude 21º14′ S, longitude 45º00′ W, and at an altitude of 841 m above mean sea level. The climate of the experimental site is classified as Cwa (subtropical climate, with cold and dry winter and hot and humid summer) (Souza et al., 2017). Seven *Pyrus communis* × *Pyrus pyrifolia* hybrid cultivars were evaluated: Cascatense, Centenária, D'água, Primorosa, Seleta, Tenra, and Triunfo (Table 1).

Table 1. List of cultivars with their origin and genealogy

Identification	Cultivar	Genealogy	Origin
1	Cascatense	Packham's Thiumph × Le Conte	IAC-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

The hybrid cultivars were grafted on to the chosen rooststock Pyrus calleryana and the graftlings were transplanted to the field in October 2010. Plants were spaced at 3.0 × 4.0 meters (833 trees/ha) and conducted in a modified central leader system. The management of trees followed modified central leader system for pruning, standard fertilization, phytosanitary control as recommended for pear tree.

2.1 Stigmatic Receptivity

Stigmatic receptivity was verified by the viscous and wetting aspect of the stigma (Almeida, 1986) and tested with 3% hydrogen peroxide (H_2O_2) (Kearns & Inouye, 1993). Flowers were collected at different floral development stages. Forty flowers of each cultivar at each floral stage were collected (E, E2, F, F2, and G—Figure 1). The material was randomly divided into four replications and ten flowers per replication.

Drops of 3% hydrogen peroxide were applied to the stigmas of the collected flowers. Those that showed bubble formation were considered as receptive. The percentage of receptive stigmas was evaluated at each floral stage by counting the number of stigmas that had bubble formation. At each floral stage, the number of receptive stigmas was multiplied by 100 and then divided by the total number of stigmas evaluated in this case, ten stigmas to calculate the percent receptivity of stigma. Data were subjected to analysis of variance, and the groups of means were compared by the Scott-Knott test at 5% probability.

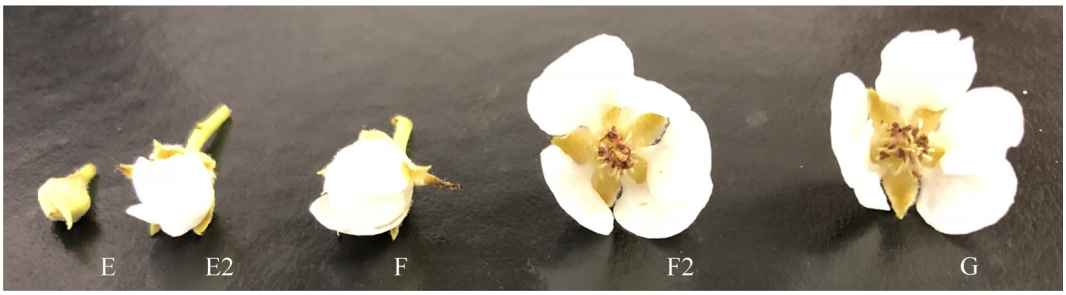

Figure 1. Major floral stages during flower development in pear tree (source: adapted from Faoro, 2009)

2.2 Number of Anthers per Flower and Pollen Grains per Anther and per Flower

The number of anthers per flower was determined by counting the anthers of ten floral buds collected from each cultivar at the pre-anthesis stage. Afterward, ten anthers were randomly separated, and each set of anthers was then stored in open microtubes at controlled temperature (27 °C) for 24 hours, in the absence of light, for the dehiscence and release of pollen grains (Nogueira et al., 2015). After 24 h, a 1,000 µl lactic acid solution was added to the tubes. After 48 hours, a 10 µl sample of each Eppendorf was placed on Neubauer slides to count the number of pollen grains, using an optical microscope (100×).

This experiment was conducted with five replications, each one consisting of 16 readings of the Neubauer slide. The amount of pollen grains per anther was calculated by multiplying the mean number of pollen grains of each sample by the volume of lactic acid in the solution (1,000 µl) and dividing that value by the product between the volume of lactic acid in the sample (10 µl) and the number of anthers of each microtube (ten). The number of pollen grains per flower was calculated by multiplying the mean number of pollen grains per anther by the mean number of anthers per flower. Data were subject to analysis of variance, and the groups of means were compared by the Scott-Knott's test at 5% probability.

2.3 Percentage of Pollen Grain Germination

Ten flowers of each cultivar were collected at the balloon stage to quantify the pollen grains germination. Anthers were removed using a tweezers and stored in uncovered petri dishes, at a controlled temperature (27 °C) for 12 hours to enable the anthesis, complete dehiscence, and release of pollen grains, based on the methodology of Zambon et al., (2014). Using a fine-bristle brush, the pollen was distributed on the surface of the petri dishes containing 20 ml of the previously established culture medium (containing 90 g L^{-1} sucrose, 700 mg L^{-1} boric acid, 145 mg L^{-1} calcium nitrate, pH of 5.2, and 10 g L^{-1} agar), as determined by Nogueira et al. (2016). Subsequently, the petri dishes were covered and stored in the absence of light for 24 hours. Each block consisted of four petri dishes.

Pollen grains were visualized in a monocular microscope (10×) to quantify the germinated pollen grains. The pollen grain, whose pollen tube length exceeded twice its diameter was considered as germinated, according to Figueiredo et al. (2013).

Four blocks per cultivar with five fields of view were used, where each petri dish represented one block. Data were subjected to analysis of variance, and groups of means were compared by the Scott-Knott's test at 5% probability.

3. Results and Discussion

3.1 Stigmatic Receptivity

Using 3% hydrogen peroxide, bubble formation was detected in the stigmatic surface indicating the activity of the peroxide enzyme, both at pre-anthesis flowers stage and completely opened flowers. The presence of this enzyme indicates the stigmatic receptivity (Kearns & Inouye, 1993).

The pear tree has a wet stigma, and its receptivity implies the production of exudates rich in proteins, free amino acids, lipids, and carbohydrates, which establish an adequate environment for the hydration and germination of the pollen grain and initial growth of the pollen tube (Sanzol et al., 2003).

Figure 2 shows that in all the cultivars studied, the stigmas were 100% receptive at the F2 and G stages, after anthesis. For cultivars D'água, Primorosa, Seleta, Tenra, and Triunfo, stigmas were 100% receptive at the F stage when the flower was at the balloon stage (pre-anthesis). This result demonstrates that manual pollination can be

performed before the flower opening, *i.e.*, at the pre-anthesis stage in breeding programs of these cultivars. Cultivar Triunfo showed 100% receptive stigma from the E2 stage (Figure 2).

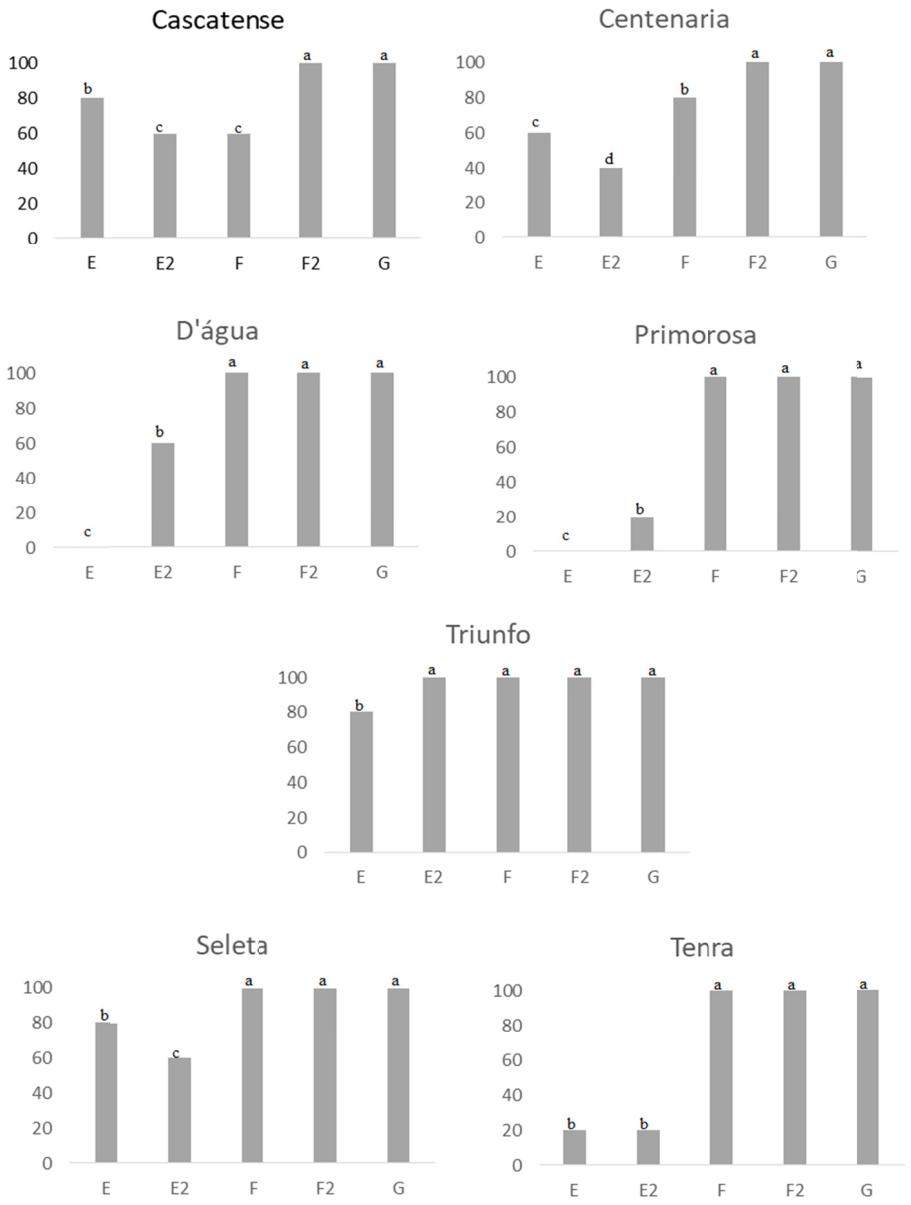

Figure 2. Stigmatic receptivity of flowers of different grafted pear hybrids at different phenological stages

Note. * means followed by the same letter did not differ from each other by the Scott-Knott's test at 5% probability.

Sanzol et al. (2003) studied pollen grain behavior after late pollination and identified three different stigmatic stages: immature, mature, and degenerated. Only the mature stigma is considered as receptive. According to the authors, the mechanisms by which the stigma loses receptivity are still incipient. However, it is believed that the stigmatic receptivity and stigma degeneration are the result of alterations in the secretion of exudates, rather than physical changes on the receptive surface.

D'água and Primorosa showed a shorter period of stigmatic receptivity, reaching 0% receptivity at the E stage. In the present study, the shortest period of the stigmatic receptivity of the cultivars might have occurred due to the high temperatures of the subtropical region where the studies were carried out. Irenaeus and Mitra (2014) studying fruit crops concluded that shortening of stigma receptivity occurs under temperature stress. Also,

studies on cherry trees have shown that the period of stigmatic receptivity can be shortened under high-temperature conditions and extended under low-temperature conditions (Hedhly et al., 2003).

For successful pollination, the pollen transferred to the receptive stigma must germinate, leading to fertilization. In many cases, fertilization may also occur when the pollen grain is deposited before the stigmatic receptivity period, provided that it remains viable to germinate as soon as the flower becomes receptive (Ramos et al., 2002). Under stress conditions, the stigmatic receptivity period may be affected due to a decrease in the amount of exudates (Srinivasan et al., 1999).

3.2 Number of Anthers per Flower and Pollen Grains per Anther and per Flower

Statistical analyses revealed a significant difference between cultivars regarding the number of anthers per flower, pollen grains per anther, and pollen grains per flower (Table 2). Accuracy estimates indicate the precision with which the experiments were conducted and the existence of variability. Appropriate accuracy values are close to the unit or 100% (Resende & Duarte, 2007). In the present study, for all evaluated traits, the accuracy was higher than 95%, confirming the accuracy of the evaluated data.

Table 2. Mean number of anthers per flower, mean number of pollen grains per anther and per flower in different grafted pear tree hybrid cultivars

Cultivar	No. of anthers per flower	No. of pollen grains per anther	No. of pollen grains per flower
Cascatense	22.0 a	1.127.50 a	24.805.00 a
Centenaria	19.2 b	860.00 a	16.512.00 b
Dagua	19.4 b	780.00 a	15.132.00 b
Primorosa	14.4 c	795.00 a	11.448.00 b
Tenra	19.8 b	11.00 b	217.80 c
Triunfo	21.6 a	3.50 b	43.20 c
Seleta	16.4 c	2.00 b	57.40 c
Accuracy	95	95	96

Note. *Means followed by the same letter in the column belong to the same group by the Scott-Knott's test at 5% probability. The abbreviated term "No." indicates "number".

Cascatense has the largest number of anthers per flower, pollen grains per anther, and pollen grains per flower, simultaneously, showing potential to be used as a pollinizer. Triunfo has a large number of anthers per flower but a very small number of pollen grains per anther, resulting in a low quantity of pollen grains per flower. Pear trees require cross-pollination due to the gametophytic incompatibility. The production of large amounts of pollen grains is a desirable trait since it increases the probability of cross-pollination (Sezerino & Orth, 2015).

Cultivars Centenaria, D'água, Primorosa, Tenra, and Seleta showed less than 20 anthers per flower (Table 2). This result may be related to a trait inherited from one of their parents. Nogueira et al. (2015), working with cultivar Packham's Triumph, detected 12 anthers per flower. Cultivars Seleta and Tenra showed a small number of pollen grains per anther and per flower. This result may be related to the lower adaptation of these cultivars to high-temperature regions. Nava et al. (2009) studied peach trees and verified a decrease in the production of pollen grains per anther due to high temperatures and water stress, which may have influenced the floral organogenesis and microsporogenesis stages.

The difference in the number of pollen grains per flower might be related to the fact that temperate-climate fruit trees require high chill hours. Therefore, when these cultivars are cultivated in regions with low chill hours, they can produce smaller flowers, smaller anthers, and fewer pollen grains than those cultivated under extended periods of cold or low temperatures during pollen development in temperate climatic zones compared to subtropical regions (Nogueira et al., 2016).

3.3 Percentage of Pollen Grains Germination

Statistical analysis of the results revealed a significant difference between the pear tree cultivars in relation to the percentage of successfully germinated pollen grains (Figure 3). The clustering of cultivars based on mean data on this per cent germination trait, resulted in four distinct clusters. The highest percentages of pollen grains germinated were observed in Cascatense (72.75%), followed by Primorosa (59.90%) (Figure 3). The percentage of pollen grains germinatedin Tenra and Triunfo was not detected and the data was not presented in the figure 3

and inboth of these cultivars, low percentages of pollen grains were germinated, and not enough pollen was obtained for the tests conducted. Therefore, these cultivars are not recommended as pollinizers.

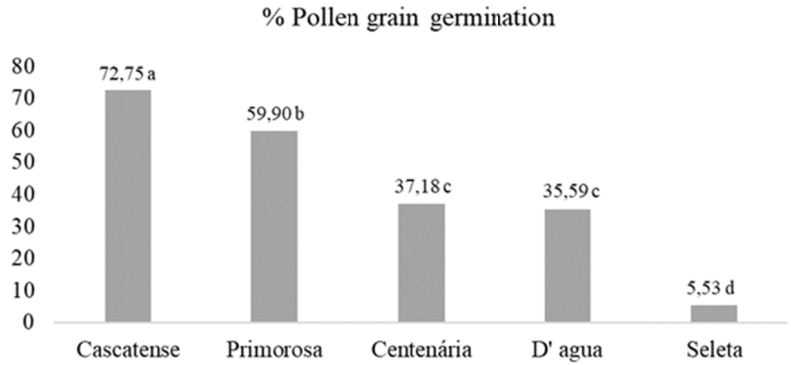

Figure 3. Percentage of pollen grain germination in different grafted pear tree hybrid cultivars

Note. * Means followed by the same letter did not differ from each other by the Scott-Knott's test at 5% probability.

For a cultivar to be indicated as a pollinizer, it must have adequate pollen germination per centage and produce a large number of viable pollen grains. The success of seed formation usually depends on the pollen grain viability (He et al., 2017). When the pollination process and/or pollen quality is inadequate, the ovule fertilization will be impaired leading to aborted seeds or malformation. This phenomenon will result in a larger number of empty locule in the pericarp (Nava et al., 2009).

Tatari et al. (2017) reported 78% and 88% of grain germination of different pear tree cultivars in Iran. In general, their results were superior to those of the present work. However, several factors, either genetic and/or environmental, can influence the percentage of pollen germination. In addition, temperature stress is considered in causing the most severe effects during pollen maturation (Zinn et al., 2010).

Considering that the winter temperatures in subtropical regions are higher, this fact might have been the reason for the low percentage of pollen grains germinated in cultivar Seleta (Figure 3). Thus, the pollen grain germination depends on the cultivar and the optimal temperature during pollen grain germination. Moreover, the development of the pollen tube varies according to the species and cultivar (Mert, 2009; Loupassaki et al., 1997).

Temperature stress may limit fertilization by inhibiting the development of male and female gametophytes, the pollen grain germination, and the pollen tube growth (Hedhly, 2011). Thus, studies on the effect of temperature stress on the reproductive process of different cultivars may be crucial in the choice of the appropriate cultivar for a determined pear crop growing region, due to influence of the microclimate, and complex reproductive physiology and biochemical mechanisms involved in understanding of the correlation between low fruiting and extreme temperature incidence before and during the flowering period (Irenaeus & Mitra, 2014).

Pollen grain production and germination can be suggested as a good criterion to determine plant's response to the high temperatures. These parameters can also be used as a selection criterion of pollinizer tree cultivars in breeding program of heat-tolerant pear genotypes (Irenaeus & Mitra, 2014).

4. Conclusions

Cultivars Seleta and Triunfo have high stigmatic receptivity for more extended periods during their flowering stage, showing potential to be used as female parents.

Cultivar Cascatense show a higher percentage of pollen germination and a larger number of anthers per flower, pollen grains per anther, and pollen grains per flower. Therefore, cultivar Cascatense has high potential to be used as a pollinizer tree, both in breeding programs for pear trees and commercial orchards.

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