Selection of pollinator cultivars for pear trees in a subtropical climate

Natália Ferreira Suárez¹ (b), Renata Amato Moreira¹ (b), Rafael Azevedo Arruda de Abreu¹ (b), Maira Ferreira de Melo Rossi¹ (b), Carolina Ruiz Zambon² (b), Anderson Oliveira Latini³ (b), Paula Nogueira Curi¹ (b), Rafael Pio^{1,*} (b)

1. Universidade Federal de Lavras 🧰 – Departamento de Agricultura – Lavras (MG), Brazil.

2. Empresa de Pesquisa Agropecuária de Minas Gerais Rie – Maria da Fé (MG), Brazil.

3. Universidade Federal de São João Del Rei 🏟 – Departamento de Ciências Exatas e Biológicas – Sete Lagoas (MG), Brazil.

Received: Sept. 28, 2023 | Accepted: Nov. 28, 2023

Section Editor: Gabriel Constantino Blain 🝺

*Corresponding author: rafaelpio@ufla.br

How to cite: Suárez, N. F., Moreira, R. A., Abreu, R. A. A., Rossi, M. F. M., Zambon, C. R., Latini, A. O., Curi, P. N. and Pio, R. (2024). Selection of pollinator cultivars for pear trees in a subtropical climate. Bragantia, 83, e20230209. https://doi.org/10.1590/1678-4499.20230209

ABSTRACT: The economic exploration of pear in the subtropical areas is possible when using hybrid cultivars obtained by crossing European (*Pyrus communis*) and Asian cultivars (*Pyrus pyrifolia*). Genetic improvement programs in Brazil have launched some cultivars in recent decades. Pear requires cross-pollination to improve fruit set in commercial plantations. In the case of these hybrid pear trees, the combinations of pollen receptor and donor cultivars that are best for cultivation in subtropical regions are not known. The objective of this study was to select cultivars with the potential to be pollinators of pear trees in subtropical regions. Four cultivars that received pollen grains (mother plants) ('Packham's Triumph', 'Tenra', 'Triunfo', and 'Seleta') and three donors ('Cascatense', 'Primorosa', and 'Shinseiki') were used. Stigmatic receptivity was observed in all cultivars during all stages of reproductive development. All cultivars had highly viable pollen grains. The cultivar 'Seleta' was the best recipient cultivar. The 'Seleta' × 'Shinseiki' cross showed the highest percentages of fruiting. The highest percentages of pistils with pollen tubes reaching the ovary were observed in the crosses 'Triunfo' × 'Shinseiki' and 'Seleta' × 'Shinseiki'. The 'Shinseiki' pear is an option as a pollinator of crops in subtropical regions.

Key words: *Pyrus communis* × *P. Pyrifolia*, pollination, pollen grain.

INTRODUCTION

Pear trees (*Pyrus* spp.) originate from regions with temperate climates. European cultivars (*Pyrus communis*) have a greater need for cold, and their fruits are pyriform, soft, and of high organoleptic quality. Asian cultivars (*Pyrus pyrifolia*) have less need of cold and can be grown in the tropics; their fruits are firm and round (Curi et al. 2017). The exploitation of pear in subtropical regions was made possible by genetic improvement studies that yielded hybrid pear trees from crosses between *P. communis* and *P. pyrifolia* (Pio et al. 2019). These hybrid pear trees have the quality of European fruits and the low cold requirement of Asian pears (Barbosa et al. 2018).

Brazilian pear production is not very significant, and consumption is eight times its volume, which makes the country one of the world's main importers of the fruit. Therefore, pear tree cultivation represents an important market opportunity to be explored nationally. However, the lack of cultivars adapted to soil and climate conditions is one of the main factors limiting the advancement of cultivated areas in Brazil (Nogueira et al., 2016).

Low fruiting is one of the limiting factors for the expansion of pear cultivation in the tropics (Bettiol Neto et al. 2014). There are no standard pollinator plants for hybrid cultivars in subtropical regions, which is the reason for their low fruiting. Low fruiting is related to the pollination process. The absence of fertilization of the ovule in pear flowers results in fewer fruits and causes reduction in production (Bisi et al. 2019b).

Most pear tree cultivars have gametophytic self-incompatibility, causing the plant to reject its own pollen (Bisi et al. 2021). Therefore, they depend on cross-pollination for fruit production. In general, the use of two or three pear cultivars with a coincident flowering period is recommended (Tatari et al. 2017). Given the need for cross-pollination for satisfactory fruit production, knowledge of the floral biology of these cultivars and the definition of the compatibility between them are determining factors for successful production (Pio et al. 2023).

The objective of present study was to select pollinator cultivars for pear trees for cultivation in a subtropical climate.

MATERIALS AND METHODS

Five cultivars of hybrid pear (*P. communis* × *P. pyrifolia*) were used: 'Cascatense' (Packham's Triumph' × 'Le Conte' – Empresa Brasileira de Pesquisa Agropecuária, Brazil), 'Primorosa' (Hood' × 'Packham's Triumph' – Instituto Agronômico de Campinas, Brazil), 'Seleta' ('Hood' × 'Packham's Triumph' – Instituto Agronômico de Campinas, Brazil), 'Tenra' ('Madame Sieboldt' × 'Packham's Triumph' – Instituto Agronômico de Campinas, Brazil), and 'Triunfo' ('Hood' × 'Packham's Triumph' – Instituto Agronômico de Campinas, Brazil). One European cultivar, 'Packham's Triumph', and one Asian cultivar, 'Shinseiki', were also studied. The choice of cultivars was based on adaptability to subtropical conditions (Bisi et al. 2019a), in two cycles: 2019/2020 and 2020/2021.

To assess stigmatic receptivity, preanthesis flowers were protected with organza bags. Ten flowers per stage were collected from each cultivar, four cultivars being used as pollen grain receptors ('Packham's Triumph', 'Seleta', 'Tenra', and 'Triunfo').

The stigmatic receptivity was verified by the viscous and wet aspect of the stigma and tested by adding 3% hydrogen peroxide (H_2O_2) to detect the action of the peroxidase enzyme, whose presence was indicated by the formation of small bubbles in receptive stigmas (Bisi et al. 2019b).

To characterize the viability of pollen grains by color, flowers of the cultivars used as pollen grain donors ('Cascatense', 'Primorosa', and 'Shinseiki') were collected, set in ethyl alcohol:acetic acid:propionic acid (6:3:2), and stored at -4°C. The anthers were excised under a stereomicroscope, and then the slides were prepared by the crush technique and stained with 2% propionic carmine and 2% Alexander dyes.

For staining with Alexander dye, pollen grains that exhibited a purple color without deformations were considered viable. They were considered nonviable when stained with green. For staining with the use of propionic carmine dye, pollen grains with red-stained cytoplasm were considered viable, and colorless pollen grains were considered nonviable (Silva et al. 2020). Five slides from each dye-based method were assessed, and 200 pollen grains were viewed per slide. The percentage of viable pollen grains out of the total number of pollen grains assessed was calculated. All slides were observed under a light microscope (Carl Zeiss, AxioLabA1) equipped with a microcamera (AxioCam ICc1) for image capture.

Field hybridizations were performed between three pollen donor cultivars ('Cascatense', 'Primorosa', and 'Shinseiki') and four recipient cultivars ('Packham's Triumph', 'Seleta', 'Tenra', and 'Triunfo'), totaling 12 crosses.

The anthers of the flower buds of the pollinator cultivars were removed, placed in Petri dishes, and dried on silica gel for 24 h to release the pollen grains. Flower buds in the balloon stage were emasculated, and then the stigmas were touched with a fine brush to transport the pollen grains of the donor cultivars. Then, the branches were bagged with organza bags to avoid contamination for approximately 40 days, after which they were assessed for effective fruiting rate, which equaled the difference between the number of pollinated flowers and the number of fruits formed (Paula et al. 2015).

The growth/development of the pollen tube was assessed in the same way as the controlled hybridizations in the field were assessed, and in the same 12 crosses. However, instead of assessing the effective fruiting, the flowers were collected 6, 12, 24, 48, 72, 96, and 120 h after pollination. Immediately after collecting the flowers, the styles were fixed in Carnoy's solution (ethanol:acetic acid, 3:1) and sent to the Cytogenetics Laboratory at Universidade Federal de Lavras. The fluorescence technique was used to assess the development of pollen tubes. The pistils were cut longitudinally at the base, keeping the ovaries intact, submerged in NaOH (8 N) on a watch glass, and put in an oven at 25°C for 10–15 minutes. They were then washed with distilled water three times and transferred to a slide with a drop of 0.1% aniline blue solution in K₃PO₄ (0.1 M). The observations were made in an inverted Olympus IMT-2 microscope with an epifluorescence apparatus and

450-nm filter. Five slides were assessed for each treatment to detect any pollen grains deposited on the stigmatic surface, any pollen tubes in the styles transmission tissue, and any pollen tubes in the ovaries.

For effective fruiting, the transformation $\sqrt{(x+0.5)}$ was applied to meet the assumptions of homogeneity of variances and normality. However, to assess the viability of pollen grains between cultivars and dyes, and their interactions, generalized linear models were used, which considered the lack of normality in the distribution of residues and corrected errors in the statistical decision. Subsequent comparisons were performed using confidence intervals (CI) of 95%, identifying significant differences when 95%CI of any treatment did not overlap with the mean of another treatment.

RESULTS AND DISCUSSION

Despite the morphological similarities of the androecium, several studies show that the amount and viability of pollen in pear flowers can vary greatly between species and cultivars (Nogueira et al. 2016). Bisi et al. (2019b) stated that these variations in pollen quantity and viability may be related to characteristics inherited from their progenitors and/or may be associated with the adaptation of cultivars to the environment where they live.

Stigmatic receptivity did not differ between the cultivars ($F_{(3.324)} = 2.40$; p = 0.067), between the phenological stages ($F_{(8.324)} = 0.71$, p = 0.685), or by the cultivar × phenological stage interaction ($F_{(24.324)} = 1.05$; p = 0.401). All stages of reproductive development showed the formation of bubbles in the stigma cavity when subjected to the 3% hydrogen peroxide test, indicating the activity of the peroxidase enzyme.

Bisi et al. (2019b) observed 100% receptivity in the stages after floral anthesis and in the balloon stage (preanthesis) in the cultivars 'Seleta', 'Tenra', and 'Triunfo'. This result demonstrates that controlled crosses of these cultivars can be performed before the opening of the flower in genetic improvement programs. However, Bisi et al. (2019b) found a shorter period of stigmatic receptivity than done here, which may be due to the high temperatures reported by those authors. Stigmatic receptivity is a key factor in the assessment of compatibility between cultivars, as the suitability of flowers in the fertilization process is directly related to their receptivity to pollen grain (Zambon et al. 2018). The stigmatic receptivity at all stages of reproductive development favors controlled hybridizations in genetic improvement programs. Receptive flowers in the preanthesis stage allow controlled hybridizations to be performed without contamination by pollen grains from materials other than those selected (Zambon et al. 2018). It is recommended to choose flower buds closer to anthesis. Although the stigma is receptive, only near the anthesis stage of the flowers the transmission of the style will begin to break down the starch, thus providing nutrients for the development of the pollen tube (Suàrez et al. 2012).

Pollen viability was significantly affected by cultivar [Wald (df = 2) = 16.7; p < 0.001], dye [Wald (df = 1) = 19.8; p < 0.001], and the cultivar × dye interaction [Wald (df = 2) = 7.5; p < 0.05]. For both dyes, the cultivar 'Shinseiki' showed the greatest viability of pollen grains: 95 and 90% with the use of propionic carmine and Alexander dye, respectively. The cultivar 'Cascatense' showed the lowest viability with the use of propionic carmine dye (87.5%), while the cultivar 'Primorosa' showed the lowest viability of pollen grains with Alexander dye (77.5%) (Fig. 1). The cultivars 'Cascatense' and 'Primorosa' had statistically equal viability when either dye was used.

Bisi et al. (2019b) observed higher percentages of in-vitro germination of pollen grains for the cultivars 'Cascatense' (72.75%) and 'Primorosa' (59.90%). The present study obtained higher rates of pollen viability, 87.5% with propionic carmine and 82.5% with Alexander dye for the 'Cascatense' cultivar and 92.5 and 77.5% for 'Primorosa', respectively. The lower pollen viability rates observed by Bisi et al. (2019b) may be associated with the influence of environmental factors, as thermal stress has its most severe effect if it occurs during pollen maturation.

The viability of pollen grains is of paramount importance in the selection of parents for use in controlled hybridizations of genetic improvement programs, and the pollinator must produce enough high-quality, viable pollen to ensure satisfactory fruit production (Bettiol Neto et al. 2014). To ensure success in hybridizations, pollen must have a high rate of viability and germination (Zambon et al. 2018). Deficient pollination processes and/or pollen quality impair egg fertilization, causing miscarriage or seed malformation, resulting in a greater number of empty locules in the pericarp (Bisi et al. 2021).

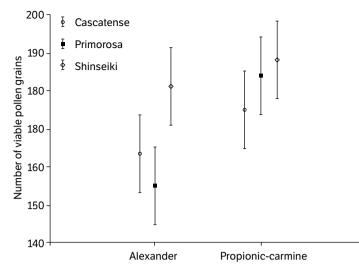


Figure 1. Number of viable pollen grains between cultivars ('Cascatense', 'Primorosa', and 'Shinseiki') and between dyes (propionic carmine and Alexander). The dispersion measures represent confidence intervals at 95%, and an overlap of the mean of one group by the interval of another indicates statistical similarity.

Pollination did not differ with the season $[F_{(1.216)} = 2.57; p = 0.110]$, the pollen donor cultivar $[F_{(2.216)} = 0.09, p = 0.916]$, the interaction between season and pollen donor cultivar $[F_{(2.216)} = 0.15; p = 0.857]$, the interaction between pollen donor cultivar and recipient cultivar $[F_{(6.216)} = 1.26; p = 0.279]$, or the triple interaction between season, pollen grain donor cultivar, and pollen grain recipient cultivar $[F_{(6.216)} = 0.25; p = 0.958]$. There was a significant difference between the receiving cultivars $[F_{(3.216)} = 16.93; p < 0.001]$ and under the interaction between the season and the receiving cultivar of the pollen grains $[F_{(3.216)} = 4.46; p < 0.05]$.

Only the cultivar 'Seleta' showed a difference between seasons, with effective fruiting of 50.6 (2019/2020) and 27.2% (2020/2021). 'Seleta' had the highest effective fruiting (for all combinations of crosses) in the 2019/2020 harvest (50.6%), followed by the cultivars 'Tenra' (16.8%) and 'Triunfo' (21.6%), which did not differ from each other. 'Packham's Triumph' had the lowest effective fruiting rate (11.2%) (Fig. 2).

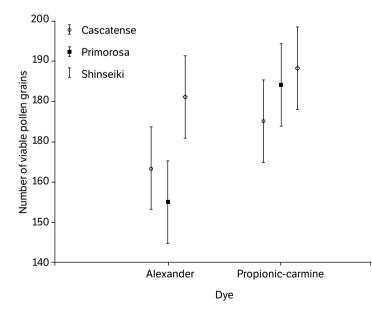


Figure 2. Effective fruiting rate among pollen-receiving cultivars ('Seleta', 'Triunfo', 'Packham's Triumph', and 'Tenra'). The dispersion measures represent confidence intervals at 95%, and an overlap of the mean of one group by the interval of another indicates statistical similarity.

In the 2020/2021 harvest, the highest effective fruiting rates were observed for the cultivars 'Seleta' (27.2%), 'Tenra' (22.9%), and 'Triunfo' (20.9%), followed by 'Packham's Triumph', which had the lowest fruit set rate (8.5%). The highest percentage of fruiting was recorded for the combination 'Seleta' × 'Shinseiki' in the 2019/2020 (60%) and 2020/2021 (34.1%) harvests. In contrast, the 'Packham's Triumph' × 'Shinseiki' cross had the lowest fruiting percentages in both the 2019/2020 (6%) and 2020/2021 (4%) harvests.

The production of large quantities of pollen grains is a desirable characteristic, because it increases the probability of cross-pollination (Sezerino and Orth, 2015). The 'Seleta' and 'Tenra' cultivars have few pollen grains per anther and per flower in subtropical regions and are considered better recipients than donors of pollen grain (Bisi et al. 2019b). Our results agree with this, as the recipient cultivar 'Seleta' showed the greatest effective fruiting (in all crosses) in the 2019/2020 harvest, followed by the cultivars 'Tenra' and 'Triunfo', which did not differ between each other. The higher fruiting rates observed for the recipient cultivar 'Seleta' may be due to the greater adaptation of this cultivar to climates with mild winters. Barbosa et al. (2018) reported that the cultivar 'Seleta' stands out from the other hybrid cultivars with its higher average fruit yield per plant and greater number of bags (fruiting structure) during its cycle.

The pollen donor cultivar 'Shinseiki' was the parent of the crosses that resulted in the highest and lowest percentages of fruiting: 'Seleta' × 'Shinseiki' and 'Packham's Triumph' × 'Shinseiki', respectively. This implies that lower fruiting is associated with lower compatibility between cultivars, since the cultivar 'Shinseiki' had the highest percentages of viable pollen grains (95 and 90% with the propionic carmine and Alexander dyes, respectively).

The cultivars 'Shinseiki' and 'Seleta' show good adaptation to cultivation in the tropics, with high production parameters and number of fruits per plant (Barbosa et al. 2018). To find better-performing cultivars, a balance between the vegetative and reproductive structures is necessary, as adequate accumulation of hours of cold is. According to the cultivar's need, the more hours of cold it experiences, the more intense its flowering, the lower the rate of bud abortion, and, consequently, the greater the fruit production (Barbosa et al. 2018).

The pollen donor cultivars in this study, 'Cascatense' and 'Primorosa', make large amounts of pollen (Bisi et al. 2019b), which makes them excellent pollinators. In addition to their high pollen production, both have high pollen viability (>75%). As the number of viable pollen grains deposited on the stigma of flowers increases, so do the size of the fruits, the number of seeds, and the percentage of fruit set (Bisi et al. 2021).

Most pear cultivars are considered self-incompatible, i.e., they reject their own pollen, relying on cross-pollination for satisfactory fruit production (Vieira et al. 2019). Thus, for pear trees to overcome gametophytic incompatibility and to ensure adequate fertilization, with a good effective fruiting rate, pollen grains from genotypes compatible with the S allele series must be transferred by pollinating insects (Sezerino and Orth, 2015).

Pollen grains without germination in the pistils were significantly likely in the crosses 'Seleta' \times 'Primorosa', 'Packham's Triumph' \times 'Primorosa', and 'Triunfo' \times 'Cascatense', which had an absence of pistils without a pollen tube germination starting at 6, 12, and 24 h after pollination, respectively. In the crosses 'Seleta' \times 'Shinseiki' and 'Packham's Triumph' \times 'Shinseiki', however, pistils without pollen tube germination were present from 24 and 120 h after pollination onward, respectively (Table 1).

Table 1. Results of logistic regression for occurrence of	pollen grains without germination and time after i	pollination: 6, 12, 24, 48, 72, 96, and 120 h*.

Pollen grains without germination		Logistic regression result		
Cross between cultivars	Times after pollinations (h)	N(n)	χ²	р
Seleta x Primorosa	6	35(2)	8.602	0.003
Packham's Triumph × Primorosa	12	35(3)	6.611	0.010
Triunfo x Cascatense	24	35(4)	8.679	0.003
Seleta x Shinseiki	24	35(29)	14.965	< 0.001
Packham's Triumph × Shinseiki	120	35(1)	4.077	0.043

N: the number of flowers evaluated; n: the number of flowers with non-germinated pollen grains; χ^2 : logistic regression result values; p: statistical probability; *among the 60 analyses, the table only presents significant products. The presence of pollen tubes in the style was significant in the crosses 'Seleta' × 'Primorosa', 'Packham's Triumph' × 'Cascatense', 'Packham's Triumph' × 'Shinseiki', 'Tenra' × 'Cascatense', 'Tenra' × 'Primorosa', 'Tenra' × 'Shinseiki', 'Triunfo' × 'Cascatense', and 'Triunfo' × 'Shinseiki', with the presence of a pollen tube in the styles from 12, 48, 24, 48, 48, 72, 72, and 24 h after pollination onward, respectively. There was also a significant relationship in the crosses 'Seleta' × 'Shinseiki', 'Packham's Triumph' × 'Primorosa', and 'Triunfo' × 'Primorosa', and 'Triunfo' × 'Primorosa', and 'Triunfo' × 'Primorosa', 'Tenra' × 'Shinseiki', 'Tenra' × 'Shin

Pollen tubes in the style		Logistic regression result		
Cross between cultivars	Times after pollinations (h)	N(n)	χ²	р
Seleta × Primorosa	12	35(27)	23.007	< 0.001
Packham's Triumph × Cascatense	48	35(21)	28.344	< 0.001
Packham's Triumph × Shinseiki	24	35(22)	32.335	< 0.001
Tenra × Cascatense	48	35(23)	17.134	< 0.001
Tenra × Primorosa	48	35(20)	31.391	< 0.001
Tenra × Shinseiki	72	35(21)	16.561	< 0.001
Triunfo × Cascatense	72	35(23	14.073	< 0.001
Triunfo × Shinseiki	24	35(25)	20.629	< 0.001
Seleta × Shinseiki	24	35(8)	12.822	< 0.001
Packham's Triumph × Primorosa	48	35(7)	7.984	< 0.001
Triunfo × Primorosa	48	35(11)	13.615	< 0.001

Table 2. Results of logistic regression for occurrence of pollen tubes in the style and time after pollination: 6, 12, 24, 48, 72, 96, and 120 h*.

N: the number of flowers evaluated; n: the number of flowers with pollen tubes in the style; χ^2 : logistic regression result values; p: the statistical probability; *among the 60 analyses, the table only presents significant products.

The presence of pollen tubes in the receptacle was significant only for the 'Seleta' \times 'Cascatense' cross (Table 3), which had no pollen tubes in the receptacle starting at 12 h.

Table 3. Results of logistic regression for occurrence of pollen tubes in the receptacle and time after pollination: 6, 12, 24, 48, 72, 96, and 120 h*.

Pollen tubes in the receptacle		Logistic regression result		
Cross between cultivars	Times after pollinations (h)	N(n)	χ²	р
Seleta × Cascatense	12	35(3)	5.4563	0.019

N: the number of flowers evaluated; n: the number of flowers with pollen tubes in the receptacle; χ^2 : logistic regression result values; p: the statistical probability; *among the 60 analyses, the table only presents significant products.

The presence of pollen tubes in the ovary was significant in the crosses 'Seleta' × 'Cascatense', 'Seleta' × 'Primorosa', 'Seleta' × 'Shinseiki', 'Tenra' × 'Cascatense', 'Packham's Triumph' × 'Shinseiki', 'Tenra' × 'Cascatense', 'Tenra' × 'Primorosa', 'Tenra' × 'Shinseiki', 'Triunfo' × 'Cascatense', 'Triunfo' × 'Primorosa', and 'Triunfo' × 'Shinseiki', with the presence of pollen tube in the ovary from 72, 24, 24, 72, 48, 72, 72, 72, 72, 48, and 48 h onward, respectively. There was also a significant relationship in the cross 'Packham's Triumph' × 'Primorosa' (Table 4), but pollen tubes were absent in its ovaries starting at 48 h.

The growth of the pollen tube in the pistil began with the germination of pollen grains on the stigmatic surface. The pollen tubes penetrated clusters in the style until reaching the ovary. In the first hours after pollination, pistils without pollen germination were observed, being 20% (6 h), 5% (12 h), 13.33% (24 h), and 3.33% (48 h). At 72 and 96 h after pollination, in all cultivars, the pollen grains had already germinated, but at 120 h after pollination a small percentage of pollen grains had not germinated (1.67%) only in the 'Packham's Triumph' × 'Shinseiki' cross.

After the germination of the pollen grains, the pollen tubes showed growth in the style (Figs. 3a and 3b), with gradual reduction in the percentages over time to 61.67% (6 h), 76.67% (12 h), 55% (24 h), 25% (48 h), and 10% (72 h). After 72 h, the pollen tubes already showed growth toward the ovary (Fig. 3c). The percentages of observations with pollen tube growth up to the receptacle region were lower, with reduction at the last assessment time from 10% (6 h) to 1.67% (120 h).

Pollen tubes in the ovary		Logistic regression result		
Cross between cultivars	Times after pollinations (h)	N(n)	χ²	р
Seleta × Cascatense	72	35(17)	9.112	0.002
Seleta × Primorosa	24	35(18)	23.246	< 0.001
Seleta × Shinseiki	24	35(21)	42.083	< 0.001
Packham's Triumph × Cascatense	72	35(16)	30.974	< 0.001
Packham's Triumph × Shinseiki	48	35(17)	18.418	< 0.001
Tenra × Cascatense	72	35(19)	24.815	< 0.001
Tenra × Primorosa	72	35(18)	30.051	< 0.001
Tenra × Shinseiki	72	35(18)	20.522	< 0.001
Triunfo × Cascatense	72	35(17)	30.255	< 0.001
Triunfo × Primorosa	48	35(19)	27.401	< 0.001
Triunfo × Shinseiki	48	35(23)	17.134	< 0.001
Packham's Triumph × Primorosa	48	35(15)	16.344	< 0.001

Table 4. Results of logistic regression for occurrence of pollen tubes in the ovary and time after pollination: 6, 12, 24, 48, 72, 96, and 120 h*.

N: the number of flowers evaluated; n: the number of flowers with pollen tubes in the ovary; 2: logistic regression result values; p: the statistical probability; *among the 60 analyses, the table only presents significant products.

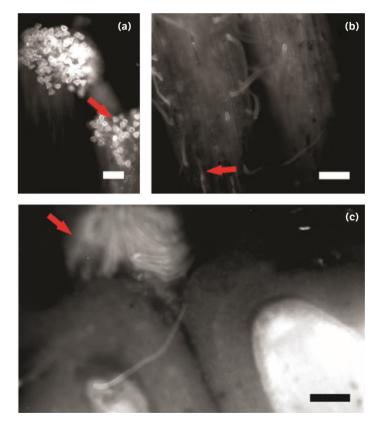


Figure 3. (a) Stigmas with pollen grains. (b) Growth of pollen tubes in the style of 'Seleta' × 'Shinseiki' 12 h after pollination. (c) Pollen tube reaching the ovary in 'Seleta' × 'Shinseiki' 72 h after pollination.

Crosses with the donor cultivar 'Cascatense' had the lowest percentage of pollen grains reaching the ovary, even after 120 h, demonstrating the low compatibility of this cultivar with the others or a longer time to reach the ovary. According to Bisi et al. (2021), the cultivar 'Cascatense' has a negative effect on seed viability, which may be related to the gametophytic

incompatibility of this cultivar with the others, which results in lower fruit mass, length, and diameter (Bisi et al. 2021). In contrast, the highest percentages of pistils with pollen tubes reaching the ovary were observed in crosses with the donor cultivar 'Shinseiki', which can be explained by the higher pollen viability of the cultivar.

At 72 and 96 h after pollination, the pollen grains of all cultivars had already germinated. The low percentage of nongerminated pollen grains 120 h after pollination in the 'Packham's Triumph' \times 'Shinseiki' cross may be related to the weaker adaptation of the cultivar to mild winter regions, since this cross was also responsible for the lower percentages of fruiting in both seasons assessed.

Over time after pollination, all pollen tubes already showed growth toward the ovary, with high percentages of pollen tubes in the ovary in the last hours assessed (> 95%). Paula et al. (2015) reported a much lower percentage of pollen tubes in the ovary (10.3%) in 'Packham's Triumph' × 'Williams' cross, although they found much higher percentages inside the pistil (> 60%), explaining the need for a longer assessment period for the pollen tube to reach the ovary.

There is the need for more regional studies or even compliance with the need for coincidences in the concentration period of cultivars, guaranteeing the supply of pollen in the orchard, as well as management techniques adapted to the cultivation locations.

CONCLUSION

The highest percentages of pistils with pollen tubes reaching the ovary were observed in crosses with the donor cultivar 'Shinseiki', which can be explained by the higher pollen viability of the cultivar.

The Asian pear cultivar 'Shinseiki' showed the best potential as a pollinator pear tree in subtropical climates, especially with the use of 'Triunfo' and 'Seleta' hybrid pear trees as acceptors.

CONFLICT OF INTEREST

Not applicable.

AUTHORS' CONTRIBUTION

Conceptualization: Suárez, N. F., Moreira, R. A., Rossi, M. F. M. and Pio R.; **Data curation:** Suárez, N. F. and Pio, R.; **Formal analysis:** Suárez, N. F., Moreira, R. A., Zambon, C. R. and Pio, R.; **Funding acquisition:** Suárez, N. F. and Pio, R.; **Investigation:** Suárez, N. F., Abreu, R. A. A., Rossi, M. F. M., Zambon, C. R., Curi, P. N. and Pio, R.; **Methodology:** Suárez, N. F., Rossi, M. F. M., Zambon, C. R., Curi, P. N. and Pio, R.; **Wethodology:** Suárez, N. F., Rossi, M. F. M., Zambon, C. R., Curi, P. N. and Pio, R.; **Witing acquisition:** Curi, P. N. and Pio, R.; **Project administration:** Pio, R.; **Resources:** Pio, R.; **Supervision:** Pio, R.; **Validation:** Pio, R.; **Writing – original draft:** Pio, R.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

FUNDING

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior 🏁 Finance Code 001 Conselho de Desenvolvimento Científico e Tecnológico 🏟 Grant No. 305849/2022-0

Fundação de Amparo à Pesquisa do Estado de Minas Gerais 🦇 Grant No. APQ-03781-22

ACKNOWLEDGMENTS

Not applicable.

REFERENCES

Barbosa, C. M. A., Pio, R., Souza, F. B. M., Bisi, R. B., Bettiol Neto, J. E. and Farias, D. H. (2018). Phenological evaluation for determination of pruning strategies on pear trees in the tropics. Scientia Horticulturae, 240, 326-332. https://doi.org/10.1016/j.scienta.2018.06.006

Bettiol Neto, J. E., Chagas, E. A., Sanches J., Pio, R., Antoniali, S. and Cia, P. (2014). Production and postharvest quality of pear tree cultivars in subtropical conditions at eastern of São Paulo state, Brazil. Ciência Rural, 44, 1740-1746. https://doi.org/10.1590/0103-8478cr20131574

Bisi, R. B., Pio, R., Farias, D. H., Locatelli, G., Barbosa, C. M. A. and Pereira, W. A. (2019a). Molecular characterization of the S-alleles and compatibility among hybrid pear tree cultivars for subtropical regions. HortScience, 54, 2104-2110. https://doi.org/10.21273/ HORTSCI14261-19

Bisi, R. B., Pio, R., Locatelli, G., Farias, D. H. and Botelho, F. B. S. (2021). General and specific combining ability in the selection of polliniser cultivars of hybrid pear trees (*Pyrus communis* x *P. pyrifolia*). Scientia Horticulturae, 277, 109797. https://doi.org/10.1016/j.scienta.2020.109797

Bisi, R. B., Pio, R., Locatelli, G., Tadeu, M. H., Zambon, C. R. and Silva, D. M. (2019b). Determination of potential pollinizer grafted pear hybrids (*Pyrus communis x Pyrus pyrifolia*) for subtropical regions in Brazil. Journal Agriculturae Science, 11, 191-198. https://doi. org/10.5539/jas.v11n6p191

Curi, P. N., Bisi, R. B., Salgado, D. L., Barbosa, C. M. A., Pio, R. and Souza, V. R. (2017). Hybrid cultivars of pear in subtropics regions: processing ability in the form of jelly. Ciência Rural, 47, e20170331. https://doi.org/10.1590/0103-8478cr20170331

Nogueira, P. V., Coutinho, G., Pio, R., Silva, D. F. and Zambon, C. R. (2016). Establishment of growth medium and quantification of pollen grains and germination of pear tree cultivars. Revista Ciência Agronômica, 47, 380-386. https://doi.org/10.5935/1806-6690.20160045

Paula, L. A., Rufato, A. D. R., Oliveira, P. R. D. D. O. and Tallamini, M. R. (2015). Hybridizations inter and intraespecific controlled for breeding of pear rootstocks. Revista Brasileira de Fruticultura, 37, 811-818. https://doi.org/10.1590/0100-2945-138/14

Pio, R., Faria, D. H., Peche, P. M., Bisi, R. B., Fazenda, L. H. V. and Silva, A. D. (2023). Production stability of pear cultivars for cultivation in the subtropical altitude climate. Bragantia, 82, e20230167. https://doi.org/10.1590/1678-4499-2023-0167

Pio, R., Souza, F. B. M., Kalcsits, L., Bisi, R. B. and Farias, D. H. (2019). Advances in the production of temperate fruits in the tropics. Acta Scientiarum Agronomy, 41, e39549. https://doi.org/10.4025/actasciagron.v41i1.39549

Sezerino, A. A. and Orth, A. I. (2015). Pollination of the portuguese pear in Bom Retiro-SC Brazil. Revista Brasileira de Fruticultura, 37, 943-951. https://doi.org/10.1590/0100-2945-209/14

Silva, D. M., Zambon, C. R., Techio, V. H. and Pio, R. (2020). Floral characterization and pollen germination protocol for Castanea crenata Siebold & Zucc. South African Journal of Botany, 130, 389-395. https://doi.org/10.1016/j.sajb.2020.01.027

Suàrez, C., Castro, A. J. and Rapoport, H. F. (2012). Morphological, histological and ultrastructural changes in the olive pistil during flowering. Sexual Plant Reproduction, 25, 133-146. https://doi.org/10.1007/s00497-012-0186-3

Tatari, M., Ghasemi, A., Mousavi, A. and Bahrami, H. (2017). Study on pollination and selection of the most suitable pollinizers for commercial pear cultivars (*Pyrus communis* L.) in Iran. Journal of Horticultural Research, 25, 49-57. https://doi.org/10.1515/johr-2017-0021

Vieira, J., Rocha, S., Vázquez, N., López-Fernández, H., Fdez-Riverola, F., Reboiro-Jato, M. and Vieira, C. P. (2019). Predicting specificities under the non-self gametophytic self-incompatibility recognition model. Frontiers Plant Science, 10, 879. https://doi.org/10.3389/ fpls.2019.00879

Zambon, C. R., Silva, L. F. O., Pio, R., Bianchini, F. G. and Oliveira, A. F. (2018). Storage of pollen and properties of olive stigma for breeding purposes. Revista Ciência Agronômica, 49, 291-297. https://doi.org/10.5935/1806-6690.20180033