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# Study of flowering dynamics and the effects of self- and open-pollination on fruit set of a commercial seedless barberry clone in Iran

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ABSTRACT

This study aimed to investigate the dynamics of flowering and evaluate the effect of open and self-pollination on the fertility of commercial seedless barberry clone shrubs in Iran. During the bud swelling stage, ten shrubs were selected, and on each shrub, one branch bearing at least 100 flower buds was chosen to monitor flowering dynamics through daily recordings of bud and open flower counts. To assess fertility, two branches bearing at least 100 flower buds were chosen on each shrub. One branch was isolated with cotton fabric bags for self-pollination treatment, while the other remained unisolated for open-pollination, with the initial and final fruit set (IFF, FFF) subsequently recorded for both. Concurrently, pollen viability was evaluated by measuring pollen germination. The flowering period ranged from 16-19 days. Under open-pollination conditions, the IFF was 70-80%, and FFF was 50-60%. Under controlled self-pollination conditions in bagged shoots, IFF was 50-60%, and FFF was 40-50%. The high fruit set in isolated branches suggests the capability for parthenocarpic fruit development stimulated by self-pollination. However, the significantly higher fruit set in open-pollination underscores the critical role of pollinator insects in enhancing pollination efficiency. Fruit drop was 20.11% and 13.37% for open- and self-pollinated treatments, respectively. This research had no specific limitations. This research provides practical and valuable information on pollination and fertility in this seedless barberry, supporting the adoption of optimal orchard management practices to enhance fertility outcomes for future applications.

## Highlights

- Temperature changes affect pollen germination and fruit formation in seedless barberry orchards.
- The difference in the initiation, peak, and duration of seedless barberry flowering is regulated by heat accumulation caused by temperature.
- In seedless barberry, open pollination also results in more initial and final fruit formation compared to self-pollination.
- A commercial clone of seedless barberry showed significant fruiting under self-pollination, indicating an inherent ability for parthenocarpy.

## 1. Introduction

The seedless barberry clone is a unique horticultural crop produced almost exclusively in Iran. The primary production regions include Qaenat, Zirkuh, Darmian, Sarbisheh, and Birjand (Tehranifar, 2002; Alemardan et al., 2013; Ganji et al., 2024; Jafari et al., 2024). Different barberry varieties are valued globally for their multiple uses, including ornamental, medicinal, and edible purposes (Arayne et al., 2007). The genus *Berberis*, the largest genus in the Berberidaceae family, includes about 631 species (Tiwari et al., 2012). Botanists recognize five barberry species in Iran: *Berberis vulgaris*, *B. orthobotrys*, *B. khorassanica*, *B. crataegina*, and *B. integerrima* (Kafi et al., 2002). However, taxonomic inconsistencies persist, especially for *B. integerrima*, *B. crataegina*, and *B. khorassanica* (Kafi et al., 2002). Some studies suggest that the cultivated seedless barberry belongs to *B. integerrima* (Heidary et al., 2009; Rezaei et al., 2010; Sodaghar et al., 2012). Nevertheless, Cadic (1992) reported that the *Berberis* genus is self-fertile and predominantly

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autogamous. The seismonastic property of the anthers, along with the behavior of bees when collecting nectar, helps facilitate self-pollination (Anderson et al., 2001). In this mechanism, anthers rapidly dehisce by touch or movement caused by wind or insects, releasing pollen directly onto the stigma and thereby promoting self-pollination (Anderson et al., 2001). However, cross-pollination has also been cited as an important reproductive mechanism in some barberry species (Verma et al., 2021).

The seedless barberry clone is one of the important economic products in Iran, yet despite centuries of cultivation, many aspects of its biology remain poorly understood. Flowering dynamics and fruit-set mechanisms remain among the least understood aspects of this clone, yet they are highly important for developing a breeding program. To date, few studies have been published regarding the pollination and fertility of seedless barberry. However, Ebadi et al. (2010), in a study on the seedlessness mechanism of Iranian seedless barberries, concluded that the primary cause of the seed absence in this clone is gametophytic self-incompatibility, while stimulative parthenocarpy is responsible for fruit set in this species. In their study, different artificial pollination treatments using a mixture of pollen grains from several wild-seeded barberry species were tested to induce seeded fruits in the seedless barberry, but the effect of the pollen from individual genotypes was not assessed. Additionally, the effect of seedless barberry pollen on wild seeded varieties remained unexplored. Heidari et al. (2010) also conducted field pollination treatments on both seeded and seedless barberries. Their results showed that neither the seeded barberry genotype *B. integerrima* nor the seedless barberry produced any seeded fruit under self-pollination treatment. Under artificial cross-pollination using pollen from a seeded genotype, the seedless barberry produced fewer seeded fruits compared to *B. integerrima*.

Despite previous findings, key gaps remain in understanding pollination dynamics and fruit-set mechanisms of seedless barberry. Clarifying these processes provides new insights into the basis of seedlessness and supports strategies to improve crop production in the seedless barberry clone. The aim of this research, in addition to studying the flowering dynamics of seedless barberry in different years, was to investigate the degree of dependence of the fertility of this plant species on the activity of pollinating insects under the influence of open pollination and self-pollination treatments.

## 2. Materials and methods

### 2.1. General project specifications

This experiment was conducted from 2020 to 2024 (only in fruiting years) (Khayyat et al., 2018) in a seedless barberry orchard at the Agricultural and Natural Resources Research and Education Center of South Khorasan, in Iran (32°05' latitude, 56°27' longitude, 1310 altitude). The region has a hot and dry climate, with an average annual rainfall of about 140 millimeters (mm). All the shrubs were uniform in age and structure, with an average age of 15 years. The orchard soil was sandy loam, and irrigation was applied by flood irrigation every 8–10 days. All seedless barberry shrubs were propagated and planted using suckers. Annual horticultural operations were carried out uniformly for all shrubs. The experiment used a split-plot in time arrangement within a randomized complete block design (RCBD) with 10 replications.

### 2.2. Study of the flowering process

To assess flowering dynamics, one branch with at least 100 flower buds was selected from each of 10 uniform, same-aged shrubs at the bud-swelling stage, before anthesis. Branches were chosen from different orientations around each shrub to ensure representative sampling.

Flowers were monitored daily between 10:00 and 12:00, and the number of unopened buds and newly opened flowers was recorded.

### 2.3. Fertility study

#### 2.3.1. Pollen grain germination test

To ensure the fertility potential of pollen grains, flowers of seedless barberry were selected at the final stage of bud swelling, before opening, and their anthers were separated in the laboratory. For the germination assay, pollen grains were placed in a solution containing 10% sucrose and 20 ppm boric acid and kept under controlled conditions **(**25°C and normal room light**)**. Pollen tube growth was examined under a binocular microscope at 24 and 48 hours after incubation to determine germination rates (Imani, 2005).

#### 2.3.2. Open pollination study

In this experiment, 10 uniform shrubs were selected, and one branch from each shrub was chosen, labeled, and left exposed to natural pollinators to allow open pollination. Fruit set was assessed at multiple developmental stages.

#### 2.3.3. Self-pollination study

To prevent contamination by foreign pollen, one branch bearing at least 100 flower buds was selected on each shrub at the late bud-swelling stage and enclosed in a breathable cotton fabric bag (55 × 40 cm) until petal fall was complete. The fabric of the insulators was such that it allowed air exchange and prevented insects from entering. The selected branch was marked, and the fertility percentage was determined at different stages. Data analysis was performed using SAS 9.1 software.

## 3. Results and discussion

### 3.1. Flowering dynamics and temperature relationship

The flowering dynamics exhibited clear temporal variations across the experimental years 2020, 2022, and 2024 (Figures 1- 3). The onset, peak, and duration of flowering were distinctly influenced by ambient temperature variations, consistent with previous findings for woody perennials in temperate zones (Chuine, 2010; Basler and Körner, 2014). In particular, the fulfillment of chilling requirements prior to anthesis was a prerequisite, as demonstrated by the dormancy release theory (Cooke et al., 2012). Once these chilling requirements were satisfied, the accumulation of heat units, commonly referred to as growing degree days (GDD), regulated the timing of flower

opening. Our observations showed that flowering began earliest in 2022 (April 2, at an average temperature of 17°C), followed by 2020 (April 12, 19°C), and latest in 2024 (April 19, 18°C). This variation aligns with established models of temperature-dependent phenological development (Menzel et al., 2006; Jeong et al., 2011), in which cooler temperatures delay flowering onset. The year-to-year differences are likely attributable to interannual climate variability affecting heat accumulation post-dormancy release. Flowering duration ranged from 16 to 19 days and was inversely related to the average temperature during the flowering period. Higher temperatures accelerated flower opening and shortened the overall flowering window, a pattern consistent with studies in other temperate shrubs (Anderson et al., 2013; Basler, 2016). This acceleration may be linked to increased metabolic activity and hormonal activities, particularly elevated gibberellin and auxin biosynthesis, which promote flower development under warmer conditions (Dennis, 2003). Conversely, lower temperatures prolong anthesis by slowing floral organ maturation. These temperature-driven phenological shifts have significant ecological and agronomic implications. A shorter flowering period at higher temperatures may not only synchronize flowering with pollinator activity but also reduce the temporal window for effective pollination, potentially impacting fruit set. In contrast, prolonged flowering under cooler conditions may increase opportunities for pollination but can increase exposure of flowers to adverse weather conditions. These findings align with those of Chuine and Régnière (2017), who demonstrated the dual role of temperature in regulating phenology. The influence of temperature on flowering phenology is a critical factor for reproductive success and yield stability in seedless barberry, especially under climate change scenarios that forecast increased temperature variability (IPCC, 2021). These findings are consistent with other studies showing that climate change and rising global temperatures can affect the flowering season and the duration of flowering in plants. In particular, climate can affect the timing and quality of flowering, potentially causing changes in fruit ripening (Guo et al., 2014; Anjum et al., 2025; Łysiak and Szot, 2023).

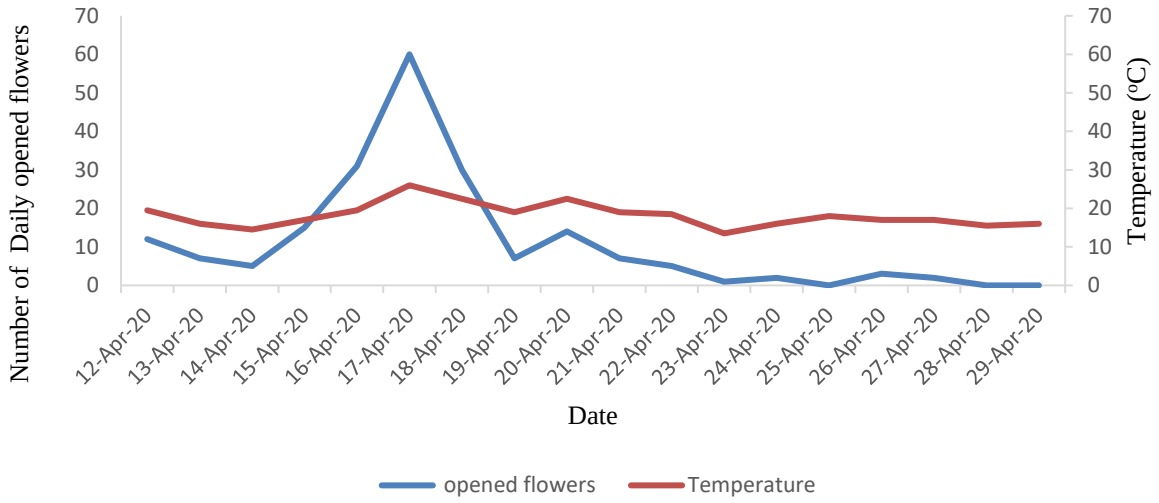



**Figure 1. Relationship between flowering dynamics and temperature in the seedless barberry commercial clone in 2020**

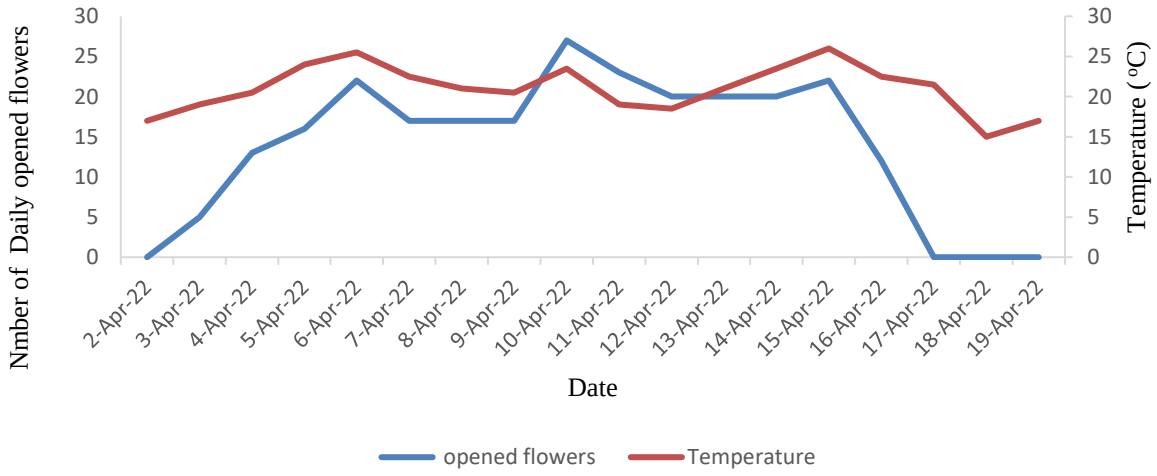



**Figure 2. Relationship between flowering dynamics and temperature in the seedless barberry commercial clone in 2022**

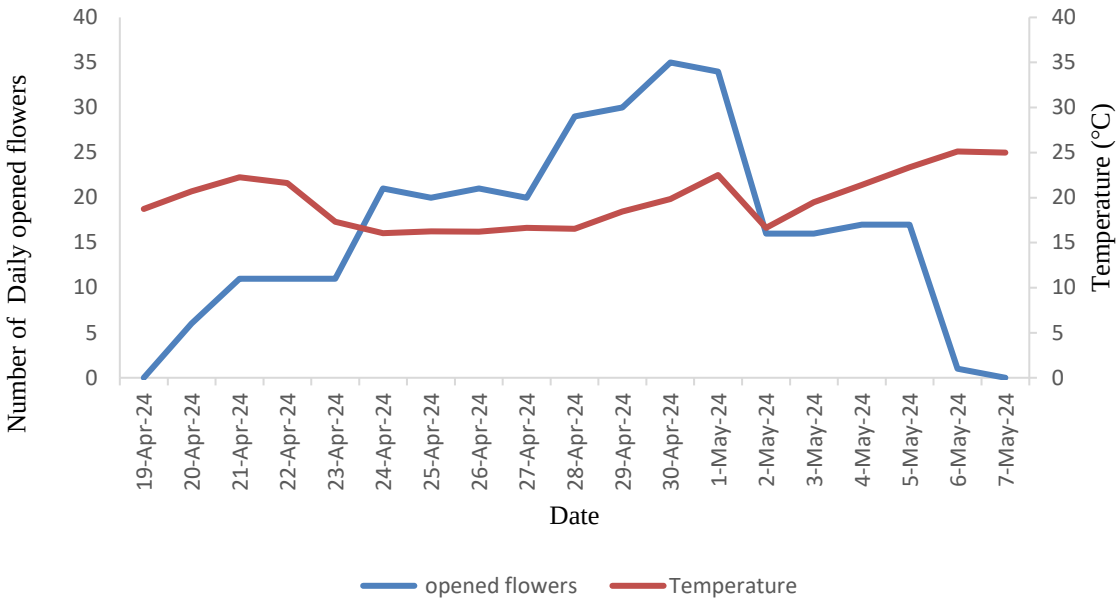



**Figure 3. Relationship between flowering dynamics and temperature in the seedless barberry commercial clone in 2024**

### 3.2. Fertility study

#### 3.2.1. Pollen grain germination test

The pollen germination test showed a moderate germination rate. Of approximately 280 pollen grains evaluated, 55% germinated after 24 hours and 70% after 48 hours under optimal conditions, with pollen tube formation observed (Figures 4 and 5).

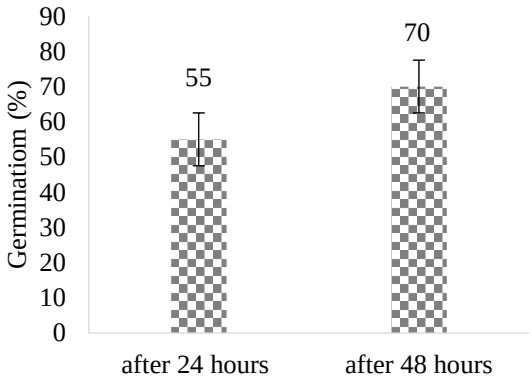



**Figure 4. Percentage of germinated pollen grains after 24 and 48 hours**



**Figure 5. Germinated pollen grains after 48 hours**

It has been reported that the average pollen germination in seedless barberry is 54%, compared with 69% in seeded barberry (Ebadi et al., 2010), which is consistent with current results. These values are somewhat lower than those reported for other fruit species like apple (*Malus domestica*) and pear (*Pyrus* spp.), where germination often exceeds 80% (Radović et al., 2023; Bound, 2022). The sticky nature of seedless barberry pollen, which restricts wind dispersal and relies on insect vectors, further emphasizes the specialized pollination ecology of this species.

#### 3.2.2. Initial fruit set (IFF)

Our results showed that the effect of pollination type (open pollination and self-pollination) on the initial fruit set trait was significant at the 1% level (Table 1).

Mean comparison results (Table 2; Figure 6) revealed that the IFF in this clone under open pollination was 77.19% in 2020, 73.09% in 2022, and 74.09% in 2024. Thus, IFF under open pollination ranged from 70 to 80 percent, with an overall mean of 74.79%. Under self-pollination, IFF values were 65.44% in 2020, 54.2% in 2022, and 57.06% in 2024, corresponding to a range of 50 to 60 percent and an average of 58.9%. Comparison of these means indicates that open pollination increases IFF by an average of 15.89% (range: 10–20%) compared to self-pollination. The relatively high fruit formation under self-pollination treatment indicates that seedless barberry is capable of self-pollinating and can produce 50-60 percent fruit without insect pollinators. However, the higher IFF observed under open pollination suggests that additional pollination by insects can increase fruit set by approximately10-20%.

**Table 1. Variance analysis of open and self-pollination in seedless barberry across three years**

| | | Mean squares | |
|---|---|---|---|
| **S.O.V** | **df** | **Initial Fruit Fertility(IFF)** | **Final Fruit Fertility(FFF)** |
| Block | 9 | 134.484$^{ns}$ | 100.268$^{ns}$ |
| pollination | 1 | 3788.970$^{**}$ | 1876.004$^{*}$ |
| Block×pollination | 9 | 255.004$^{ns}$ | 365.801$^{ns}$ |
| Years | 2 | 318.465$^{ns}$ | 112.171$^{ns}$ |
| Pollination× years | 2 | 68.655$^{ns}$ | 24.216$^{ns}$ |
| Block×years | 18 | 256.397$^{ns}$ | 223.547$^{ns}$ |
| Error | 18 | 202.614 | 151.082 |
| CV% | | 21.294 | 24.043 |

* and ** show significance at the 5% and 1% levels, and ns means no significant difference.

**Table 2. Means comparison of primary fruit fertility (IFF) and final fruit fertility (FFF) on barberry fertility**

| **Pollination** | **Mean initial fertility(iff)** | **Mean final fertility (fff)** |
|---|---|---|
| Open pollination | 74.79 ±5.72 $^{a}$ | 56.71 ± 4.03$^{a}$ |
| Self pollination | 58.9 ± 5.72 $^{b}$ | 45.53± 4.03 $^{b}$ |

Means followed by a similar letter in the same column indicate a non-significant difference**.**

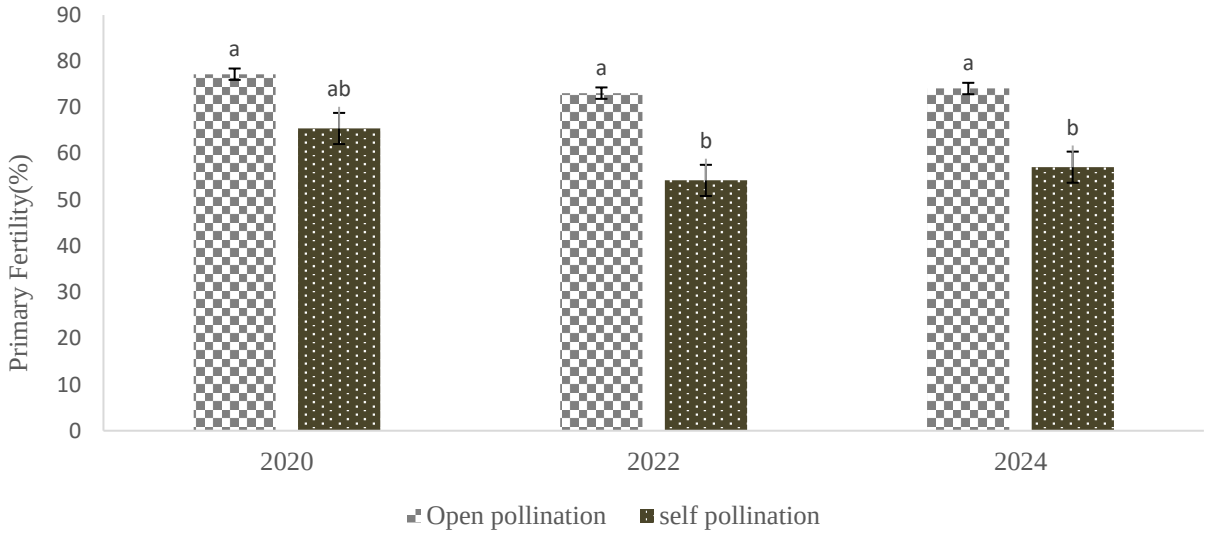



**Figure 6. Effect of open and self-pollination on primary fruit fertility in seedless barberry**

### 3.2.3. Final fruit set (FFF)

The analysis of variance results (Table 1) indicated that pollination type (open pollination vs. self-pollination) significantly impacted the FFF trait of the seedless barberry shrub at the 5% level. Also, mean comparison results (Table 2; Figure 7) revealed that the FFF percentage under open pollination was 59.33% in 2020, 56.83% in 2022, and 53.98% in 2024. These findings suggest that under open pollination, the average final fertility rate in barberry flowers ranges from 50 to 60 percent (mean, 56.71%).

Under self-pollination, the FFF was 48.33% in 2020, 43.36% in 2022, and 44.9% in 2024, resulting in an average FFF of 45.53% (range: 40–50%). Comparing the average FFF under open pollination and self-pollination revealed that open pollination increases FFF by 11.18% (range: 10-15%) compared to self-pollination. Across all experimental years, FFF under open pollination exceeded that under self-pollination, underscoring the critical role of pollinating insects in the fertility of seedless barberry shrubs. Specifically, the three-year average IFF showed a 15.89% difference between open pollination (74.79%) and self-pollination (58.90%), while the three-year average FFF exhibited an 11.18% difference between open pollination (56.71%) and self-pollination (45.53%). Also, findings from a study by Ebadi et al. (2010) reported an FFF of approximately 70% under open pollination and about 40% under self-pollination, which was consistent with the results of the current experiment.

Although theoretically, the difference between IFF and FFF in pollination treatments is expected to persist until harvest, in practice, it was observed that this difference decreases as fruit harvest approaches. The smaller numerical difference between IFF and FFF under open pollination compared to self-pollination indicates that fruit thinning (i.e., the balance between number of fruit and nutrient reserves) plays an important role in the seedless barberry commercial clone.

The difference between IFF and FFF, representing fruit drop in this clone, was 20.11% under open pollination and 13.37% under self-pollination treatment. The greater fruit drop observed under open pollination may be attributed to several factors. A primary factor is increased competition for nutrient uptake during early fruit development; the greater number of fruits formed under open pollination intensifies competition for photosynthates and nutrients, resulting in a higher rate of fruit abscission. Additionally,

hormonal imbalances caused by heterogeneous pollen grains may contribute to genetic incompatibility, further increasing fruit drop during the initial stages of fruit development.

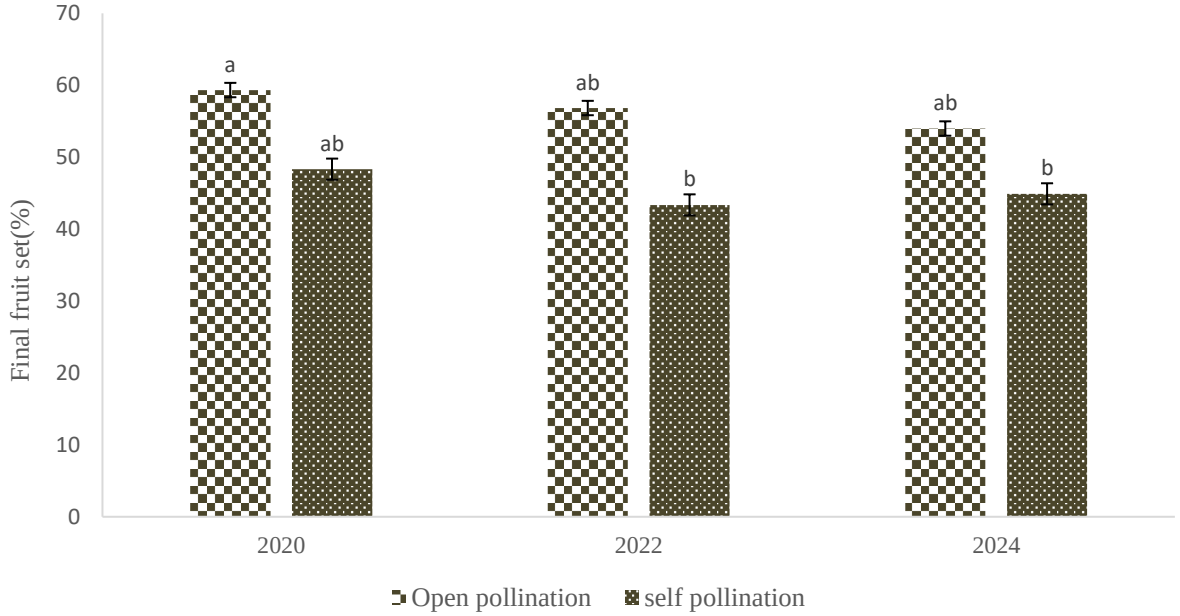



**Figure 7. Effect of open and self-pollination on final fruit fertility in seedless barberry commercial clone**

Pollination treatments confirmed that the seedless barberry commercial clone exhibits partial self-compatibility, with self-pollination achieving IFF between 50–60%, while open pollination yielded significantly higher fertility (70–80%). The 10–20% increase in fruit set due to insect-mediated cross-pollination underlines the critical role of pollinators, likely bees and flies, in maximizing reproductive success (Gómez et al., 2009; Garibaldi et al., 2013). Sahani et al. (2023) have pointed out that enhancing the activity of pollinating insects in fields and orchards can significantly increase fertility and fruit production, especially in plants that have self-fertilizing systems. Experiments showed that the contribution of self-pollination to the total pollen received by the stigma is very limited (only about 6% of the pollen transferred during a bee visit). In self-pollination treatments, the percentage of fruit formation was much lower than in open pollination, although the number of seeds per fruit was not significantly different from that in cross-pollination. Pollinators transfer pollen from other individuals to the stigma of the desired flower. Mobile stamens transferred pollen in a targeted manner on the insect body and increased the probability of pollen reaching the stigma of other flowers (Li et al., 2022). Stamens of Berberis and Mahonia are inserted between paired nectar glands, and when touched by an insect's tongue, rapidly snap forward so that their valvate anthers press pollen onto the insect's tongue or face (Li et al., 2022). In the *Berberis julianae* species, although self-pollination is possible, cross-pollination plays a major and effective role in increasing fruit formation and effective pollen transfer (Li et al., 2022). The reduction from IFF to FFF, particularly noticeable in self-pollination, suggests post-pollination mechanisms such as embryo abortion or resource limitations play an important role in determining final fruit retention. This phenomenon, known as selective fruit thinning, is well-documented in fruit crops and is believed to optimize resource allocation to viable seeds and developing fruits (Obeso, 2002). Notably, the smaller difference between IFF and FFF in open pollination treatments suggests more effective fertilization and lower embryo abortion rates, possibly due to higher genetic diversity and heterozygosity associated with cross-pollination, which enhance seed viability (Charlesworth and Willis, 2009). These findings align with the pollen quality hypothesis, which proposes that outcrossing improves seed development (Lloyd and Schoen, 1992).

Ebadi et al. (2010) reported that over 60% of seedless barberry ovules had a healthy and complete embryo sac, and more than 56.8% of pollen grains germinated on the stigma, with pollen tubes successfully reaching the ovule, but gamete fusion did not occur**.** The high percentage of pollen germination and pollen tube growth observed in the present study (50-70%), along with the relatively high fertility obtained under self-pollination (50-60%) and the fruit formation without seeds, not only supports the presence of stimulated parthenocarpy in seedless barberry but also suggests the occurrence of gametophytic incompatibility or early embryo abortion during the early stages of egg-cell development. Another study on *Berberis microphylla* in the harsh climate of Patagonia compared the effects of open pollination, self-pollination, and cross-pollination. Contrary to the initial assumption that the presence of sensitive stamens and moist stigmas indicated of self-compatibility, the results of the experiments showed that in pollination with pollen from the same plant, although the pollen germinates on the stigma, the pollen tube does not reach the ovule (Radice and Arena, 2019).

## 4. Conclusion

The present results suggest that the flowering period in the commercial clone of seedless barberry varied between 16 and 19 days, and its duration is strongly influenced by mean environmental temperature. Although temperature also affects the onset of flowering, additional factors, such as the fulfillment of chilling requirements, play a critical role. Substantial fruit set under self-pollination, as indicated by both IFF and FFF, demonstrates that the seedless barberry commercial clone is highly self-compatible and capable of producing fruit without insect pollinators. However, the consistently higher fertility observed under open pollination treatments underscores the importance of insect pollinators. Additionally, insect-mediated pollination increased both IFF and FFF by 10–20%, highlighting its potential to enhance reproductive success and fruit set in this clone. Future studies should further investigate the effects of parthenocarpy on barberry flowering and fruiting, repeat experiments across multiple years for validation, and investigate pollen tube growth using advanced laboratory methods. Such efforts will deepen understanding of the reproductive physiology of seedless barberry and support more effective crop management strategies.

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