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Research Article

The Stimulating Effect of Polyamine and Brassinolide on Sweet Cherry cv. '0900 Ziraat' Pollen

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Pollen germination,
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Prunus avium L,
Putrescin

Abstract: Sweet cherry exhibited self-incompatibility and the group incompatibility cultivar '0900 Ziraat' is known to be a self-incompatible cultivar. Fruit set and yield to realize, it is obligatory to the overlap blooming periods between the main and pollinizer cultivars, as well as to consider factors such as self and cross-pollination, environmental terms, and pollen quality. In the study, Pollen germination (PG) and pollen tube length (PTL) were evaluated after following applications (0.05, 0.25, 0.50, 2.50 mM and control) of 24-epibrassanoid (24-epiBL) and Putrescine (PUT). PG and PTL rates were significantly impressed by the application of two bioregulators (24-epiBL and PUT), and the longest PG and PTL formed in the 0.25 mM application of both bioregulators. Whereas higher doses particularly, 0.50 and 2.5 mM presented restrictive effects on both PG and PTL. In comparing two bioregulator applications on PG and PTL, a higher stimulant effect was observed at low doses in PUT, and the higher inhibition effect was monitored in 24-epiBL with 2.5 mM application. In conclusion, on PG and PTL of sweet cherry cultivar '0900 Ziraat' high concentrations (0.50 mM and 2.50 mM) of two bioregulators showed toxic effects contrary, lower concentrations (0.05 mM and 0.25 mM) have been positively efficient.

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1. Introduction

Türkiye is a leading country in cherry production, ranking first globally with an annual output of 736 791 tons (FAO, 2025). The 0900 Ziraat cultivar is the most preferred among cherry growers in Turkey due to its late ripening, high fruit quality (which includes a dark color, large size, and sweet flavor), resistance to cracking, extended shelf life, and strong export value. In cherry cultivation, the main problems are fertilization, self-incompatibility, group incompatibility, and double fruit formation (Sarısu et al., 2014). Successful fertilization is ever related to cultivar genetic properties and climatic variations (Calic and Radojevic 2017, Muradoğlu et al. 2017, Aronne et al., 2021). The synchronized blooming of main and pollinizer cultivars, their compatibility status, rainfall patterns, chilling requirements, spring frost events, winter damage, and pollination success are crucial factors for preventing fruit drop (Engin and Ünal, 2002; Bekefi 2004; Cittadini et al., 2006; Beyhan and Karakaş, 2009). The 0900 Ziraat cherry variety faces several yield-related issues, with self-incompatibility being the most significant factor contributing to low yields, along with late flowering, pollination, and fertilization (Özçağıran, 1977). The effective fruit sets often require cross-pollination, which depends on the availability of pollinizers and useful pollen grains. Therefore, pollen is the key event in sexual fruit species acquiring adequate harvest. For effective pollination and fertilization, it is essential to have viable and compatible pollen sources and grains, the pollen tube growth within the style, the quantity of pollen grains, and the timely transfer of sufficient pollen onto the stigma (Thompson, 2004; Alcaraz and Hormaza, 2021; Jain et al., 2023). The studies were conducted to identify pollen quality (germination and tube length etc.) from various plant species and used basic mediums containing boric acid, some mineral salts, or plant growth regulators by the researcher (Tosun and Koyuncu, 2007; Sütyemez, 2011; Muradoğlu et al., 2024). In recent years studies have presented that PG and PTL were seriously stimulated or inhibited by plant growth regulators (Tosun and Koyuncu, 2007; Muradoğlu et al., 2010 and 2024). One of the important plant growth regulators used for agricultural production is BRs and PAs groups. BRs, whose important forms are 24-epibrassinolide and 28-homobrassinolide are polyhydroxy steroidal phytohormones. Moreover, polyamines (PAs) have especially been known as putrescine and spermine. Brassinosteroids (BRs) and polyamines (PAs) are crucial phytohormones that rapidly assimilate and metabolize, occur in all plant organs, and affect plant growth, development, involved in a diverse arrangement of biological processes, flowering, growth of reproductive regulate reproductive growth and promote pollen and tube growth (Hewitt et al., 1985; Singh and Shono, 2003; Kang and Guo, 2011; Sotomayor et al., 2012; Romero et al., 2018; Muradoğlu et al., 2024). Recent studies have focused on understanding the mechanisms behind plant bioregulators and have been observed to significantly enhance various biological processes in plants. The application of exogenous brassinolide has been reported to improve anther and pollen development, as well as pollen tube germination and growth by Vogler et al., (2014), Maita and Sotomayor (2015), and Muradoğlu et al., (2024). However, there is a lack of sufficient reports regarding the effects of 24-epibrassinolide (BRs) and putrescine (PAs) on pollen grain (PG) and pollen tube length (PTL) in sweet cherries. We aim to investigate the pollen viability, germination rate, and tube length of the "0900 Ziraat" cultivar. Additionally, we will study the effects of exogenously applied 24-epibrassinolide and putrescine, and the influence of concentrations on pollen germination and tube length. This information is valuable for optimizing the exogenous use of 24-epibrassinolide and putrescine doses to enhance pollination and fruit sets in sweet cherry.

2. Material and Methods

The cultivar of ‘0900 Ziraat’ was growing in a sweet cherry growers’ orchard grafted on 'Idris' rootstock with 4x4 planting, approximately sixteen years located in Seben district, Bolu province constitutes the study material. Appromaxially a hundred flower buds were harvested at the balloon period on different positions of branches and were kept in a paper envelope and as soon as possible times transported to the laboratory. Flower buds were kept at room temperature (25 C and 24 hours) until the anthers opened and scattered pollens. Viable pollens rates were calculated using a 1.0% TTC (2-3-5-triphenyl tetrazolium chloride) test reported by (Eti, 1991) and they were categorized by their color such as dark red as "viable," pink as "semi-viable," and colorless or dark-colored as "non-viable". Also, pollen viability rating was calculated following the formula as %= viable pollen+ semi-viable pollen/2. The liquid culture medium (15% sakkaroz and 0.01 boric acid) was prepared according to Brewbaker and Kwack (1963). The experiment utilized three slides, with two designated areas on each slide, firstly, 50 µl liquid culture medium, then 50 µl 24 epiBL or PUT solutions onto slides. 24 epiBL or PUT solutions (0.05, 0.25, 0.50, and 2.50 mM) were prepared and distilled water (50 µl) was used as control. Pollens were spread out onto medium with the hanging drop method on the slide helping a needle under a light microscope. Following, the slides were kept in Petri dishes on moist glass rods in a dark medium (22±2 °C, 3 hours). After this duration, 10 % ethanol was dropped onto the slide and closed with a coverslip, and PG percentage and PTL were evaluated under a light microscope (Shivanna and Rangaswamy, 1992).

2.1. Statistical Analysis

The average data were analyzed using SPSS 23.0 statistical software, with results presented as means. A one-way ANOVA and Duncan's multiple range test were applied to compare the applications, with a statistical significance set at 5%. Pollen viability, PG, and PTL were measured in triplicate, with 50 measurements per replicate.

3. Results

Viable and non-viable pollen ratios obtained from the TTC test performed are presented in Figure 1. Pollen viability of '0900 Ziraat cv' was observed at 48.3%, the total viable (30.4%) and semi-viable (66.2%). Moreover, non-viable pollen rations also were observed at meager rates of 3.4%.

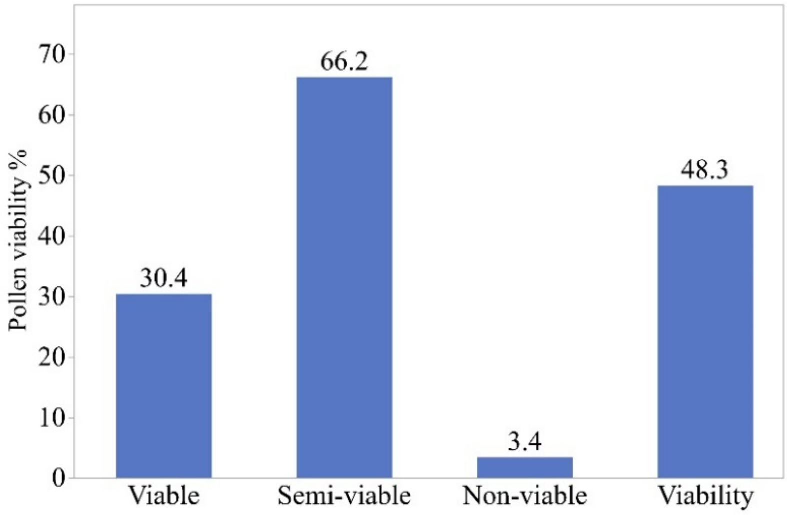

Figure 1. The percent pollen viability using the TTC test.

The pollen germination rates were counted to examine the effects of 24-epiBL and PUT after applications. The total PG significantly increased by 33.76-30.27%, 39.97- 66.92%, and 1.15-39.69% using 0.05, 0.25, and 0.50 mM 24-epiBL and PUT compared with the control respectively. On the contrary, significant declines were observed in the use dose of 2.50 mM 24-epiBL (28.15%) and PUT (13.50%). The highest PG rate was observed with 0.25 mM applications of two biostimulators compared to control and other applications. A serious decline was observed in the PG rate after 0.25 mM application compared with the control (Figure 2).

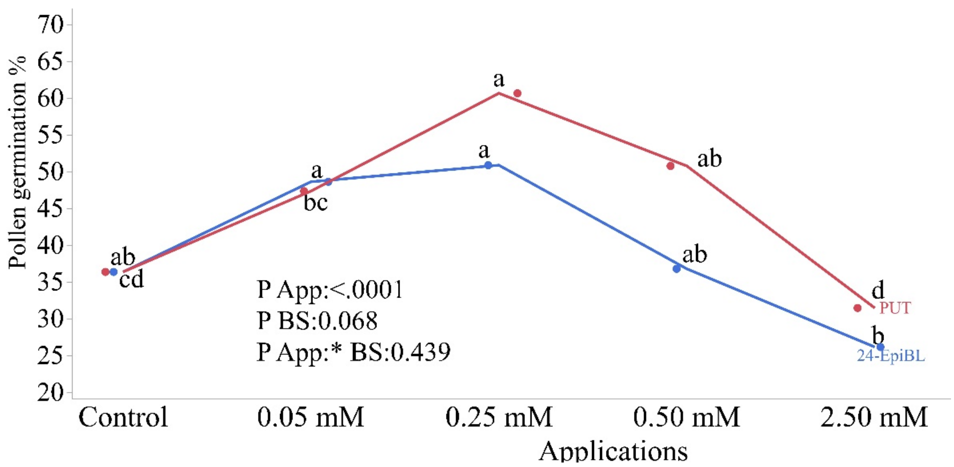

Figure 2. Effects of 24-epiBL and PUT applications on the pollen germination of sweet cherry '0900 Ziraat cv'. App. Applications, BS: biostimulators (24-epiBL and PUT).

The PTL was measured to examine the effects of PUT and 24-epiBL after applications. When, compared with the control, the PTL was promoted by 18.78% and 24.71% in 24-epİBL applications (0.05 mM and 0.25 mM) respectively. In addition, PUT applications (0.05 mM, 0.25 mM, and 0.50 mM) also raised PTL by 24.76%, 31.56%, and 18,11%. Conversely, in PTL significant inhibition was observed by 23.42% and 46.51% in high 24-epiBLapplications (0.50 mM) compared with the control. In PTL the highest inhibitions were executed by 46.51% and 5.99% with 2.50 mM application in both 24-epiBL and PUT (Figure 3). PTL showed differences depending on the used biostimulants and applications and the highest inhibitions were observed 24-epiBL in applications compared to PUT applications.

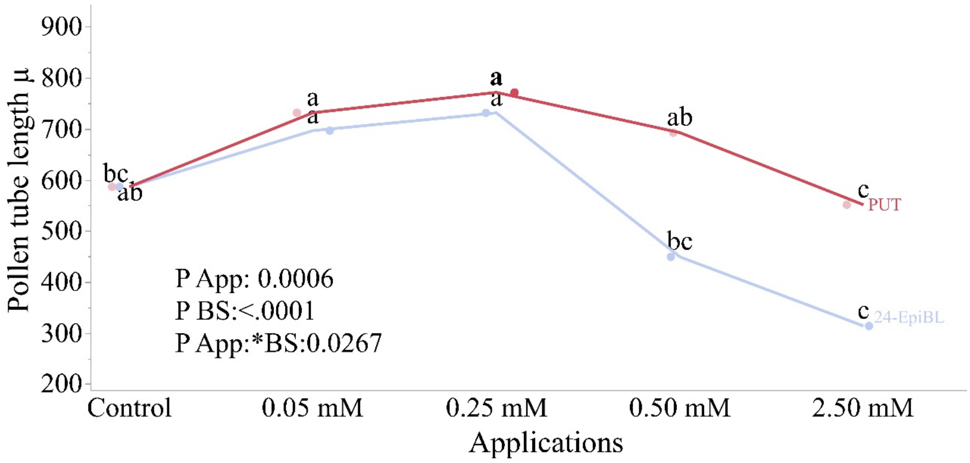

Figure 3. Effects of 24-epiBL and PUT applications on pollen tube length of sweet cherry '0900 ziraat cv'. App. Applications, BS: biostimulators (24-epiBL and PUT).

4. Discussion

Fruit set in plants significantly relies on pollen quality, which is defined not only by pollen viability, but also by the flowering biology of plants, pollen number, pollen germination capacity, and pollen tube length (Bykova et al., 2012; Cerovi´c et al., 2014). In our data, the '0900 Ziraat' cultivar pollens exhibited performance of exceptional pollen viable quality (30.4% viable and 66.2% sem-viable) and nominal level non-viable (3.4%) using 15% sucrose concentration. Similar results have been reported in sweet cherry cultivars by Pal et al. (2015) between 32 to 80%, by Beyhan and Karakaş (2009) between 66.38 to 96.31, by Tosun and Koyuncu (2007) between 79% to 93%, and by Sütyemez (2011) between 78 to 85%. Differences in pollen viability rates are thought due to differences in the variety, sucrose content, and method used.

The new approaches for enhancing plant pollen quality occur in the exogenous application of different plant regulations. Previously research concerning plant hormones showed that they respond to stimulating or inhibiting pollen germination and tube length in many plants. Hewitt et al. (1985) reported pollen germination was stimulated in *Prunus avium and Prunus armeniaca* by auxin and gibberellic. Similar Arabidopsis pollens were stimulated with brassinosteroid (Vogler et al., 2014). Contrary, exogenous jasmonic acid (0.5mM) decreased pollen germination in strawberries (Yıldız and Yilmaz, 2002), methyl jasmonate (0.5 mM and higher) inhibited PG and PTL in Prunus armeniaca (Muradoğlu et al., 2010). Reporting showed increasing concentrations of GA3 reduced pollen germination in many fruits such as peach (Xue et al., 2008), male pistachio flowers (Acar et al., 2010), and kiwifruit Qi et al. (2010). According to Aloisi et al. (2016) and Çetinbaş-Genç (2020), pollen germination and tube length stimulation or inhibition depend on plant regulation types and used doses.

Researchers reported BRs (brassanoid) and PAs (putrescine and spermine) effective on PG and PT elongation that was improved by exogenous application (Vogler et al., 2014; Aloisi et al., 2016; Benko et al., 2020). Matsuura-Tokita et al. (2024) reported that brassinosteroids (BRs) play a significant role as both male and female reproductive hormones throughout plant fertilization. Ye et al. (2010) and Jia et al. (2020) declared that BRs signaling is important in anther/pollen and ovule development. BRs influence on enhancing PG and PTL has also been previously reported in sweet cherry and *Camelia* *japonica* by Hewitt et al. (1985), tomato by Singh and Shono (2003), Arabidopsis by Vogler et al., (2014), almond by Maita and Sotomayor (2015), and *Cydonia oblanga* (Muradoğlu et al., 2024).

Our results showed that 24 epiBL and PUT had a distinct effect on PG and PTL and the stimulation effect of PG and PTL in sweet cherry depended on the use of 24-epiBL and PUT doses. The optimum stimulation on PG and PTL of' Prunus avium ‘0900 ziraat cultivar' appeared at 0.25 mM of 24-epiBL and PUT. PG and PTL showed an encouragement effect when supplemented with 0.05 mM and 0.25 mM of 24-epiBL. Besides Specifically, from 0.05 mM to 0.50 mM PUT applications on PG and PTL levels increased and the most significant increase was monitored with the 0.50 mM application. On the contrary, high concentrations of 24-epiBL and PUT (2.50 mM) applications proved crucial inhibitory effects on PG and PTL. Similar results have been reported treatments with exogenous spermidine influence PT elongation across various species, including *Malus domestica* (Del Duca et al., 1997), *Lycopersicon esculentum* (Song et al., 1999), *Prunus mume* (Wolukau et al., 2004), *Prunus dulcis* (Sorkheh et al., 2011), *Camellia sinensis* (Çetinbaş-Genç et al., 2020), *Citrus* (Karabıyık, 2024) and *olea europea* (Dölek and Karabıyık, 2024). Yin et al. (2019), declared that even at very low doses, BRs can impact various physiological processes in plants. Karabıyık, (2024) declared high concentrations (0.1 mM) of putrescine inhibited pollen germination in *citrus lemon*. It was also noted excessive concentrations of polyamines inhibited pollen germination as concentrations lower and higher than optimal were less effective and effects largely depend on different factors such as plant species, growth stage, interactions with other hormones, and dosage.

Conclusion

Our findings revealed pollen germination and elongation of tubes in sweet cherry '0900 Ziraat cv' were notably enhanced when exogenous putrescine and 24-epibrassinolide were added. Specifically, 0.50 mM application of two bioregulators (24-epiBL and PUT) had a more stimulating effect on PG and PTL than other concentrations. However, higher concentrations (2.5 mM) of both bioregulators inhibited the PG and PTL. Notably, PUT applications have more pronounced stimulation on PG and PTL than 24-epiBL applications were observed. These observations, present a new light on understanding the mechanisms of PUT and 24-epiBL applications on the inhibition or stimulation of PG and PTL in '0900 Ziraat' cultivars of sweet cherry.

Ethical Statement

Ethical approval is not required for this study.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

Conceptualization: F.M.; Ş.B.; H.K. Investigation: F.M., Ş.B., M.H. Methodology: F.M.; M.H. Formal analysis: F.M.; Ş.B., M.H. Software: F.M.; Visualization: F.M. Writing–original draft: F.M., Ş.B.; H.K.; G.P.; M.H. Writing–reviewand edit: F.M., Ş.B., H.K.; G.P.; M.H.

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