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ORIGINAL ARTICLE

Alleviation of Cadmium Stress on Pollens of Quince Varieties Through Epibrassinolide

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Abstract

Environmental factors significantly impact plant physiological processes, especially during pollination and fertilization. Pollen germination (PG) and pollen tube length (PTL), crucial in plant reproduction, are particularly vulnerable to environmental contaminants that affect fruit sets adversely. This study investigated the impact of varying cadmium (Cd) doses, a significant toxic heavy metal, and the alleviative potential of external 24-epibrassinolide (24-epiBL) applications on cadmium stress, determined through PG and PTL. Quince pollen viability rates, assessed using the TTC (2-3-5-triphenyl tetrazolium chloride) viability test, varied between 38.58% and 55.80%. PG and PTL rates decreased in response to higher Cd doses. The application of epiBL increased PG and PTL, but a notable decline was observed at the 2.00 µM application. A positive relationship between the application dosage of epiBL and its alleviation effect on Cd stress on PG and PTL was observed except for the 2.00 mM. Results revealed variability in PG and PTL rates among quince varieties, and PG and PTL rates decreased with higher Cd doses. This study suggests that epiBL up to 0.50 mM is an efficient tool for alleviating Cd stress in quince pollens.

Keywords 24-Epibrassinolide · Heavy metal · *Cydonia vulgaris* · Plant bioregulators · Pollen germination

Introduction

Quince, scientifically known as *Cydonia oblonga* Mill. (syn. *Cydonia vulgaris* Pers.), is one of the ancient fruit species in the *Rosaceae* family, subfamily *Maloideae* (*Pomoidae*) and has been cultivated for over 4000 years. There are approximately 1000 species and 30 genera, including *Cydonia* (Bell and Leitao 2011; Sykes 1972). Quince is acknowledged for its diverse therapeutic effects, encompassing anti-inflammatory, antioxidant, antimicrobial, anticancer, and anti-ulcerative properties (Isra and Maha 2022). The foremost key challenge in quince cultivation face relates to flower and fruit drop during initial growth stages, leading to reduced economic efficiency. Factors such as water availability and quality, pruning, soil condition, nutrition, and environmental factors before and after flowering significantly influence flower and fruit shedding, ultimately impacting negatively yield. Besides these factors, the primary determinants influencing fruit yield are pollination and fertilization which are attributed to flower incompatibility or inadequate pollination and reasons for fruit drop in quince (Boskovic and Tobutt 2001; Ortega and Dicenta 2004; Balta and Muradoğlu 2006).

Quince is commonly characterized as self-compatible in pollination in references (Maniei 1995). Nevertheless, quince varieties are categorized into four groups in terms of pollination: self-compatible varieties with more than 10% fruit set, semi-compatible varieties with 3–8% fruit set, and self-incompatible varieties displaying a 1–2% fruit set. However, entirely incompatible variations exhibit a fruit set of less than 1% (Nagy-Deri et al. 2013). According to this classification, self-incompatible and semi-compatible varieties necessitate companion pollinator varieties to achieve proper fruit sets. The male reproductive organ responsible

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for pollination and fertilization plays a crucial role in various studies encompassing morphological, physiological, biotechnological, ecological, biochemical, and molecular genetic investigations (Dane et al. 2004). Hence, assessing pollen viability is integral in fruit breeding. Successful fertilization requires a high percentage of pollen germination and rapid tube growth. Insufficient pollen germination rates and limited tube length may result in reduced fruit formation (Sharafi 2011). Pollen germination is influenced by various factors such as heavy metals (Muradoğlu et al. 2017), temperature (Jackson and Linskens 1982; Petropoulou and Alston 1998), pollinator, anther number, pollen quantity, nutrient levels, and plant variations (Albuquerque Jr. et al. 2010). Additionally, growth regulators have been identified as positive factors impacting pollen germination (Qiu et al. 2005). Studies have indicated that externally applied plant hormones can alter plant physiological and biochemical properties, alleviating stress caused by toxic metals. In response to diverse stresses, plants utilize a myriad of signaling molecules, including hormones, to mediate their reactions (Zhao et al. 2019). Phytohormones have been extensively studied as stimulants for plant growth and development and declared to ensure vigorous plant life and play a crucial role in resisting multiple stresses (Verma et al. 2016). One of the recognized phytohormone groups is brassinosteroids (BRs), which represent a recognized group of phytohormones derived from polyhydroxy steroids. BRs are an imperative group of plant hormones that elaborate in regulating the growth and development of plants, aiding plants in adapting to their environment, maintaining equilibrium, and enabling adaptation to environmental fluctuations throughout their life cycle. They provide tolerance to various stresses by facilitating cell elongation and root division, xylem differentiation, enzyme activity, photosynthesis rate regulation, fruit development, and adaptation to salinity and heavy metal stress (Sharma et al. 2017). Moreover, they regulate the numerous growth and development processes of plants. BRs crosstalk with diverse hormones to standardize plant development and physiology (Li et al. 2021). Research on the alleviation of the adverse impacts of cadmium (Cd) on the germination of pollen in fruit species is insufficient. While it is recognized that plant hormones respond to stress conditions in various plants, no investigations focusing on pollen germination and tube elongation of quince under cadmium-induced stress have been identified.

This study aimed to explore the detrimental effects of cadmium on pollen germination and tube elongation in three quince varieties and one genotype. Additionally, it sought to ascertain the impact of externally applied varying doses of 24-epiBL on pollen germination and tube elongation, as well as to evaluate tolerance level to cadmium-induced stress.

Materials and Methods

Materials

The plant material of the study was comprised of quince varieties 'Eşme' variety grafted on quince seedling rootstock, approximately 10 years old, 'Ege 22' variety grafted on Quince A rootstock, 'Ege 25' variety grafted on BA 29 rootstock, and one genotype grown from seed obtained from a producer orchard in Sakarya province, Turkey.

Methods

Pollen to be used in the present studies was gathered from buds at the balloon stage (before the petals opened) on branches of different directions and heights of the determined trees. The petals of the flower buds were retained at room temperature for 24 h before opening, and the pollen viability rates of the varieties were determined by the TTC (2-3-5-triphenyltetrazolium chloride) viability test ascertained by Eti (1991). Pollen assessments were conducted under a light microscope (Olympus CX23), categorizing dark red pollen as "viable," pink as "semi-viable," and colorless or dark-colored as "non-viable" (Fig. 1).

Pollen germination culture medium was prepared according to Brewbaker and Kwack (1963). Three slides, culture media, and heavy metal solutions were utilized to determine the germination rates and tube length of pollen. As the heavy metal solution, 50, 100, 250, 300, 400, and 500 µM were used for cadmium ($3CdSo_4*8\ H_2O$), whereas 0.05, 0.25, 0.5, and 2.5 mM were used for 24-epiBL (HytoTech labs, Kansas, USA). Furthermore, combinations of 300 µM Cd and epiBL doses were used. First, 50 µl of culture medium was dropped onto the slide, and then 50 µl of Cd solution or epiBL dose was applied. Pure water was used in the control group. Pollen was dispersed onto the culture and heavy metal medium on the slide using a needle under

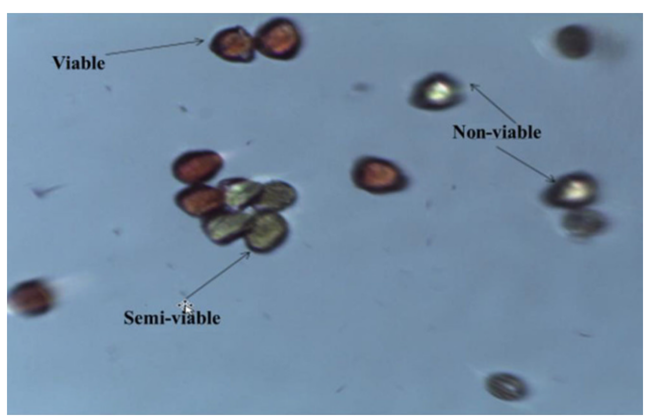

Fig. 1 Viability status of 2-3-5-triphenyltetrazolium chloride-treated pollen

a light microscope. Subsequently, these slides were retained in Petri dishes on moist glass rods in a dark environment at 22 ± 2 °C for 3 h. At the end of this period, a few drops of 10% ethanol were dropped onto the slide and the coverslip was secured. Obligatory measurements of pollen germination percentage and pollen tube lengths were prepared using an ocular micrometer under a light microscope (Shivanna and Rangaswamy 1992). The study was planned according to the randomized parcel trial design. Pollen viability, germination, and tube length measurements were accomplished in triplicate were made in each repetition. SPPS (22.0) package program was used in the statistical analysis of the data attained.

Results and Discussion

The notable statistical variances were determined in the pollen viability levels of the varieties and genotypes to which the TTC solution was applied. The pollen germination, semi-viability and non-viability rates were much higher in the genotype compared to 'Ege 22' and 'Ege 25' varieties, respectively (Fig. 2). The vitality rates of pollen are on a variety basis: in the genotype, the living pollen rate is 55.80%, the semi-viable rate is 41.93% and the non-viable pollen rate is 2.27%. In the 'Eşme' variety, the viable pollen rate is 38.58%, the semi-viable proportion is 47.11%, and the non-viable pollen rate is 14.31%. In the 'Ege 22' variety, the viable pollen rate was 40.11%, the semi-viable percentage was 48.32% and the non-viable pollen proportion was 11.56%, and in the 'Ege 25' variety, the viable pollen percentage was 43.00%, the semi-viable rate was 36.08% and the non-viable pollen proportion was 20.92%.

In a parallel study accompanied by the determination of quince pollen viability using the TTC method and 15% sugar medium, pollen germination in lemon and quince varieties showed fluctuation conferring to the collection period. Pollen germination in the early balloon, late balloon period, 1st day, 2nd day, and 3rd day, as 82.9%, 84.0%, 60.6%, 71.7%, and 40.0% for the lemon variety and as 74.3%, 90.2%, 91.5%, 68.0%, and 38.8% for the 'Eşme' variety were determined, respectively by Öztürk Erdem and Çekiç (2016). In another study conducted on seven quince varieties using the IKI (iodine potassium iodide) method, pollen viability rates were declared to be between 90.8 and 92.1% (Dalkılıç and Mestav 2011). Pollen germination and tube growth have a substantial influence on reproduction in plants, and a high pollen germination rate and rapid tube growth are substantial for successful fertilization. Low pollen germination and short tube length may cause deprived fertilization and low fruit formation (Wu et al. 2008; Sharafi 2011). In control, revealed pollen germination and tube lengths for 'Eşme' showed 40.67% and 551.35 µ, 'Ege 22' exhibited 53.21% and 463.31 µ, 'Ege 25' variety displayed 50.32% and 551.59 µ, and the genotype showed 67.50% and 580.33 µ (refer to Fig. 2). The outcomes of this experiment were aligned with previous studies on quince pollen germination. In a study involving fifteen quince varieties at a 15% sugar concentration, pollen germination rates ranged from 18.90% to 89.80% (Çetin and Soylu 2006). Similarly, Radovic et al. (2020) found, using an in vitro method with eight quince varieties, pollen germination rates ranging from 64.62% to 86.43%. Discrepancies between these studies and the present results can be attributed to varying techniques in pollen germination assessment, sugar concentrations, variety differences, and the timing of pollen collection.

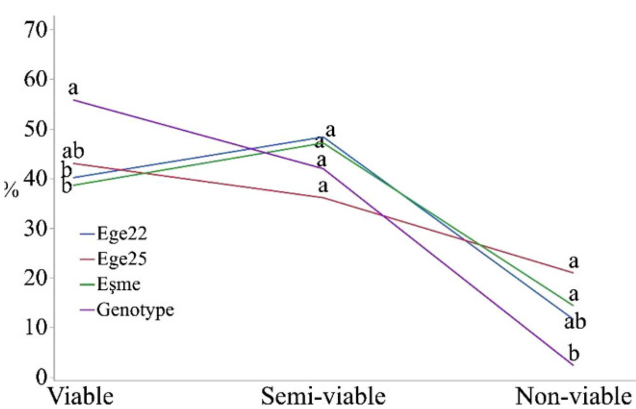

Fig. 2 Pollen viability rates of 2-3-5-triphenyltetrazolium chloride-treated varieties and genotype. Different letters on top of the treatments indicate significant differences at $p \leq 0.05$ in the LSD test

Cd has been reported to hinder growth, reduce the content of photosynthetic pigments, lower the activities of antioxidant enzymes, and elevate levels of malondialdehyde and reactive oxygen species (ROS) (Muradoğlu et al. 2015; Ali et al. 2014). Furthermore, it negatively impacts plant reproduction by inhibiting pollen grain and ovule growth, resulting in the development of inviable flowers and shriveled grains (Sabrine et al. 2010; Wang et al. 2014; Muradoğlu et al. 2017). Consistent with prior studies, our findings demonstrate a decrease in PG and pollen tube length (PTL) of quince pollen corresponding to increasing Cd doses (Fig. 3). The decline rates for PG and PTL ranged from 5.75–26.76% to 96.53–99.78% in the 'Eşme', from 8.53–8.64% to 85.08–85.06% in the 'Ege 22', from 5.66–28.78% to 89.07–97.90% in the 'Ege 25' variety, and from 9.73–19.58% to 85.33–93.98% in the genotype response to Cd applications, respectively (Fig. 3). In alignment with these outcomes, research has indicated the detrimental effects of Cd on both the vegetative and generative organs of plants.

Toxic substances are a widespread concern, and a substantial amount of literature is delving into the impact of heavy metals on plants. One potential method to mitigate

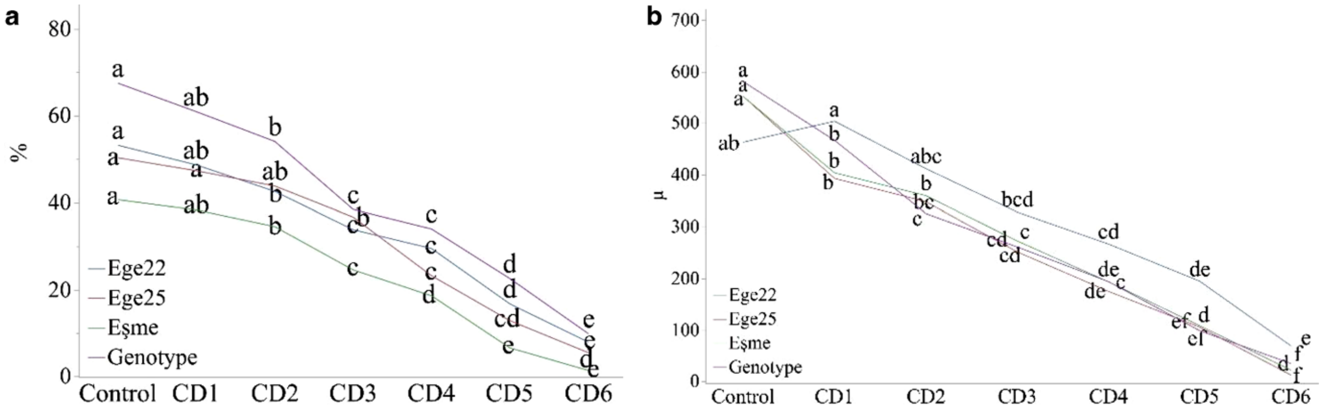

Fig. 3 Effects of cadmium doses on pollen germination (**a**) and tube length (**b**). *CD:* Cadmium, *CD1:* 50 µM, *CD2:* 100 µM, *CD3:* 250 µM, *CD4:* 300 µM, *CD5:* 400 µM, *CD6:* 500 µM cadmium. Different letters on top of the treatments indicate significant differences at $p \leq 0.05$ in the LSD test

the adverse effects of toxic metals on plant growth involves the application of certain plant growth regulators known as BRSs (Brassinostereoids). Despite BRSs' capacity to ameliorate stress conditions, there is quite limited literature concerning their effects on pollen. In this study, raising doses of 24-epiBL applications was observed to positively correlate with pollen germination and tube length. Conversely, keen reductions were observed in high-dose applications compared to the control (Fig. 4). The application of 24-epiBL at 0.50 µM augmented pollen germination and tube length rates by 21.12–23.58% for 'Eşme,' 31.59–35.38% for 'Ege 22,' 17.77–19.80% for 'Ege 25,' and 18.90–22.07% for the genotype. Conversely, high-dose 24-epiBL (2.00 µM) application led to reduced pollen germination and tube length by 20.45–35.09% in 'Eşme,' 13.02–31.44% in 'Ege 22,' 30.54–48.54% in 'Ege 25,' and 30.54–48.54% in the genotype compared to the control. Notably, the 24-epiBL application enhanced PG and PTL up to the rate of 0.50 µM concentration, after this concentration a substantial decline was monitored. Among varieties and genotype, the 'Ege 25' variety exhibited the lowest pollen germination and tube length in response to high (2.00 µM) 24-epiBL application (Fig. 4). In recent times, similar studies have been performed by Hewitt et al. (1985) on *Prunus avium* and *Camellia japonica*, by Sotomayor et al. (2012) on *Prunus dulcis*, Singh and Shono (2005) on tomato and Thussagunpanit et al. (2012) on rice and who declared brassinoids to increase the germination and development of pollen. In another result related to our study outcomes, it was quantified that the addition of 10 µm epiBL to the pollen germination medium in *Arabidopsis* plants significantly increased in vitro pollen germination and growth; in contrast, epiBL concentrations above 20 µm were declared to have an inhibitory effect by Vogler et al. (2014).

Cadmium is one of the heavy metals well-known to cause toxicity even at very low concentrations. In recent years, various strategies and technologies have been established to tolerate stress in cadmium-stressed plants, among which the application of plant hormones has emerged as the most effective and least detrimental approach. Specifically, significant attention has been directed toward BRs, known for their role in enabling plants to withstand abiotic stresses like heavy metals (Allagulova et al. 2015; Ramakrishna and Rao 2015). BRs have been instrumental in regulating essential physiological processes such as cell elongation, division, ATP activity, and safeguarding against the loss of photosynthetic pigments. They have demonstrated a pos-

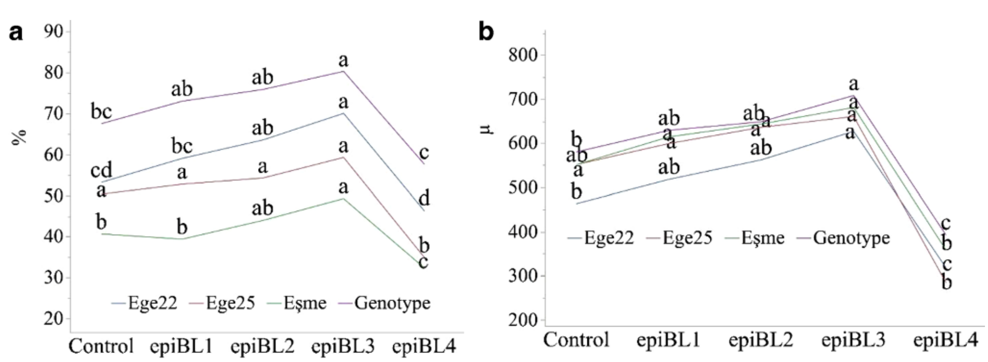

Fig. 4 Effects of epibrassinolide doses on pollen germination (**a**) and tube length (**b**). *epiBL* 24-Epibrassinolide, *epiBL1* 0.05 mM, *epiBL2* 0.25 mM, *epiBL3* 0.5 mM, and *epiBL4* 2.00 mM. Different letters on top of the treatments indicate significant differences at $p \leq 0.05$ in the LSD test

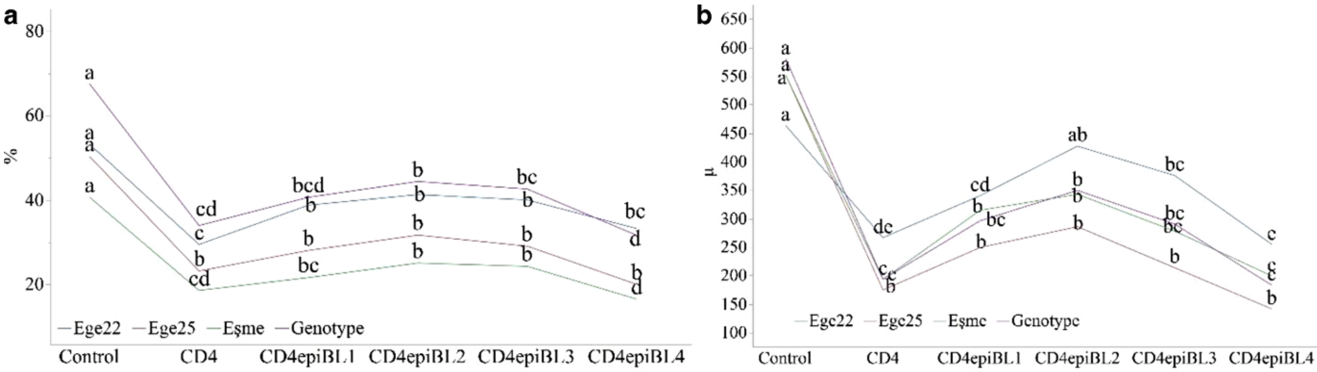

Fig. 5 Effects of cadmium and epibrassinolide doses on pollen germination (**a**) and tube length. (**b**). *CD4* 300 µM cadmium, *CDepiBL1* 300 µM cadmium+ 0.05 mM epibrassinolide, *CDepiBL2* 300 µM cadmium+ 0.25 mM epibrassinolide, *CDepiBL3* 300 µM cadmium+ 0.5 mM epibrassinolide, and *CDepiBL4* 300 µM cadmium+ 2.00 mM epibrassinolid. Different letters on top of the treatments indicate significant differences at $p \leq 0.05$ in the LSD test

itive contribution to growth even in challenging environmental conditions (Sasse 1997; Musing 2005; Hasan et al. 2011; Hayat et al. 2012). The results showed that the impact of 300 µM Cd application notably decreased PG and PTL compared to the control (Fig. 3). Particularly, the 'Eşme' variety displayed the highest sensitivity with a decrease ranging from 54.26% to 64.96%, followed by 'Ege 22' with a decrease of 44.58–42.49%, 'Ege 25' with a decline of 53.86–68.66%, and the genotype exhibiting a decrease of 49.66–66.63%. Conversely, under 300 µM Cd stress, the external application of epiBL exhibited a positive influence on PG and PTL in a dose-dependent manner. However, a substantial decline was noted after the application of 2.0 mM epiBL compared to the control (Fig. 5).

The highest PG and PTL in the 'Ege 22' variety with epiBL (0.05, 0.25 and 0.50 mM) applications under 300 µM Cd stress improved as 31.57–27.69%, 40.08–60.26% and 35.77–40.92%, respectively. But a 12.85% increase in PG and 4.52% decline in PTL was noticed with 2.0 24-epiBL application, while the lowest increased 16.39–62.84%, 34.83–77.36% and 30.75–43.85% with 24-epiBL applications for the 'Eşme' variety, respectively. In contrast, a 10.97% and 3.08% decline was observed in PG and PTL with 2.0 24-epiBL application. Studies indicate that the effects of BRs largely depend on various factors, including dose, plant species, growth stage, environmental conditions, types, and duration of stress, as well as their interaction with other hormones, growth regulators, and signaling molecules (Nolan et al. 2019; Yin et al. 2019). The optimal concentration range of BRs for agricultural crop production has been indicated to fall between 5 to 50 mg per hectare, as suggested by Khripach (2000). Similar findings have been reported where predominantly, at a very low dose (nM to mM), BRs can distress different plant physiological processes; however, plant responses might vary even within a narrow concentration range. For instance, in cucumber plants, a high BR concentration (0.2–1.0 µM 24-epibrassinolide, EBR, a bioactive BR) suppressed CO_2 assimilation capacity, while a moderate concentration (0.1–0.15 µM EBR) has been reported to enhance photosynthesis (Jiang et al. 2012). Similar findings on the impact of BRs in alleviating heavy metal stress align with our study. In a study accompanied by Gokbakır and Engin (2017) on the 'Kardinal' grape variety, it was observed that while the pollen germination rate of the variety was 26.08% in the control group, a low external epiBL application increased this rate to 44.40%. Conversely, higher applications (0.1 mg L-1 homobrassinolide) led to a 17.85% decline in pollen germination. Previous research has demonstrated that 24-epiBL applications significantly reduce the uptake of heavy metals in barley, sugar beet, tomato, and radish (Khripach et al. 1996). Additionally, exogenous applications of 10-6–10-4 M 24-epiBL prevented metal accumulation in algal cells under heavy metal stress (Bajguz 2000). Moreover, low doses of BR (0.1 µM EBR) facilitated stomatal opening, while higher doses (1.0 µM EBR) prompted stomatal closure in plants (Xia et al. 2014). These findings suggest that plant responses to BR concentrations are contingent upon the specific application method, plant species, growth stage, and growth conditions (Ahammed et al. 2014).

A two-way clustering analysis employing dendrograms was utilized to examine heatmaps and visually represent the data in the study (Fig. 6). These dendrograms organize the different applications utilized in our study (control, Cd1, Cd2, Cd3, Cd4, Cd5, Cd6, epiBL1, epiBL2, epiBL3, epiBL4, CD4+epiBL1, CD4+epiBL2, CD4+epiBL3, and CD4+epiBL4), as well as the studied parameters (pollen germination and tube length), based on their similarities. The lateral dendrogram illustrates a similarity between the control and the epiBLs treatment. Both the control and the epiBL1, and epiBL2 treatments are grouped in a cluster along with the epiBL3 treatment across all quince varieties. The heatmap indicates that these treatments predominantly had positive effects on pollen germination and pollen tube

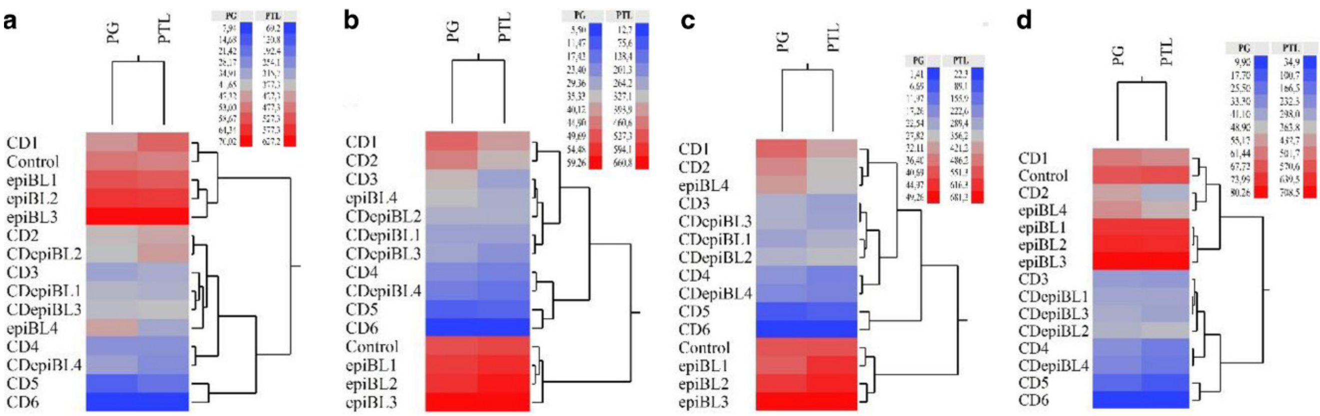

Fig. 6 Heatmap analysis based on the average values of pollen germination and tube length was studied for the three quince varieties (**a** 'Ege 22', **b** 'Ege 25', **c** 'Eşme') and one genotype (**d**). The *blue* to *red* color scale indicates values from low to high, respectively

length. Another cluster comprises treatments: Cd1, Cd2, Cd3, Cd4, Cd5, and Cd6, which exhibited adverse effects compared to the control. The treatment cluster comprising CD4+epiBL1, CD4+epiBL2, and CD4+epiBL3 demonstrated a mitigating effect on cadmium, while Cd4epiBL4 showed negative effects on pollen germination and tube length compared to cadmium applications at 300 µM. The color representation distinctly showcases an increase in pollen germination and tube length with epiBL treatment, except for epiBL4, and a decrease with cadmium treatments compared to the control. It is evident that a significant relationship exists between treatments (epibrassonoid and cadmium), treatment dosage, and pollen germination and tube length.

Conclusion

This study focused on the influence of Cd and epiBL applications and their combined impact on pollen germination and pollen tube length in quince varieties and a genotype. The rates of pollen germination and tube length exhibited fluctuations across different quince varieties. Cd had a pronounced negative impact, while epiBL demonstrated a notably positive effect, especially up to a 0.50 mM application. Furthermore, when cadmium and epiBL applications were combined, epiBL showcased a significant alleviating effect on Cd stress on pollen germination and tube length, depending on the dosage. This study suggests doses of epiBL up to 0.50 mM as an efficient tool for the alleviation of Cd stress in quince pollens.

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Conflict of interest The authors declare that they have no competing interests regarding the publication of this paper.

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