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Brassinosteroids and gibberellic acid: effects on *in vitro* pollen germination in grapevine

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Abstract

Many physiological processes related to plant growth and development are under the influence of growth regulators, which also have an impact on pollen germination. In this study, the effects of two brassinosteroid compounds, epibrassinolide and 22S,23S-homobrassinolide, and gibberellic acid (GA_3) on *in vitro* pollen germination of two table grape cultivars, 'Italia' and 'Cardinal' (*Vitis vinifera* L.), were determined. A total of 28 treatments, alone and in combination, were applied to freshly collected pollens which were sown on a basic medium with 1% agar and 20% sucrose. Petri dishes were kept at 26±1°C for 24 hours. Counting of the germinated pollens revealed that the effects of these plant hormones were cultivar- and substance-specific. The cultivar 'Italia' was not influenced by the treatments (the highest germination ratio being 44.4% from 0.001 mg L^{-1} epibrassinolide) as opposed to the cultivar 'Cardinal'. The highest germination ratio in 'Cardinal' was about 50% in pollens treated with 25 mg L^{-1} GA_3 + 0.01 mg L^{-1} epibrassinolide. The control group resulted in 32.38% germination. Combining GA_3 with epibrassinolide provided slightly higher germination ratios compared to combining GA_3 with 22S,23S-homobrassinolide.

Key words: grapevine, pollen germination, epibrassinolide, homobrassinolide, gibberellin

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Introduction

Pollen germination and pollen tube growth are two of the most important aspects pertaining to pollen quality (Sharafi and Bahmani, 2011). *In vitro* pollen germination has been investigated at the genetic, physiological and biochemical levels in different plant species. Pollen germination evaluated *in vitro* might validate germination *in vivo* (Hormaza and Herrero, 1999).

Literature on *in vitro* pollen germination of horticultural crops is abundant with studies determining basic medium constituents including boric acid, mineral salts or plant growth regulators. However, there are contradicting results on the effects of growth regulators added to the growth medium.

Following pollination, pollen germination and pollen tube growth have been shown to be dependent on the gibberellins on site (Plackett *et al.*, 2011). Pharis and King (1985) stated that reproductive development is one of the physiological events regulated by gibberellins. Chauhan and Katiyar (1998) reported that gibberellic acid (GA_3) at low concentrations, as opposed to the higher concentrations, promoted pollen germination in *Pinus kesiya*. Böll *et al.* (2009) stated that gibberellins had an influence on pollen germination in seeded vine species. Sotomayor *et al.* (2012) found important effects of gibberellin on increasing germination ratio of almond pollens. On the other hand, Radović *et al.* (2016) stated that GA_3 had less impact on pollen germination of almond compared to auxin.

Brassinosteroids, first found in the bee-collected pollens of rape plants (Grove *et al.*, 1979), are the least investigated growth regulators in terms of their effects on pollen germination and growth. Brassinosteroids were shown to improve pollen tube growth in *Camelia japonica* (Hewitt *et al.*, 1985) and tomato (Singh and Shono, 2003). On the other hand, Ylstra *et al.* (1995) reported contradicting results in tobacco using sterols. Extensive studies in Arabidopsis have shown that brassinosteroids are important in the regulation of reproductive growth (Kang and Guo, 2011). Thussagunpanit *et al.* (2013) showed increasing and decreasing effect of 0.01 µM 24-epibrassinolide on pollen viability and germination. Application of 24-epibrassinolide to Arabidopsis pollens resulted in stimulated germination and pollen tube growth (Vogler *et al.*, 2014). So, there is both a lack of information and contradicting results about the effects of brassinosteroids on pollen germination.

This study was conducted to determine the effects of two brassinosteroid compounds, epibrassinolide and 22S,23S-homobrassinolide, along with the other growth regulator group, gibberellins, on *in vitro* pollen germination and growth of grapevine cultivars.

Materials and methods

1. Plant materials

Pollens of the table grape cultivars 'Italia' and 'Cardinal' (*Vitis vinifera* L.) were used as plant materials. Cultivars are white colored and late maturing. The vines were located at the experimental vineyard in the Dardanos Campus of Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

2. Pollen collection and growth regulator treatments

Inflorescences were collected in the morning hours at the 23rd stage (full bloom) of Eichhorn and Lorenz (1977) classification. Inflorescences were brought into the lab and all unopened flowers were emasculated. Anthers were later collected in Petri dishes and allowed to burst for 24 hours at room temperature. Pollen germination capability was determined using epibrassinolide (EBR, Sigma E-1641), 22S,23S-homobrassinolide (HBR, Sigma H-1267) and gibberellic acid (GA_3, commercial grade 1 mg a.i.) at three concentrations (w/v) with basic 1% agar and 20% sucrose medium. Concentrations applied were GA_3 25, 50 and 100 mg L^{-1}; EBR 0.001, 0.01 and 0.1 mg L^{-1}; and HBR 0.001, 0.01 and 0.1 mg L^{-1}. There were also combinations of EBR and HBR with GA_3 using exact concentrations (for example, 25 mg L^{-1} GA_3 + 0.1 mg L^{-1} EBR, etc.).

3. Germination measurements

For germination studies, growth regulators were added to the solid basal medium. Appropriate amounts of freshly prepared solutions of growth regulators (individually and in combinations) were thoroughly mixed with the basal germination medium during cooling but before attaining a semisolid condition (Engin and Hepaksoy, 2003). Pollen was dusted on the media using a fine camel hair brush so as to provide a nearly uniform layer. Petri dishes after inoculation were incubated for 24 hours at 26±1°C. After the incubation period, the media surface was flooded with a fixative solution (20 parts glycerin, 5 parts formaldehyde, 3 parts glacial acetic acid and 72 parts water; Pfahler, 1967) to stop further germination. Petri dishes were stored at room temperature and observations on percent germination were recorded from four different areas (i.e. four

replicates per treatment) under a light microscope (Olympus 41X, Olympus Corp., Japan) at 10x magnification.

4. Statistics

Statistical analysis was performed using Minitab statistics package program (v16 for Windows) for one-way analysis of variance at 95% confidence level after the data was transformed to arcsine square root. Differences among the treatments were tested using Duncan's multiple comparison analysis.

Results

In this study, the response of pollens obtained from inflorescences of table grape cultivars 'Italia' and 'Cardinal' to different plant growth regulators added to the medium in Petri dishes was evaluated. Statistical analysis showed that pollen germination of cv. 'Italia' was not influenced ($p \geq 0.05$) by the plant growth regulators (Table 1). Pollens not treated with any plant growth regulators (control) showed 26.08% germination, while the highest ratio was obtained from 0.001 mg L^{-1} EBR treatment (44.40%). The lowest percentage of germination was obtained from the application of 0.1 mg L^{-1} HBR (17.85%). Although no significant differences were observed, it was seen in the mean values of the applications that as the amount of GA_3 in the combination treatments increased, the mean ratio of pollen germination slightly decreased. In addition, homobrassinolide yielded higher germination compared to epibrassinolide.

Table 1. *In vitro* pollen germination ratios (%) of table grape cultivars 'Italia' and 'Cardinal' (*Vitis vinifera* L.), exposed to different plant growth regulators

Plant growth regulators (mg L^{-1})	Italia (%)	Means of the applications	Cardinal (%)	Means of the applications
GA_3 25	25.26		27.71 bcd*	
GA_3 50	40.37	32.23	30.04 abcd	30.37
GA_3 100	31.05		33.37 abcd	
EBR 0.1	21.24		27.53 bcd	
EBR 0.01	30.54	32.06	31.57 abcd	30.38
EBR 0.001	44.40		32.05 abcd	
HBR 0.1	17.85		25.50 bcd	
HBR 0.01	28.95	28.93	15.68 cd	24.96
HBR 0.001	40.00		33.70 abc	
GA_3 25 + EBR 0.1	24.55		29.22 bcd	
GA_3 25 + EBR 0.01	35.21	31.93	49.58 a	35.04
GA_3 25 + EBR 0.001	36.04		26.32 bcd	
GA_3 50 + EBR 0.1	21.98		34.57 abc	
GA_3 50 + EBR 0.01	29.17	28.33	41.26 ab	32.96
GA_3 50 + EBR 0.001	30.35		23.05 bcd	
GA_3 100 + EBR 0.1	32.66		26.10 bcd	
GA_3 100 + EBR 0.01	24.09	27.86	39.78 ab	32.37
GA_3 100 + EBR 0.001	26.83		31.24 abcd	
GA_3 25 + HBR 0.1	35.40		40.66 ab	
GA_3 25 + HBR 0.01	33.42	36.82	38.91 ab	33.62
GA_3 25 + HBR 0.001	41.64		21.30 bcd	
GA_3 50 + HBR 0.1	38.82		27.73 bcd	
GA_3 50 + HBR 0.01	30.51	33.80	23.37 bcd	27.04
GA_3 50 + HBR 0.001	32.06		30.01 abcd	
GA_3 100 + HBR 0.1	38.90		21.40 bcd	
GA_3 100 + HBR 0.01	23.21	27.42	13.31 d	19.76
GA_3 100 + HBR 0.001	20.15		24.56 bcd	
Control	26.08	23.08	32.38 abcd	32.38

*means with different letters differ significantly at $p \leq 0.01$.

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Pollens of cv. 'Cardinal', on the other hand, responded significantly ($p \leq 0.01$) differently to hormones (Table 1). However, the magnitude of the effects was not easily distinguished from one another. The highest germination was obtained from the combined application of 25 mg L^{-1} GA_3 + 0.01 mg L^{-1} EBR (approx. 50%). The closest ratio to this was from 50 mg L^{-1} GA_3 + 0.01 mg L^{-1} EBR application (41.26%). The control group resulted in 32.38% germination. The difference between the highest (49.58%) and the lowest ratio (13.31%) was more than 300%. To generalize the responses to individual hormones, germination increased with increasing GA_3 concentrations (from 27.71% to 33.37%), although to a little extent. The same tendency was also true for EBR (from 27.53% to 32.05%), but not for HBR. When the hormones were combined, it was seen that medium concentration of EBR (0.01 mg L^{-1}) resulted in the highest percentage with all GA_3 concentrations. This was not observed with the HBR applications and the effect was mainly dependent on the GA_3 concentrations.

Discussion

In horticultural crops, fruit set and yield mainly rely on pollen quality, nutrient components and requirements for optimum pollen germination, and assessments from *in vitro* germination experiments would help growers and breeders to choose the best possible pollinator cultivars. The plant growth regulators used in this study resulted in cultivar- and substance-specific responses.

Literature is abundant with studies on effects of hormones on pollen germination *in vivo* or *in vitro*. Pollen germination is reported to be influenced by growth regulators provided by the pollen grains and the styles (Jennings and Topham, 1971). Qi *et al.* (2010) reported the inducing effects of high gibberellin concentrations in kiwifruit. The same tendency was observed in 'Cardinal', but not in 'Italia'. However, Xue *et al.* (2008) stated that high GA_3 resulted in loss of pollen germination in peach. Acar *et al.* (2010) found reduced pollen germination in male pistachio flowers with increasing concentrations of GA_3. However, in this study, the effect was mainly dependent on the genotype and clear-cut differences were not observed in 'Cardinal'. We reported similar results in a previous study testing growth hormones on wine grape cultivars (Gökbayrak and Engin, 2016a). In another study (Gökbayrak and Engin, 2016b), we tested the influence of EBR, NAA (naphthalene acetic acid) and GA_3 on the *in vitro* pollen germination of some table grapes and found that stimulating effects of the hormones were cultivar-specific and that, in general, GA_3 was the most inducing one while EBR had weak effects. Sotomayor *et al.* (2012) reported greatest pollen germination from 0.1 mg L^{-1} brassinolide and 1 mL L^{-1} GA_3 in almond cultivars. Maita and Sotomayor (2015) found significant positive effects of brassinolide, GA_3 and kinetin in pollen germination of almonds.

Conclusion

The influence of hormones is mainly dependent on genotypes. However, the composition of nutrient medium and/or the concentrations of hormones may also play a critical role in inducing germination. Comparing the means of the applications showed that in the cultivars 'Italia' and 'Cardinal', hormones did not result in considerably better germination. An assumption could be made that growth substances for normal germination were already in the pollens to begin with, and application of hormones proved impractical in a general sense for these cultivars under these conditions. Finally, the two brassinosteroid compounds gave different results, with HBR being the less inducing compound in terms of increasing germination.

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