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Original Scientific Paper

Pollen of *Arbutus unedo*: Effects of plant growth regulators

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ABSTRACT:
Arbutus unedo is a species mainly used for landscaping purposes and lately for honey production. Its antioxidant characteristics have also been a subject of of research interest. The germination of pollen grains freshly collected from flowers was tested *in vitro,* and 30% sucrose gave the highest pollen germination. Subsequently, three growth regulators belonging to the categories of gibberellins and brassinosteroids [24-epibrassinolide (Ebl) and 22S,23S-homobrassinolide (Hbl)] were added to the growth medium singly or in double combinations (gibberellin + brassinosteroid), and the petri dishes were kept for 24 hours at 26±1°C. The results showed that the highest germination rates were obtained from treatments with 0.01 ppm Hbl (45.47%) and 0.001 ppm Hbl (26.74%). They were followed by treatments with 0.001 ppm Ebl, 25 ppm GA_3 and 0.1 ppm Ebl. As the concentration of GA_3 increased, the germination rate declined considerably. Statistical analysis of the combined treatments showed that combinations of growth regulators lowered the germination rates compared to their individual application. Moreover, action of the lowest GA_3 concentration (25 ppm) best matched the effects of brassinosteroids, and increasing the GA_3 concentration in combined treatments did not improve germination. The highest germination was obtained with 0.1 ppm Hbl, followed by all Ebl treatments. Brassinosteroids might be an inducer of pollen germination in plants depending on their type and concentrations. When individually included in the growth medium, the extent of this induction was greater with the homobrassinolide than with the epibrassinolide. Their interactions with gibberellins are shown to be mostly dependent on the concentration of gibberellic acid used. The tested epibrassinolide was more consistent than the homobrassinolide in maintaining higher germination levels. The findings of this study indicate that gathering more information from studies involving other plant species is needed to clarify the role of brassinosteroids in *in vitro* and *in vivo* germination.

Keywords:
anther, epibrassinolide, flower, gibberellin, strawberry tree

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INTRODUCTION

Arbutus unedo, a member of the Ericaceae family, is also known as strawberry tree. It is widely distributed in the Mediterranean basin. Its habitat is maquis and evergreen shrub. It flowers from September to December and bears fruit from August to November (Soro & Paxton 1999). *Arbutus unedo* is a natural plant found in the coastal flora of Turkey, along with its close relative *A. andrachne*. With its shiny leaves and impressive red fruits, it is widely used for landscaping purposes.

The flowers, with recurved lobes, are bell-shaped, 8–9 mm long, white and often pale-pink (Maleš *et al.* 2006). The flowers of *A. unedo* flowers are a significant source of nectar and pollen for bees (Gomes 2011). Its pollen grains are relatively large (60 μm) and anthers hang below nectaries in its drooping flowers (D'Albore & Persano Oddo 1978).

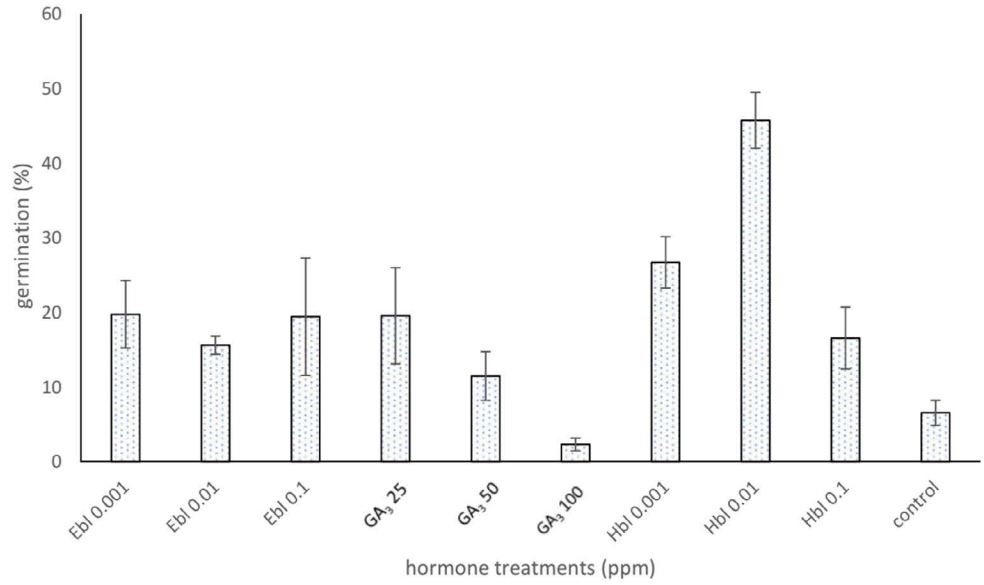

Figure 1. Effects of plant growth regulators individually applied on *in vitro* germination ability of *A. unedo* pollen on a medium containing 1% agar and 30% sucrose. The control represents treatment with no phytohormones.

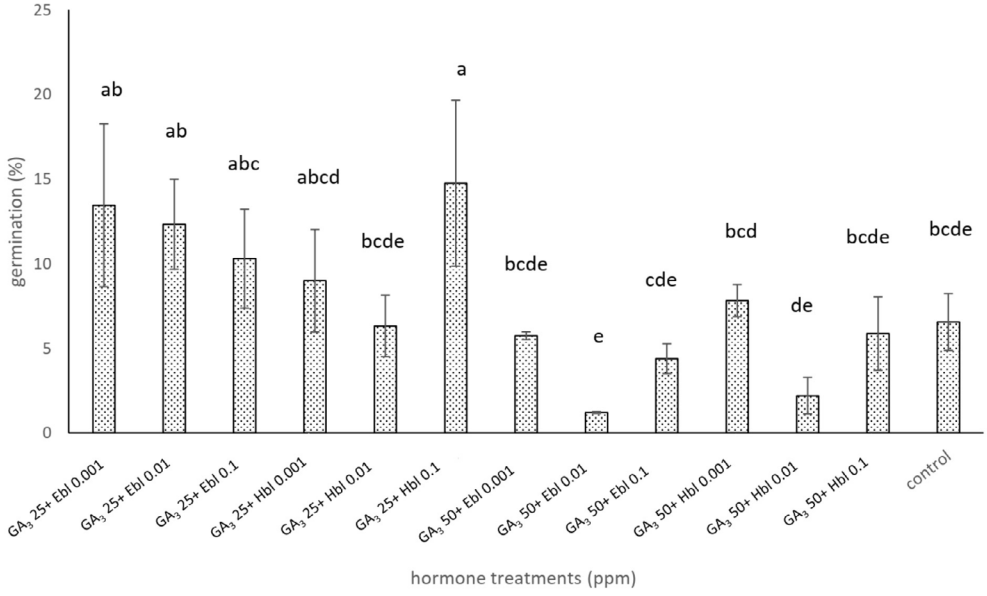

Figure 2. Effects of plant growth regulators applied in combination on *in vitro* germination ability of *A. unedo* pollen on a medium containing 1% agar and 30% sucrose. The control represents treatment with no phytohormones.

Extensive search of the literature regarding *Arbutus unedo* pollen revealed that it has been a food source for honeybees or constituent of honey composition. Pollen germination, *in vivo* or *in vitro*, has not been a subject of interest. This is the first attempt to determine the *in vitro* germination ability of *Arbutus unedo* pollen and effects of various plant growth regulators on induction of its germination.

MATERIALS AND METHODS

Flowers from *Arbutus unedo* plants were collected from hilly lands in Çanakkale, Turkey in the first week of November 2015. Anthers were removed with tweezers and put in petri dishes. They were kept at 22°C for 24 hours to allow them to burst open. Pollen grains were collected and used immediately for the experiment.

In order to determine the best sugar concentration in a basal growth medium containing 1% agar (Gökbayrak & Engin 2016a), several concentrations ranging from 10 to 100% were tested. After determining the best supporting sucrose concentration, individual applications of two brassinosteroid compounds [24-epibrassinolide (Ebl) and 22S,23S-homobrassinolide, (Hbl)] and gibberellic acid (GA_3) were tested, in addition to combinations of gibberellic acid with Hbl and Ebl. The types and concentrations were as follows: Hbl (Sigma H-1267) and Ebl (Sigma E-1641) in concentrations of 0.1, 0.01 and 0.001 ppm; and GA_3 (commercial grade, 1 mg a.i.) in concentrations of 25, 50 and 100 ppm.

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.

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 h at 26±1°C. After the incubation period, the surface of the media was flooded with a fixative solution. This solution contained 20 parts glycerin, 5 parts formaldehyde, 3 parts glacial acetic acid and 72 parts water (Pfahler 1967). The Petri dishes were stored at room temperature and observations on the percent of germination were recorded from four different areas under a light microscope (Olympus 41X, Olympus Corp., Japan) at 10× magnification.

Data collected from the counting were evaluated using the MINITAB statistics package (ver. 16 for Windows) at a 95% confidence level after arcsin square root transformation, and significant differences between the groups were tested with Tukey's test. When the effects of combinations of concentrations were tested, the 100 ppm GA_3 + Ebl/Hbl combination was excluded due to the fact that no germination at all was obtained.

RESULTS AND DISCUSSION

Preliminary tests were done in order to determine the best sugar concentrations in the basal growth medium. They showed that 30% sucrose in the medium provided better *in vitro* pollen germination (15%) in this study (data not shown). Sucrose concentrations of 50% and greater yielded absolutely no germination. Gözlekçi & Onursal (2009) tested the *in vitro* germination ability of *Arbutus andrachne* pollen grown in a medium containing 0.5% agar, 10% sucrose and 0.001% boric acid, and they observed a 30.6% germination level. Although *A. andrachne* is in the same sub-family of Arbutiodae, it is clear that *A. unedo* reacted to different concentrations of sucrose in the growth medium and the concentration of agar might even play an important part in supporting pollen germination. Addition of boric acid in the *A. andrachne* study might also have had an inducing effect, which was absent in the current study.

Subsequently, pollen grains were allowed to germinate on a medium containing 30% sucrose and 1% agar with applications of Ebl, Hbl and GA_3 (Fig. 1). The results showed that the highest germination rates were obtained from treatment with 0.01 ppm Hbl (45.47%) and 0.001 ppm Hbl (26.74%). They were followed by treatment with 0.001 ppm Ebl, 25 ppm GA_3 and 0.1 ppm Ebl. As the concentration of GA_3 increased, the germination rate declined considerably. The control group displayed the second lowest germination rate.

In addition to the above-described experiment, the effects of growth regulators applied in combinations were also tested (Fig. 2). It was clear from the obtained results that 100 ppm GA_3 with either Ebl or Hbl did not induce pollen germination at all and they were thereafter excluded. This might be either because the amount of gibberellic acid in the medium was excessive or because in combination with brassinosteroids GA_3 exerted antagonistic effects to the point of halting germination. Statistical analysis repeated with the remaining treatments (i.e., excluding 100 ppm GA_3) showed that combination of treatments lowered the germination rates compared to individual application, that the lowest GA_3 concentration (25 ppm) best matched the effects of brassinosteroids and that increasing the GA_3 concentration in combined treatments did not improve germination. The highest germination was obtained with 0.1 ppm Hbl, followed by all Ebl treatments.

Effects of brassinosteroids were studied by Gökbayrak & Engin (2016a) in some table grapevines, and they found that gibberellic acid, followed by epibrassinolide, had an inducing effect. In another study by Gökbayrak & Engin (2016b), it was found that individual growth regulators added to the growth medium increased pollen germination in Cabernet Sauvignon and Merlot (*Vitis vinifera* L.). However, in Cabernet Sauvignon this effect was not insignificant when combinations of gibberellic acid and brassinosteroids (Ebl or Hbl) were applied. In Merlot, on the other hand, they produced percentages similar to those of the control group, while 100 ppm GA_3 with 0.01 ppm Ebl was found to cause a significant increase. The same tendency in gibberellic acid action was observed in our study, where it was shown that gibberellins did not significantly increase the germination rate of *A. unedo* pollens. However, the tested homobrassinolide, as opposed to the epibrassinolide, had a greater inducing effect on the pollen, this effect depending closely on the concentration applied. Combining plant growth regulators greatly reduced the percent of germination. This might have been due to interactions between the brassinosteroids and gibberellins. Some publications have suggested that interaction occurs between brassinosteroids and gibberellins at the signaling level (Bai *et al.* 2012; Gallego-Bartolomé *et al.* 2012; Li *et al.* 2012). On the other hand, Tong *et al.* (2014) and Unterholzner *et al.* (2015) developed theories that brassinosteroids can regulate plant growth by modulating gibberellin levels. Gökbayrak & Engin (2016b) speculated that the effects of gibberellic acid on germination of grape pollen could be dependent on the concentration and type of the brassinosteroids added to the growth medium.

CONCLUSION

Brassinosteroids, originally isolated from a pollen source, can be an inducer of pollen germination, depending on their type and concentrations. When individually included in the basal growth medium, the extent of this induction was greater with the tested homobrassinolide than with the epibrassinolide. Their interactions with gibberellins, a group of plant growth regulators universally known for inductive effects on pollen germination, are shown to be mostly dependent on the concentration of gibberellic acid used. The tested epibrassinolide was more consistent than

the homobrassinolide in maintaining higher germination levels. The findings of this study indicate that gathering more information from studies involving other plant species is needed to clarify the role of brassinosteroids in *in vitro* and *in vivo* germination.

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REZIME

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Polen *Arbutus unedo*: efekti regulatora rasta biljaka

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Arbutus unedo je vrsta koja je uglavnom korišćena u pejzažne svrhe, kasnije i za proizvodnju meda. Njegove antioksidantne osobine su, takođe, bile predmet istraživanja. Klijavost polena, sveže pokupljenog sa cvetova, testirana je *in vitro* i najveću klijavost je obezbedila 30% saharoza. Kasnije, tri regulatora rasta, koja pripadaju giberelinima i brasinosteroidima (24-epibrassinolide, Ebl and 22S,23S-homobrassinolide, Hbl), dodata su u medijum za rast samostalno ili u kombinaciji (giberelin+brasinosteroid) i petri kutije su čuvane 24 h na 26±1°C. Rezultati pokazuju da je najveći odnos klijanja obezbeđen pri tretmanu sa 0.01 ppm Hbl (45.47%) i 0.001 ppm Hbl (26.74%). Zatim slede 0.001 ppm Ebl, 25 ppm GA_3, i 0.1 ppm Ebl. Sa rastom GA_3, odnos značajno opada. Statistička analiza kombinovanih tretmana pokazuje da kombinovanje regulatora rasta smanjuje odnos u poređenju sa individualnom primenom i da najniže koncentracije GA_3 (25 ppm) najbolje odgovaraju brasinosteroidima i povećanje koncentracije GA_3 u kombinovanim tretmanima ne podstiče klijanje. Najintenzivnije klijanje je zabeleženo sa 0.1 ppm Hbl, nakon čega slede svi Ebl tretmani. Brasinosteroidi mogu indukovati klijanje kod biljaka zavisno od njihovog tipa i koncentracije. Kada se samostalno dodaju u medijum za rast, obim ove indukcije je veći sa homobrasinolkidima nego sa epibrasinolidima. Pokazano je da njihova reakcija sa giberelinima najviše zavisi od koncentracije korišćene giberelinske kiseline. Epibrasinolid je u poređenju sa homobrasinolidom konzistentniji u održavanju viših nivoa klijanja. Rezultati ove studije pokazuju da uloga brasinosteroida u *in vitro* i *in vivo* klijanju zahteva više informacija iz studija izvedenih na drugim vrstama biljaka.

Ključne reči: antere, epibrasinolid, cvet, giberelin, jagodnjak