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SHORT COMMUNICATION

Influence of plant growth regulators on sex differentiation and floral characteristics of pomegranate flowers: a case of brassinosteroids

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Abstract

The objective of the present study was to determine the effects of brassinosteroid application on the sex determination and floral growth of pomegranate flowers. Whole tress of *Punica granatum* 'Mayhoş-8' were sprayed with 0.001, 0.01, and 0.1 mg L^{-1} 28-homobrassinolide (Hbr) at bud break. The selection of flowers was based on the appearance of a visible pistil/stigma and shape of the ovary of intact flowers. Grouping of bisexual flowers was based on their flower position, i.e., single, terminal, and lateral flowers, during the first flush of flowering in mid-June. Approximately 20 days and 40 days after spraying, 100 randomly chosen bisexual flowers per collection time were collected and measurements of floral parts were immediately taken. The differences between bisexual and functional male flowers were not distinct enough to assert any significant effects from the different Hbr treatments. Irrespective of treatments, the percentage of male flowers was higher than that of bisexual flowers. Hbr treatments applied at the bud break period had significant effects only on bisexual flowers that developed as single flowers. Applying 0.01 mg L^{-1} Hbr produced comparably lower percentages of single bisexual flowers (53.4%). Although the 0.1 and 0.001 mg L^{-1} Hbr treatments resulted in increased percentages of bisexual flowers, the difference was not significant. For the first collection time (mid-June), the effects of Hbr concentration on the size of the floral parts for bisexual flowers were only significant in ovary width and stigma diameter. As the concentration decreased, smaller ovaries were obtained. During the second flowering, application of 0.01 mg L^{-1} Hbr considerably increased the overall sizes of floral parts in bisexual flowers. Hbr might affect the ratio of flower formation in certain positions on a branch and could influence the growth of floral parts in bisexual flowers. Further elucidation of these effects would help to better understand floral organ development in plants.

Keywords

brassinosteroids; *Punica granatum* L.; sex development; flowers

Introduction

The destination of the flower primordium to produce a certain type of sexual morphology can be changed by the application of hormones at the right time and correct stage of floral development [1].

Gibberellins have mainly been associated with the development of male organs [2]. However, this effect is not universal and it has been found that gibberellin was more effective in increasing female flower formation in *Jatropha curcas* [3]. Conversely, cytokinins have been reported to alter flower sex in favor of femaleness [4]. Auxins [5], ethylene [6], and abscisic acid [7] have been found to influence sex differentiation

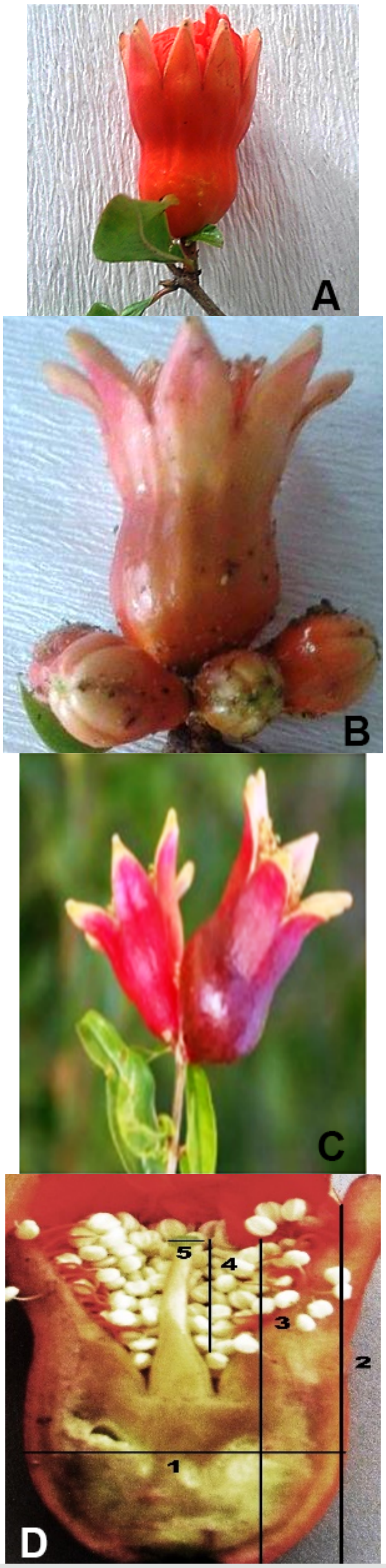

Fig. 1 Pomegranate flower types. (**A**) Single flower at the open petal stage. (**B**) Terminal flower. Flower cluster with a central flower subtended by closed buds and the terminal flower is past bloom, petals have abscised, and the ovary has enlarged. (**C**) Lateral flower. Flower cluster with lateral flower that is past bloom and petals have abscised. (**D**) Flower characteristics measured: 1 – ovary width; 2 – base to sepal notch (stigma + style + stylopodial length); 3 – total pistil length; 4 – stigma+style length; 5 – stigma diameter.

in plants. When *Carica papaya*, a dioecious plant, was treated separately with morphactin, ethephon, and triiodobenzoic acid (TIBA), it became more female [8]. Brassinosteroids have also been shown to play a role in the control of morphological changes and sex differentiation in plants [9,10]. Although they have been tested on the pollen germination of pomegranates and epibrassinolide and were found to increase in vitro pollen germination in pomegranate cultivars [11,12], their potential effects on flower sex formation are yet to be tested.

Therefore, the present study aim was to determine the effects of brassinosteroid application on the sex determination of pomegranate, an andromonoecious fruit species that has flowers that are bisexual or hermaphroditic, which develop into fruit, and functional male flowers that characteristically abort development after pollination.

Material and methods

Plant material

The orchard site was located at the Horticulture Experimental Farm of Çanakkale Onsekiz Mart University, 5 m above sea level. A collection of 13-year-old trees of pomegranate (*Punica granatum* L.) 'Mayhoş-8', planted at a distance of 3 × 5 m, were selected for the experiment. Trees were managed under conventional methods.

Plant growth regulator treatments

A compound of class brassinosteroid, 28-homobrassinolide (Hbr), was used for the treatments. The concentrations of Hbr applied were as follows: 0.001, 0.01, and 0.1 mg L^{-1}. At bud break (third week of May, 2015), the plant growth regulator was applied on the whole canopy of trees with a handgun sprayer until runoff. Application was undertaken on a windless day using an average of 1.4 ±0.34 L of solution per tree. Pure water was sprayed on the control trees. Every treatment included 0.1% Tween-20 to facilitate adherence. Each application was performed on three trees (replicates).

Data collection

At the end of June, the selection of flowers was undertaken on intact flowers based on the appearance of a visible pistil/stigma and shape of the ovary. Male flowers lacked a visible pistil/stigma and had a vase shaped ovary, whereas bisexual flowers contained a clearly visible pistil/stigma and an urn shaped ovary. Grouping of bisexual flowers based on their flower position was also undertaken on the trees without removing them from the branches only during the first flush of flowering in mid-June. Bisexual flowers were counted and spatially classified into three groups as follows: single (Fig. 1A), terminal (Fig. 1B), and lateral (Fig. 1C) flowers. Each tree constituted one replicate.

Approximately 20 days (mid-June, first wave of flowering) and 40 days (mid-July, second wave of flowering) after spraying, 100 randomly chosen bisexual flowers per treatment per flush period were collected and transported to the laboratory. Measurements immediately taken on these flowers were (***i***) ovary width, (***ii***) base to sepal notch length (stigma + style + stylopodial), (***iii***) total pistil length, (***iv***) stigma+style length, and (***v***) stigma diameter as shown in Fig. 1D. Each of the four replicates in one treatment contained 25 flowers.

Statistical analysis

The experiment was carried out as a completely random design with three replications containing three trees per treatment and four replicates containing 25 flowers per treatment. Statistical analysis was performed using statistical analysis software program MINITAB (Minitab Inc., ver. 16), and the significant means were compared using Tukey's test.

Results and discussion

When assessing the flowers based on their pistil presence and shape of ovaries, the differences between the bisexual and functional male flowers were not distinct enough to assert any significant effects of the Hbr treatments. Functional male flowers were always almost twofold higher than that of the percentage of bisexual flowers (Tab. 1). The percentages of bisexual flowers at different positions on the shoots are shown in Tab. 1. Hbr treatments applied during the bud break period only had significant effects on bisexual flowers that developed as single flowers. Applying 0.01 mg L^{-1} Hbr gave comparably lower percentages of single bisexual flowers (53.4%) than that of the other treatments, including the control. Although 0.1 and 0.001 mg L^{-1} Hbr treatments resulted in increased percentages of bisexual flowers (65.2% and 61.9%, respectively), the difference was not significant. In the production of lateral flowers with hermaphrodites, 0.1 mg L^{-1} Hbr was effective (26.1%); however, this effect was not significant compared to the other treatments. The ratio of hermaphrodite flowers that developed on the terminal positions was not affected by the different treatments. However, the results indicated that the flowers were responsive to the applications depending on the concentration. Although no significant effects of Hbr on sex determination in pomegranate flowers were determined, Dellaporta and Calderon-Urrea [13] stated that any action of hormones in changing floral sex morphology was species dependent. Besides the genotype, the application time and concentration might have also played a role in obtaining sex conversion in the flowers. Irrespective of the treatments, the percentage of male flowers were higher than that of the bisexual flowers, as also mentioned in a study by Chaudhari and Desai [14].

In general, this cultivar produced flowers in descending order of single (72.1%), lateral (16.5%), and terminal (11.3%) flowers in the control group. When treated with Hbr, single female flowers were followed by terminal and lateral ones, except in the 0.1 mg L^{-1} Hbr treatment. Although the relationship between flower size and fruit size was not studied in the present study, Wetzstein et al. [15] stated that terminal and single flowers were bigger with their wider ovaries, which might lead to bigger fruit development. They also indicated that lateral flowers had high occurrence of poor ovule development. It is speculated from the present study that it could be beneficial to apply Hbr to decrease the ratio of lateral flowers in pomegranate trees compared to the single and terminal flowers depending on the concentration applied.

For the first collection time (mid-June), the effects of Hbr on the size of the floral parts of the bisexual flowers were only significant for ovary width and stigma diameter (Tab. 2). As the concentration of Hbr decreased, smaller ovaries were obtained. The influence of Hbr on the stigma diameter was similar, except that the difference between 0.1 and 0.01 mg L^{-1} Hbr treatments was not significant. Although there were no statistical differences, the application of 0.001 mg L^{-1} Hbr produced longer base to sepal notch (25.1 mm) and total pistil length (27.9 mm). Conversely, 0.1 mg L^{-1} Hbr had the opposite effect and produced bisexual flowers with the shortest lengths of sepal notch and total pistil length (19.6 and 21.6 mm, respectively). Stigma+style length did not change among treatments, with only the 0.1 mg L^{-1} Hbr treatment shortening this length by approximately 2 mm compared to the control.

Tab. 1 Effects of 28-homobrassinolide (Hbr) in flowers of ‘Mayhoş-8’ pomegranate cultivar.

	Flowers (%)				Bisexuals at flower positions (%)					
	Bisexual		Functional male		Terminal		Lateral		Single	
Treatments (mg L^{-1})	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Hbr 0.1	36.7 ±8.5 ^{a*}	14.6–58.8	63.2 ±10.5 ^{a}	31.6–94.8	8.7 ±0.0 ^{a}	7.9–9.5	26.1 ±3.0 ^{a}	17.3–34.9	65.2 ±1.5 ^{a}	59.1–71.3
Hbr 0.01	29.5 ±11.0 ^{a}	6.1–52.9	70.5 ±13.0 ^{a}	49.4–91.6	28.4 ±7.5 ^{a}	14.2–42.6	18.1 ±6.0 ^{a}	6.1–30.1	53.4 ±0.5 ^{b}	51.4–55.4
Hbr 0.001	39.8 ±19.5 ^{a}	6.4–73.2	60.2 ±16.0 ^{a}	34.7–85.7	22.1 ±5.5 ^{a}	14.7–29.5	15.9 ±5.0 ^{a}	9.1–22.7	61.9 ±4.0 ^{a}	50.1–73.7
Control	39.2 ±11.0 ^{a}	14.3–64.1	60.8 ±7.50 ^{a}	40.5–81.1	11.3 ±1.5 ^{a}	8.6–13.9	16.5 ±8.0 ^{a}	4.2–28.8	72.1 ±3.0 ^{a}	65.7–78.5

* Mean ±*SE*. Means within a column followed by different letters are significantly different at $p \leq 0.05$.

Tab. 2 Effects of 28-homobrassinolide (Hbr) in flowers of ‘Mayhoş-8’ pomegranate cultivar.

	Ovary width (mm)		Base to sepal notch length (mm)		Total pistil length (mm)		Stigma+style length (mm)		Stigma diameter (mm)	
Treatments (mg L^{-1})	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Size measurements of floral parts of bisexual flowers, first wave of flowering period, mid-June										
Hbr 0.1	18.8 ±0.8 ^{a*}	16.9–20.2	19.6 ±3.1 ^{a}	12.9–25.5	21.6 ±3.0 ^{a}	14.4–27.9	14.2 ±2.2 ^{a}	10.1–18.1	1.46 ±0.1 ^{a}	1.3–1.6
Hbr 0.01	16.2 ±0.3 ^{b}	12.2–20.2	22.4 ±1.0 ^{a}	14.9–29.9	25.1 ±1.8 ^{a}	16.3–33.9	15.6 ±1.0 ^{a}	8.2–23.1	1.47 ±0.2 ^{a}	1.0–2.0
Hbr 0.001	15.4 ±0.6 ^{b}	14.6–16.1	25.1 ±1.1 ^{a}	23.5–27.9	27.9 ±1.7 ^{a}	23.5–31.9	15.4 ±2.3 ^{a}	14.1–18.3	1.37 ±0.1 ^{b}	0.7–1.6
Control	15.9 ±0.5 ^{b}	12.3–17.4	22.4 ±1.0 ^{a}	16.5–26.2	25.5 ±1.3 ^{a}	15.8–31.4	16.3 ±1.3 ^{a}	8.3–25.4	1.52 ±0.1 ^{a}	1.0–2.0
Size measurements of floral parts of bisexual flowers, second wave of flowering period, mid–July										
HBr 0.1	31.9 ±1.9 ^{ab*}	25.1–38.7	22.2 ±2.1 ^{ab}	10.9–51.7	36.3 ±1.5 ^{ab}	24.7–52.3	11.1 ±0.9 ^{b}	8.4–14.1	1.0 ±0.1 ^{a}	0.6–1.7
HBr 0.01	34.9 ±2.2 ^{a}	30.4–39.4	25.9 ±2.8 ^{a}	10.0–61.3	39.2 ±2.0 ^{a}	22.9–62.6	12.3 ±0.4 ^{ab}	9.3–17.7	1.0 ±0.1 ^{ab}	0.6–1.8
HBr 0.001	21.2 ±1.4 ^{c}	12.3–30.1	13.1 ±0.7 ^{c}	9.7–17.8	26.7 ±1.8 ^{c}	15.2–36.9	13.6 ±1.1 ^{a}	3.7–17.3	0.8 ±0.1 ^{b}	0.4–1.0
Control	27.0 ±1.0 ^{b}	21.2–32.8	17.2 ±1.0 ^{bc}	11.1–29.9	33.1 ±1.0 ^{b}	24.8–45.9	12.7 ±0.3 ^{a}	9.1–15.8	0.8 ±0.0 ^{b}	0.5–1.4

* Mean ±*SE*. Means within a column followed by different letters are significantly different at $p \leq 0.05$.

For the second collection time (mid-July), the effects of the plant growth regulator were more evident in all aspects of floral parts (Tab. 2). The ovary was widest in the 0.01 mg L^{-1} Hbr treatment (34.9 mm). The lowest Hbr concentration (0.001 mg L^{-1}) resulted in the smallest ovary size (21.2 mm). The length of base to sepal notch and total pistil length changed greatly depending on the concentration of Hbr applied. Similarly, the shortest and longest values were obtained from 0.001 and 0.01 mg L^{-1} Hbr treatments, respectively. Stigma+style length of the bisexual flowers was higher in the lowest Hbr treatment; however, it was the highest concentration that provided the widest stigma in the flowers. From the influences of the Hbr treatments on the size of the bisexual flower parts, it can be deduced that they were more responsive to higher concentrations of Hbr than to lower concentrations. Grouping of the bisexual flowers based on their position on the branches was not undertaken in the present study; therefore, comparing results with the more detailed, yet lacking any hormone application, study of Wetzstein et al. [15] could not be adequately performed. However, the concentration of Hbr is important in exerting some effects on increasing ovary width, base to sepal notch length, and total pistil length. During the second wave of flowering, the 0.01 mg L^{-1} Hbr treatment considerably increased the overall sizes of floral parts in the bisexual flowers. It provided the widest and biggest flowers in terms of ovary width, base to sepal notch length, and total pistil length. Wetzstein et al. [15] stated that the total increase in vigor of ovary and number of ovules present in a flower might produce large flowers, which can lead to larger fruits. From this point of view, application of Hbr in pomegranate trees might be beneficial to enhance larger fruit production. Abubakar et al. [16] showed supporting evidence of the beneficial effects of Hbr in increasing fruit yield with a slight increase in fruit drop in the pomegranate cultivar 'Kandhari Kabuli'.

Conclusion

Brassinosteroids are a group of hormones that play a physiological and morphological role in the growth and development of plants. Hbr, applied at certain concentrations in the present study, did not cause a change in the direction of sexual morphology in pomegranate flowers. However, Hbr might affect the ratio of flower formation in certain positions on a branch. Hbr influences the growth of floral parts in bisexual flowers depending on the concentration. Further elucidation of these effects would help to better understand floral organ development in plants.

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