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ORIGINAL ARTICLE

Effects of Plant Growth Regulators on Sex Expression and Flower Development in Pomegranates

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Abstract

The effects of external treatments with gibberellin, brassinosteroid and auxin on sexual determination and flower development in pomegranate (*Punica granatum* L. cv. Çekirdeksiz) were investigated. Eigth treatments with three growth regulators, viz., epibrassinolide at 0.01 and 0.1 ppm, homobrassinolide at 0.01 and 0.1 ppm, GA3 at 10 and 25 ppm, NAA at 5 and 15 ppm and control (water spray) were sprayed at the time of initiation of new sprouts. Effects of the plant growth regulators on the percent of the flowers types were not clear. Plants produced considerably more male flowers. Although no statistical importance was detected, it was clear that all growth regulators decreased bisexual flowers and increased male flowers. There were no significant effects of growth regulators on the formation of bisexual flowers at different positions in the plants. The percentage of single bisexual flower was significantly higher than terminal or lateral bisexual flower in a cluster in the control plants. Effects of the plant growth regulators on the size of the parts of the bisexual pistils were, however, found important. The smallest ovaries were obtained from 0.1 ppm HBr, base to sepal notch was the only characteristic which was not influenced by the treatments and stayed between 15 and 18 mm. Total pistil length of the bisexual flowers was greatest with EBr applications and the shortest with the HBr treatments (about 27.5 mm). Length of the style and stigma was mostly shorter, but 0.1 ppm EBr boosted it up comparably more than the rest of the applications including the control. Stigma, on the other hand, was widest in the control flowers, closely followed by all except 0.1 ppm EBr application.

Keywords *Punica granatum* · Flower · Brassinosteroid · Gibberellic acid · NAA

Einfluss von Wachstumsregulatoren auf Geschlechtsausprägung und Blütenentwicklung bei Granatapfel

Schlüsselwörter Granatapfel *(Punica granatum)* · Blüte · Brassinosteroide · Gibberellinsäure · NAA

Introduction

In woody perennial fruit species, reproduction and crop yield solely depend on flower development. Genetics, environment and hormones, in combination, regulate sexual expression in flowers. Although plant species are believed to be predisposed to having certain type of sex and not change it throughout their lives, it is well documented that age, dry soil, high light intensity, etc., could cause sex differentiation (see Freeman et al. 1980). In addition, exogenous application of hormones can alter flower primordia to have a sex conversion in plants, depending on their time of application and concentration (Papadopoulou et al. 2005).

Gibberellins and cytokinins, hormones responsible for inducing flowering, could masculinize or feminize flowers (Khryanin 2002). Studies in mung bean (Arteca et al. 1983), tomato (Vardhini and Rao 2002) and Arabidopsis (Woeste et al. 1999) showed that ethylene and brassinosteroids also play a role in sexual expression in certain species. Abubakar et al. (2012) showed in pomegranate that homobrassinolide decreased abscission and increased return bloom, without reference to flower sexes. How much brassinosteroids are involved in sexual differentiation in plants is not clear. Pa-

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padopoulou et al. (2005) realized a number of external treatments with brassinolide in cucumber, melon and zucchini and concluded that cucumber was more sensitive than zucchini, by reducing the number of male flowers in the initial phase of development and promoting the initiation of the first female flower in the main shoot. Manzano et al. (2011) reported that cucumber plants were more sensitive to ethylene than to brassinosteroids and concluded that even though they may regulate the induction of female flower, depending on genotype, the role of brassinosteroids was not clear.

Pomegranates are andromonocious plants carrying both male and hermaphrodite flowers in one plant. This fruit species is known for its extended flowering period with overlapping stages of flower development and differing ratios of male to herpahroditic flowers from beginning to end of bloom. Growth regulators have been used to increase the flowering, to increase size of the flowers and to minimize the dropping of hermaphrodite flowers (Abubakar et al. 2012; Anawal et al. 2015). However, their effects on possibly changing ratio of flower sexes have been overlooked and not discussed. In this study, a number of external treatments with gibberellins, brassinosteroids and auxins were performed on one of the commercially improtant pomegranate cultivar in Turkey and their effects on on sexual determination and flower development were discussed.

Material and Method

Plant Material

Pomegranate (*Punica granatum* L.) cultivar 'Çekirdeksiz' (Seedless) was selected as the plant material. This cultivar is early ripening (September) and one of the many cultivars bred through selection by Alata Horticulture Research Institute, Mersin, Turkey from Antalya region in the Mediterranean region. Its rind is green yellow (ground) and little red (atop) with pink arils. It has sweet taste (Yılmaz 2007). The orchard site was located at the Horticulture Experimental Farm, Çanakkale Onsekiz Mart University, Çanakkale, Turkey, 5 m above sea level. A total of 27 trees were selected for the research. Samples were collected from 13-year-old trees, planted at distance of 5 m × 3 m. Selected trees were cultivated the same, such as pruning, fertilization, irrigation and disease control.

Treatments

Types and concentrations of the plant growth regulators used were as follows: naphthalene acetic acid (NAA) with concentrations of 5 and 15 $mg\,L^{-1}$; gibberellic acid (GA_3) with concentrations of 10 and 25 $mg\,L^{-1}$; homobrassinolide (HBr) with concentrations of 0.01 and 0.1 $mg\,L^{-1}$; and epibrassinolide (EBr) with concentrations of 0.01 and 0.1 $mg\,L^{-1}$. Four plant growth regulators were applied at bud break (May 21, 2015) with a handgun sprayer until runoff. Applications were realized on windless days using an average of 1.2 ± 0.27 L of solution per tree. Pure water was sprayed on the control trees. In order to attain high wetting, 0.1% Tween 20 was added to the solutions before spraying.

Measurements

All flowers of both types (functional male and hermaphrodite) at the open petal stage were collected from uniform branches about the same length and diameter from trees and brought to the laboratory for measurements. Bisexual (Fig. 1a, b) and functional male (Fig. 1c, d) flowers were separated based on the size of the pistil. It is shortened in the male flowers. The number of bisexual and functional male flowers per application was determined. Sex determination in each plant was determined as the percentage of bisexual flowers per plant in all applications.

Bisexual flowers were categorized into three different positions. Bisexual flowers were numbered as single flower (Fig. 2a), terminal flower (Fig. 2b) on a cluster and lateral flower (Fig. 2c) on a cluster and flower parts measured. Flower characteristics measured were 1) ovary width, 2) base to sepal notch length, 3) total pistil length, 4) stigma + style length and 5) stigma diameter (Fig. 2d).

Statistical Analysis

The experiment was carried out in a complete randomized design with three replications containing one tree per replicate per treatment. The statistical analysis was performed using statistical analysis software MINITAB software (Minitab Inc., ver.16), and the significant means were compared using Tukey's test.

Results and Discussion

Flowering of the pomegranate cultivar, 'Çekirdeksiz' occurred about 40 days after bud break on newly developed branches of the same year, mostly on spurs or short branches. Flowers of the 'Çekirdeksiz' cultivar were grouped into two types of flowers on the same tree; hermaphrodite (bisexual) and functional male flowers. Literature classified flowers depending on the length of the pistil in the flower. Wetzstein et al. (2011) expressed two types of flowers on the same tree: hermaphroditic bisexual flowers and functionally male flowers in the pomegranate. In contrast, Chaudhari and Desai (1993)

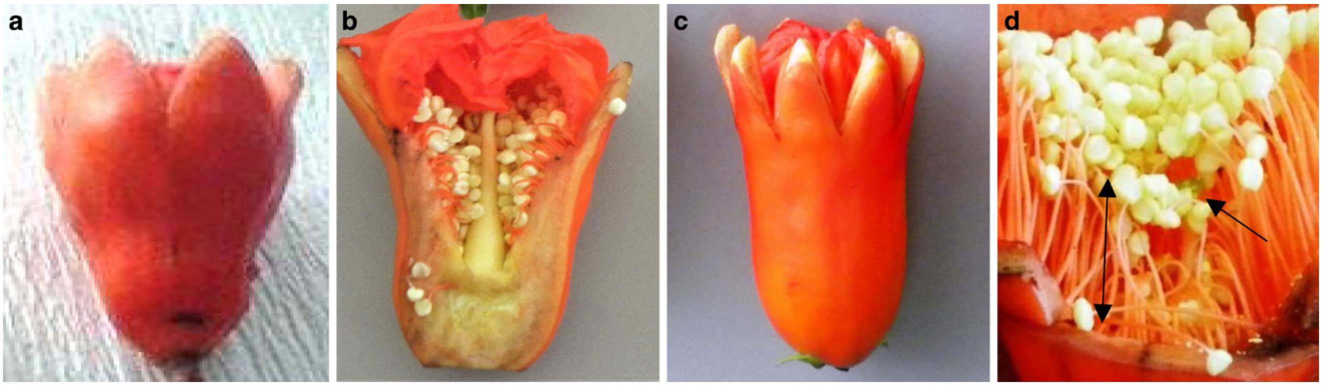

Fig. 1 Pomegranate flowers. **a** Hermaphrodite flower, **b** Hermaphrodite flower showing anthers with red filaments and well developed pistil, **c** Functional male flower, **d** Functional male flower showing anthers with red filaments inserted on the inner surface of the calyx tube and well developed stamens but an underdeveloped pistil (arrows)

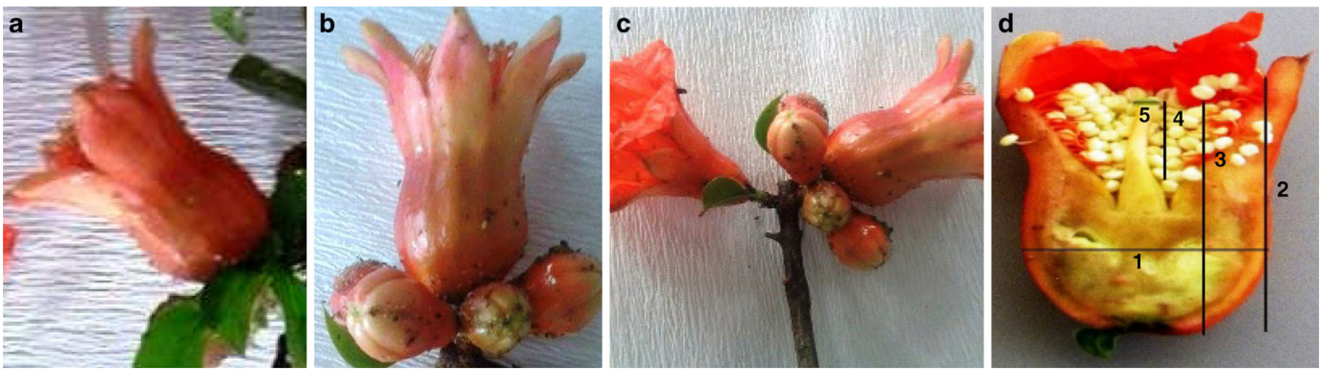

Fig. 2 Pomegranate flowers. **a** Single near sessile flower, **b** Terminal flower. Flower cluster with a central flower subtended by closed buds, **c** Lateral flower. Flower cluster with a lateral flower subtended by closed buds, **d** Longitudinal section of a bisexual flower showing measurements of ovary width, base to sepal notch length, total pistil length, stigma + style length and stigma diameter

classified pomegranate flowers into three types: male, hermaphroditic, and intermediate.

Effects of the plant growth regulators on the percent of the flowers types were not clear (Table 1). Plants produced considerably more male flowers. Although no statistical importance was detected, it was clear that all growth regulators decreased bisexual flowers and increased male flower formation. Respective evidence is supportive in terms of gibberellic acid effect. Chaudhari and Desai (1993) reported increased and decreased number of male and hermaphrodite flowers, respectively, in pomegranate after GA_3 application. Ahire et al. (1993) applied hormones 75 days after start of bloom and found that GA_3 induced more male flowers and less hermaphrodite flowers. However, Goswami et al. (2013) reported that 50 ppm NAA was found effective on increasing hermaphrodite flowers. The difference with the study at hand might have been due to genotype and/or concentration. Anawal et al. (2015) stated that budbreak application of NAA and GA_3 did not result in the production of more flowers in pomegranate.

Flowers appeared single, terminal or lateral on a cluster (Table 1). Bisexual flowers were near sessile and born either singly or in clusters of commonly one terminal flower subtended three or four lateral flowers. There were no significant effects of growth regulators on the formation of bisexual flowers at different positions in the plants. The percentage of single bisexual flower was significantly higher than terminal or lateral bisexual flower in a cluster in the control plants. However, the ratios change in the declining direction when plants were exposed to the hormones. The second highest ratios were obtained from lower GA_3, and higher concentrations of HBr and EBr applications. Control plants had the lowest amount of terminal bisexual flowers, as did the rest of the applications except for 25 ppm GA_3 treatment (55.5%). Lateral hermaphrodite flowers were mostly found in the plants exposed to higher NAA and lower HBr and EBr concentrations. The percentage of male flowers in pomegranate can be significant and more than 60 to 70% depending on variety (Engin and Gökbayrak 2017) and season (Chaudhari and Desai 1993; Mars 2000).

Effects of the plant growth regulators on the size of the parts of the bisexual pistils were on the other hand found important (Table 2). Only application that resulted in the smallest ovaries was 0.1 ppm HBr, and the others resulted

Table 1 Effects of plant growth regulators on the ratio (%) of bisexual and functional male flowers and the sex determination of bisexual flowers at different positions in 'Çekirdeksiz' pomegranate cultivars

Treatments mg L^{-1}	Sexes of flowers (%)				Bisexual flowers at different positions (%)					
	Bisexual		Functional male		Single		Terminal		Lateral	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
NAA 5	16.4	4.88–27.9	83.5	77.1–90.0	37.3	20.8–53.7	22.3 b[a]	20.8–23.8	40.2	9.49–71.1
NAA 15	10.6	5.42–15.7	89.4	81.7–97.1	30.1	4.72–55.2	20.3 b	19.5–21.1	49.5	3.25–95.9
GA_3 10	7.62	5.11–10.1	92.3	88.0–96.7	57.6	38.4–76.9	15.3 b	7.69–23.0	26.9	13.8–39.9
GA_3 25	11.1	3.91–18.3	88.8	87.6–90.1	33.3	11.1–55.5	55.5 a	21.2–89.9	11.1	9.71–12.4
HBr 0.01	13.8	7.17–20.5	86.1	76.7–95.5	41.5	3.96–79.2	11.8 b	3.44–20.3	48.5	7.97–89.0
HBr 0.1	9.09	2.17–16.0	90.9	82.7–99.1	56.0	24.5–87.5	12.8 b	4.54–21.1	30.3	10.1–50.5
EBr 0.01	14.4	6.86–22.0	85.5	73.3–97.8	36.6	17.7–55.5	17.7 b	8.88–26.6	45.5	11.1–80.0
EBr 0.1	15.8	4.11–27.6	84.1	70.0–98.2	58.7	28.5–88.9	6.34 b	1.58–11.1	34.9	7.73–62.1
Control	22.9	9.21–36.6	77.0	59.7–94.4	75.0	60.9–89.1	4.16 b	3.30–5.03	20.8	5.16–36.5

[a]Means within the column with different letters are significantly different at $p \leq 0.05$

Table 2 Size measurements of parts in bisexual flowers in the pomegranate cultivar 'Cekirdeksiz' when treated with plant growth regulators

Treatments mg L^{-1}	Measurement (mm)									
	Ovary width		Base to sepal notch		Total pistil length		Stigma + style length		Stigma diameter	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
NAA 5	29.3 a	21.5–36.5	15.4 a	11.8–19.1	30.4 b	24.3–38.8	12.0 e	10.9–13.1	1.01 ab	0.68–1.37
NAA 15	30.5 a[a]	22.6–41.5	17.7 a	10.1–24.4	29.8 ab	23.1–41.7	13.5 de	11.0–18.1	0.97 ab	0.42–1.77
GA_3 10	27.6 a	24.3–34.5	17.8 a	10.1–24.5	32.8 b	23.5–39.2	15.1 c	6.46–22.9	0.95 ab	0.36–1.73
GA_3 25	27.7 a	24.4–34.6	16.9 a	8.41–31.9	31.8 b	16.8–45.5	15.0 c	6.67–23.1	0.96 ab	0.22–1.77
HBr 0.01	27.7 a	22.4–40.9	15.9 a	11.3–31.9	27.4 c	21.8–39.9	14.5 cd	11.4–16.6	0.97 ab	0.61–1.40
HBr 0.1	21.4 b	14.6–31.1	15.2 a	8.47–25.5	28.1 c	16.8–38.7	11.9 e	6.67–16.7	1.07 ab	0.80–1.31
EBr 0.01	30.9 a	25.4–37.4	17.8 a	11.1–27.8	36.9 a	29.7–45.5	17.1 b	12.1–20.9	0.93 b	0.37–1.62
EBr 0.1	30.8 a	20.8–39.2	17.9 a	12.1–25.4	36.7 a	25.9–44.3	19.8 a	15.7–23.1	0.70 c	0.22–1.28
Control	27.6 a	24.3–34.5	16.9 a	13.4–24.1	32.8 b	23.4–39.3	15.3 c	11.7–18.5	1.18 a	0.93–1.74

[a]Means within the column followed by different letters are significantly different at $p \leq 0.05$

in similar sizes. Base to sepal notch was the only characteristic which was not influenced by the treatments and stayed between 15 and 18 mm. Total pistil length of the bisexual flowers was greatest with EBr applications (approx. 37 mm) and the shortest with the HBr treatments (about 27.5 mm). Length of the style and stigma was mostly shorter, but 0.1 ppm EBr boosted it up comparably more than the rest of the applications including the control. Stigma, on the other hand, was widest in the control flowers, closely followed by all except 0.1 ppm EBr application. These results showed that size of the pistil was affected by the hormones, however the type of the hormone had varying influences. Epibrassinolide was the main hormone that elongates the pistil, while it narrows down the surface of the stigma, depending on the concentration. Literature lack information regarding the individual effects of the hormones on components of the pistil in the bisexual flowers, rendering the results hard to be discussed. The closest study was by Anawal et al. (2015) who found that 100 ppm NAA resulted in longest and widest flowers in pomegranate.

In pomegranates sex expression may be controlled by hormonal factors. In our research, all exogenous treatments of different plant growth regulators increased the production of functional male flowers and decreased that of hermaphrodite flowers, compared to the untreated plants. Influence of the hormones were more evident with development of the pistils in bisexual flowers. The long-styled flowers with larger ovary usually developed with epibrassinolide application, whereas the short-styled bisexual flowers with narrow ovaries developed on higher homobrassinolide concentration. The role of two type of brassinosteroids in the control of sex determination of pomegranate still stays undetermined and warrant more investigation in order to help growers and breeders to shift flowering process towards the way desired.

Conflict of interest H. Engin and Z. Gökbayrak declare that they have no competing interests.

References

Abubakar AR, Ashraf N, Ashraf M (2012) Effect of plant biostimulants on flowering, fruit drop, yield, and return bloom of pomegranate cv. Kandhari Kabuli. Asian J Hortic 7(2):473–477

Ahire GZ, Desai UT, Chaudhari SW, Masalkar SD (1993) Crop regulation in pomegranate: 1. Effect of growth regulators on flower induction, sex and flower drop. Ann Arid Zone 32(2):97–98

Anawal VV, Narayanaswamy P, Kumar HS (2015) Effects of plant growth regulators on induction of flowering in pomegranate (Punica granatum L.) cv. Bhagwa. Int J Sci Res 4(12):7–9

Arteca RN, Tsai DS, Schlagnhaufer C, Mandava NB (1983) The effect of brassinosteroid on auxin-induced ethylene production by etiolated mung bean segments. Physiol Plant 59:539–544

Chaudhari SM, Desai UT (1993) Effects of plant growth regulators on flower sex in pomegranate (Punica granatum L.). Indian J Agric Sci 63:34–35

Engin H, Gökbayrak Z (2017) Flower biology of some Turkish pomegranates. Vocarstwo- Journal of Pomology 51(197–198): 47–52.

Freeman DC, Harper KT, Charnov EL (1980) Sex change in plants: old and new observations and new hypotheses. Oecologia 47:222–232

Goswami JD, Patel NM, Bhadauria HS, Wankhade VR (2013) Effect of plant growth regulators on quality traits of pomegranate cv. Sindur. Asian J Hortic 8(1):361–363

Khryanin VN (2002) Role of phytohormones in sex differentiation in plants. Russ J Plant Physiol 49:545–551

Manzano S, Martinez C, Megias Z, Gomez P, Garrido D (2011) The role of ethylene and brassinosteroids in the control of sex expression and flower development in Cucurbita pepo. Plant Growth Regul 65:213–221

Mars M (2000) Pomegranate plant material: genetic resources and breeding, a review. Options Mediterraneennes Serie A 42, pp 55–62

Papadopoulou E, Little HA, Hammar SA, Grumet R (2005) Effect of modified endogenous ethylene production on sex expression bisexual flower development and fruit production in melon (Cucumis melo L.). Sex Plant Reprod 18:131–142

Vardhini BV, Rao SSR (2002) Acceleration of ripening of tomato pericarp discs by brassinosteroids. Phytochemistry 61:843–847

Wetzstein HY, Ravid N, Wilkins E, Martinelli AP (2011) A morphological and histological characterization of bisexual and male flower types in pomegranate. J Am Soc Hortic Sci 136(2):83–92

Woeste KE, Ye C, Kieber JJ (1999) Two Arabidopsis mutants that overproduce ethylene are affected in the posttranscriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. Plant Physiol 119:521–529

Yılmaz C (2007) Nar. Hasad Yayıncılık, İstanbul, p 190 (in Turkish)