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ENDOGENOUS GIBBERELLIN AND ABSCISIC ACID INFLUENCE ALTERNATE BEARING IN PISTACHIO (*PISTACIA VERA* L.)

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Abstract

Plant growth regulators hold a strong influence on the alternate bearing, despite the occurrence of many other factors. In the present research, alterations in endogenous GAs (gibberellins) and Abscisic acid-metabolites (ABA) were analyzed in different physiological periods and organs along with the role of flower bud abscission (Alternate bearing) in Pistachio (*Pistacia vera* L.) by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). The results showed significant differences in the content of ABA and GAs between 'On' and 'Off' years in various tissues of pistachio cultivar. Nine GAs and seven ABA were identified in pistachio, namely, GA_3, GA_4, GA_7, GA_8, GA_{19}, GA_{29}, GA_{34}, GA_{44}, and GA_{53}; ABA, DPA, ABAGE, PA, 7'OH-ABA, neo-PA, t-ABA, respectively. Especially, DPA and GA_{19} were the dominant ABA and GAs analyzed in this work, respectively. The different GA and ABA-metabolites were found more in flower buds than in other organs. The maximum ABA and GAs peaks were obtained after 35 days of full blooming during May. Almost all ABA metabolites, as well as GA_{19} and GA_{44}, increased during fruit kernel development at 55 days after flowering. The data of present research support that endogenous GAs and ABA produced in different organs influence pistachio flower bud abscission and, therefore, are closely related to alternate bearing.

Key words: Abscisic acid; Alternate bearing; Flower bud abscission; Gibberellins; *Pistacia vera*.

Abbreviations: ABA Abscisic acid, ABAGE ABA-glucose ester, DPA Dihydrophaseic acid, PA phaseic acid, $GA_{1–53}$ Gibberellins 1–53

Introduction

Alternate bearing is one of the major problems in many fruit tree species, occurring in both deciduous and evergreen trees. This situation adversely affects both the producer and the national economy worldwide. The processes involved are not universal and quite different in different fruit tree species (Lavee, 2007). In contrast to other fruit species, pistachio produces an abundant number of inflorescent buds every year primarily on fruiting trees during the period of embryo growth. The initiation of flower in pistachio begins in late March or early April that continues till early May or beginning of July in fruit-bearing and non-fruit-bearing trees. Flower bud abscission occurs during the initial 5–6 weeks of fruit development and intense abscission occurs only in 'On' year trees during 10–12 weeks that coincides with fast embryo development in both 'On' and 'Off' year trees (Vemmos, 2010; Okay *et al.,* 2011; Gundesli *et al.,* 2019). Although alternate fruit-bearing trees have been known for many years, it still remains a problem in many fruit species today. Nevertheless, metabolic processes, their induction, and involved messengers are only partially known. Although different horticultural practices have well-established and used to minimize many types of alternate bearing, their impact is only partial in most cases. Monselise & Goldschmidt (1982) identified possible external and internal factors in pistachio and other fruit trees that may cause alternate bearing. These factors include genetic factors (Esmaeilpour & Khezri, 2006; Karafakioglu & Aksoy, 2019), plant nutrition (Vemmos, 1999; Baninasab & Rahemi, 2006), and plant growth regulators (Lovatt & Ferguson, 1998, 2001; Okay *et al.,* 2011; Okatan, 2018). However, the physiological causes of this condition have not been fully determined (Lovatt & Ferguson, 2001; Kour *et al.,* 2018). On the other hand, researchers have acknowledged that alternate bearing in many fruit species is a complex developmental process involving a series of morphological and physiological stages (Monselise & Goldschmidt, 1982; Al-Shdiefat & Qrunfleh 2008; Okat *et al.,* 2011). Many earlier studies reported that some physiological events such as shoot elongation, flower initiation, abscission, embryo development, senescence, fruit set, and growth are regulated by plant growth regulators (Takeda *et al.,* 1980; Ulger *et al.,* 2004; Vemmos, 2010; Okay *et al.,* 2011; Gundesli *et al.,* 2020). Among these, gibberellins (GAs) and abscisic acid (ABA) have been found to have more direct and significant effects on seed dormancy and germination, seed ripening, primary root and shoot elongation, growth, flower bud induction, and formation, abscission, flowering and fruit-set in many species (Takeda *et al.,* 1980; Al-Shdiefat & Qrunfleh, 2008). ABA is essential for embryo growth in the early stages of seed development but higher levels of ABA at later stages of development inhibit embryo development by affecting GA signaling (Raz *et al.,* 2001). In addition, GAs is a well-known inhibitor of flowering in different plants and in turn of fruit production. GAs have been presumed to be involved in alternate bearing (Ebert & Bangerth, 1981; Goldschmidt *et al.,* 1997). When GAs were applied to flower buds of pistachio, they caused abscission even in 'On' year trees; therefore, it has been reported that GAs play a possible role in abscission and ABA has a potential effect on flowering (Rallo *et al.,* 1994; Okay *et al.,* 2011). Although many factors are effective in alternate bearing,

still we do not have any well-established information (Monselise & Goldschmidt, 1982; Lovatt & Ferguson, 1998, 2001; Lovatt *et al.,* 2006; Moosa *et al.,* 2019). However, the role of endogenous GA and ABA on this issue remains a source of uncertainty and problem. One of the challenges of intrinsic GA and ABA research is the lack of a precise and effective methodology to determine and identify the type and amount of GAs in plant tissues. Detailed understanding of the alternate bearing mechanism is necessary to develop appropriate pistachio orchard techniques to reduce flower bud abscission. Further, there is not enough information about the change and the relationship between plant growth regulators and flower bud abscission (alternate bearing).

Therefore, the present study was aimed to understand the behavior of pistachio trees in relation to alternate bearing (flower bud abscission) phenomena, by following the pattern of ABA-metabolites and GAs in the different organs and periods of 'Uzun' pistachio trees to understand how to reduce the effect of this phenomenon.

Materials and Method

Plant material: In order to determine the variation level in endogenous plant growth regulators within the cultivated pistachio, a Turkish 'Uzun' cultivar was developed at Pistachio Research Institute. The experiment was conducted in 2015 during the growing seasons at Pistachio Research Institute in Gaziantep provinces, Turkey. Thirty-three years old 'Uzun' cultivar was grafted on *Pistacia atlantica* Desf. rootstock and planted at 10 × 10 m intervals that were used as explants. In this study, shoots, leaves, and flower buds were analyzed from 'On' and 'Off' year trees.

Plant tissue sampling: The samples were collected between 08:00 and 10:00 h in the morning following 35, 45, 55, and 65 days after full blooming (DAFB) in the year 2015 (Table 1). The experiments were arranged in a randomized block design with three replications. For growth regulator analysis, one-year-old branches from different directions of the canopy (north, south, east and west), were considered per replication. Different explants including 50 young leaves and 10 shoots were excised and immediately transferred to the dry ice. They were separated into leaves, shoots, and buds then were frozen in liquid nitrogen. The samples were rinsed with sterile distilled water and lyophilized (IlShin Freeze Dryers, FD-8518, Ede, Netherlands) using a lyophilizer and homogenized using coffee grinder followed by storage at 4°C.

Table 1. Sampling dates of Gibberellins and Abscisic Acids for metabolic analyses.

Year	2015	
	Shoots, leaves, and flower buds	
Physiological periods	35 DAFB	15.05.2015
	45 DAFB	25.05.2015
	55 DAFB	04.06.2015
	65 DAFB	14.06.2015

DAFB: Days after full blooming. * Full blooming date, respectively, in the study years: 10.04.2015

Plant growth regulator analysis: Quantification of ABA and GAs in pistachio was carried out by ultra-performance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) in Plant Biotechnology Institute of National Research Council of Canada (http://www.nrc-cnrc.gc.ca/eng/solutions/advisory/plant_hormone.html). The plant growth regulators including ABA metabolites [cis and trans abscisic acid, phaseic acid (PA), dihydrophaseic acid (DPA), 7-0-hydroxy ABA, neo-PA, abscisic acid glucose ester (ABA-GE)]; Gas (GA1, 3, 4, 7, 8, 9, 19, 20, 24, 29, 34, 44, 51, and 53) were quantified. Their deuterated forms were used as internal standards and synthesized either according to Abrams *et al*., (2003) or Zaharia *et al*., (2005). The UPLC/ESI–MS/MS utilized a Waters ACQUITY UPLC system equipped with a binary solvent delivery manager and a sample manager coupled to a Waters Micromass Quattro Premier XE quadrupole tandem mass spectrometer via Z-spray interface. MassLynx™ and QuanLynx™ (Micromass, Manchester, UK) were used for data acquisition and data analysis. The samples were injected onto an ACQUITY UPLC HSS C18 SB column (2.1 9 100 mm, 1.8 lm) with an in-line filter and separated by gradient elution of water containing 0.02% formic acid against an increasing gradient of a mixture containing acetonitrile and methanol (50:50, *v/v*). The procedure for the quantification of multiple plant growth regulators has been described in detail by Chiwocha *et al*., (2003, 2005) and Lulsdorf *et al*., (2013).

Statistical analysis

A randomized complete block design was followed for all the experiments with three replications per treatment. The means ± standard error (SE) were calculated from three independent experiments and concentrations were reported in ng g^{-1} of dry weight (DW). The significant difference was compared by the least significant differences (LSD) test executed at 5% level of probability.

Results

Identification and quantification of ABA

ABA and metabolite contents in shoot samples: With the latest available technology, the abscisic acid (ABA) and metabolites in the different organs were identified and concentrated for quantification. The concentrations of ABA-metabolite (ng/g DW) in the shoot are shown in Table 2. Significant differences were recorded among the physiological periods in the contents of ABA ($p<0.05$) (Tables 2 and 3). In total, seven ABA-metabolites were identified in the shoot samples. Different trends and concentrations in ABA-metabolites were observed for both 'On' and 'Off' year samples. Among the ABA metabolites, diphasic acid (DPA: between 51481.11–79171.21 ng/g in 'On; 33418.34–85138.54 ng/g DW in 'Off' year) was the dominant metabolite among the ABA metabolites and showed the highest value of more than 85% of the total ABA-metabolites. Neophaseic acid

(neoPA: between 23.52–103.14 ng/g in 'On'; 12.56–83.56 ng/g DW in 'Off' year) was found to have the lowest value (Table 2). In a study, during the month of July when fruit bud abscission was dense, it was observed that after 36 days of flowering shoots, ABA, DPA, neoPA, and PA levels were high, but after 55 and 65 days of flowering, almost all ABA-metabolite content was lower. DPA and t-ABA levels are opposite between the trees of alternate years, i.e., "on" and "off" year. Furthermore, the shoots of 'Off' year have higher DPA, t-ABA, and 7'OH-ABA content than the trees of 'On' year.

ABA and metabolites content in leaf samples: The concentration (ng/g DW) of ABA and its metabolites in leaves are shown in Table 3. Seven ABA-metabolites were identified in the leaf samples as well. The variations in ABA metabolite concentrations were observed for both 'On' and 'Off' year samples. Diphasic acid (DPA: between 4582.71–22920.09 ng/g in 'On; 6768.18–18790.24 ng/g DW in 'Off' year) was the dominant metabolite and showed the highest value in 35 DAFB over 85% of the total ABA-metabolites whereas hydroxy-ABA (7'OH-ABA: between 8.92–19.04 ng/g in 'On'; 4.63–15.25 ng/g DW in 'Off' year) was found to have the lowest value. During early periods of full flowering in the month of May (35 DAFB), ABA, DPA, neoPA, and PA were at the highest levels with significant difference ($p<0.05$) and after 66 DAFB, they showed a gradual decrease. The ABA amount was highest in the 'On' year, and lower in the very next 'Off' year. Thus, ABAGE levels increased from May to July. ABA metabolites in leaves showed the opposite effect compared to shoots. The shoots of 'Off' year trees contained higher ABA-metabolites than 'On' year trees.

ABA and metabolite contents in flower bud samples: The concentrations (ng/g DW) of the various ABA and metabolites in flower buds are shown in Table 4. The ABA metabolites showed variation among 'On' and 'Off' year trees. Seven ABA metabolites were identified. Among the ABA-metabolites, diphasic acid (DPA: between 62337.38–128197.92 ng/g in 'On; 68774.46–122079.36 ng/g DW in 'Off' year) was the dominant metabolite and showed the highest value in 35 DAFB, i.e., over 63.10% of the total ABA metabolites whereas hydroxy-ABA (7'OH-ABA: between 453.83–1399.53 ng/g in 'On'; 403.21–1109.42 ng/g DW in 'Off' year) was found to have the lowest value. 'On' year trees had more ABA-metabolites content than 'Off' year trees.

Table 2. The concentration of ABA and metabolites detected in shoot samples during different physiological periods in the 'On' and 'Off' year trees of 'Uzun' Pistachio variety.

Bearing	ABA and metabolites (ng/g DW)													
	ABA		DPA		ABAGE		PA		7'OH-ABA		neo-PA		t-ABA	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
35 DAFB	6898.62^{a}	7582.95^{a}	85137.69^{a}	51480.63^{c}	7329.32^{b}	5534.96^{b}	9182.21^{a}	10345.71^{a}	130.13^{a}	126.08^{a}	83.45^{a}	102.68^{a}	132.90^{a}	78.33^{c}
45 DAFB	3606.76^{b}	3608.62^{b}	58475.34^{b}	70668.90^{b}	9483.72^{a}	6233.46^{b}	2328.38^{b}	1635.55^{b}	133.28^{a}	89.16^{b}	47.11^{b}	44.72^{b}	107.87^{b}	133.73^{a}
55 DAFB	1483.41^{c}	1296.95^{c}	50525.47^{c}	79170.57^{a}	5234.32^{d}	6195.17^{b}	1127.44^{c}	1206.92^{bc}	40.72^{b}	75.63^{bc}	22.22^{c}	22.56^{c}	41.36^{d}	87.51^{c}
65 DAFB	530.28^{d}	1217.86^{c}	33418.50^{d}	54496.37^{c}	6147.23^{c}	12013.03^{a}	931.50^{c}	746.99^{c}	43.82^{b}	82.23^{bc}	12.54^{d}	27.61^{c}	56.46^{c}	99.64^{b}
LSD	4826.28**	13522.74**	5964.83**	6472.57**	719.9**	791.08**	15099.27**	16650.74**	11.94**	11.63**	6.05**	7.17**	11.28**	12.44**

<LOD = Below the limit of detection, ND: No detected in any of the samples, LSD: Least significant difference, Statistical differences ($p<0.05$) between periods are indicated by different letters

Table 3. The concentration of ABA and metabolites detected in leaf samples during different physiological periods in 'On' and 'Off' year trees of 'Uzun' Pistachio variety.

Bearing	ABA and metabolites (ng/g DW)													
	ABA		DPA		ABAGE		PA		7'OH-ABA		neo-PA		t-ABA	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
35 DAFB	1983.19^{a}	1133.03^{c}	18790.37^{a}	22920.00^{a}	1172.91^{b}	1295.30^{b}	1728.05^{a}	1395.25^{a}	11.23^{b}	18.96^{a}	13.78^{a}	16.46^{a}	196.56^{a}	138.81^{b}
45 DAFB	1438.81^{c}	2976.02^{a}	12721.93^{b}	16579.28^{b}	954.79^{c}	1504.83^{a}	813.84^{b}	975.36^{b}	14.93^{a}	12.28^{c}	14.38^{a}	12.69^{b}	83.94^{b}	163.68^{a}
55 DAFB	1467.95^{c}	1787.99^{b}	6768.23^{c}	8584.91^{c}	883.94^{c}	1289.09^{b}	478.82^{d}	691.69^{c}	5.97^{c}	15.86^{b}	7.10^{c}	8.01^{c}	58.61^{c}	95.31^{c}
65 DAFB	1556.59^{b}	669.08^{d}	6849.63^{c}	4582.25^{d}	1379.80^{a}	1449.70^{a}	658.79^{c}	419.75^{d}	4.54^{d}	8.99^{d}	8.67^{b}	8.12^{c}	61.31^{c}	41.71^{d}
LSD	6.25**	184.96**	1227.98**	1490.22**	111.09**	138.28**	103.44**	93.9**	1.23**	1.77**	1.39**	1.45**	14.03**	14.55**

<LOD = Below the limit of detection, ND: No detected in any of the samples, LSD: Least significant difference, Statistical differences ($p<0.05$) between periods are indicated by different letters

Table. 4. The concentration of ABA and metabolites detected in flower bud samples during different physiological in 'On' and 'Off' year trees of 'Uzun' Pistachio variety.

Bearing	ABA and metabolites (ng/g DW)													
	ABA		DPA		ABAGE		PA		7'OH-ABA		neo-PA		t-ABA	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
35 DAFB	29923.30^{a}	31923.60^{a}	122079.36^{a}	118294.47^{b}	25551.37^{d}	43130.31^{d}	28620.40^{a}	36008.73^{a}	806.85^{b}	1005.15^{c}	175.10^{a}	224.39^{a}	211.19^{a}	161.37^{c}
45 DAFB	24896.78^{b}	34160.81^{b}	76090.46^{c}	105684.02^{c}	47034.39^{a}	66730.50^{a}	12739.55^{b}	10850.31^{b}	1109.42^{a}	1399.53^{a}	158.41^{b}	111.66^{c}	156.74^{c}	200.57^{b}
55 DAFB	10058.48^{c}	14382.36^{c}	107147.60^{b}	128197.92^{a}	41275.31^{c}	62927.57^{c}	10391.76^{c}	11268.15^{b}	712.61^{c}	1297.34^{b}	106.83^{c}	127.38^{b}	116.69^{d}	170.38^{c}
65 DAFB	3199.54^{d}	3524.60^{d}	68774.46^{d}	62337.38^{d}	43865.32^{b}	58776.39^{b}	5402.89^{d}	6671.34^{c}	403.21^{d}	453.83^{d}	115.95^{c}	111.41^{c}	192.87^{b}	241.40^{a}
LSD	775.02**	1743.81**	6716.20**	6808.36**	1943.35**	2999.73**	1464.00**	1823.31**	58.97**	76.76**	11.91**	14.50**	14.41**	12.94**

<LOD = Below the limit of detection, ND: No detected in any of the samples, LSD: Least significant difference, Statistical differences ($p<0.05$) between periods are indicated by different letters

Identification and quantification of GAs

GAs content in shoot samples: Table 5 shows the content (ng/g DW) of various GAs in the shoot. Generally the amount of different GAs were significantly different among different flowering periods ($p < 0.05$) (Tables 5-7). The GA content differed in the 'On' and 'Off' year trees. Eight GA metabolites were identified in the shoot samples. GA_{19} (between 13.70–161.04 ng/g in 'On; 5.58–171.71 ng/g DW in 'Off' year) was the dominant metabolite with the highest value whereas GA_8 (between 4.43–12.73 ng/g in 'On'; 5.96–17.42 ng/g DW in 'Off' year) was found to have the lowest value. The shoots of 'On' year trees had higher GA_8 and GA_{44} content than 'Off' year trees. In contrast, the shoots of "off-year trees had higher GA_{19} content. During the month of July after 35 days of flowering, GA_{19} and GA_{44} levels were high. Also, GA contents in the shoots of 'On' year trees were more than 'Off' year trees (Table 7).

GAs content in leaf samples: The concentration (ng/g DW) of the various GAs in leaves is shown in Table 6. Six GAs were identified in the leaf samples with considerable variations in both 'On' and 'Off' year trees. Among the GAs**,** GA_{19} (between 4.45–32.95 ng/g in 'On; 9.24–88.64 ng/g DW in 'Off' year) was the dominant metabolite and showed the highest value in 35 DAFB, whereas GA_8 (between 8.92–19.04 ng/g 'On'; 9.62–14.28 ng/g DW in 'Off' year) was found to have the lowest value. During the early periods of full flowering in the month of May (35 DAFB), GA_{19} and GA_{29} were maximum and showed a gradual decrease after 65 DAFB. Almost all the GAs in the leaves of 'On' year trees have a higher level than 'Off' year trees.

GAs content in flower bud samples: The concentration (ng/g DW) of the various GAs in flower buds is shown in Table 7. The GAs differed in the 'On' and 'Off' year trees and seven GAs were identified in the flower bud samples. Among them**,** GA_{19} (between 13.23 –177.14 ng/g in 'On; 8.03–126.20 ng/g DW in 'Off' year) was the dominant metabolite with highest value in 35 DAFB whereas GA_8 (between 453.83 –1399.53 ng/g in 'On'; 7.00–24.32 ng/g DW in 'Off' year) was found to have the lowest value. Particularly the value of GA_{19} and GA_{29} in flower bud was more than shoot and leaf (Table 7). Thus a high value in the flower of the fruit-bearing trees was confirmed. This reveals the direct association with the flower bud abscission (alternate bearing).

Table 5. The concentration of gibberellins detected in shoot samples during different physiological periods in 'On' and 'Off' year trees of 'Uzun' Pistachio variety.

Bearing	Gibberellin (ng/g DW)															
	GA_8		GA_{19}		GA_{44}		GA_3		GA_4		GA_7		GA_{34}		GA_{53}	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
DAFB 35	4.55^{d}	12.73^{a}	171.71^{a}	161.02^{a}	22.46^{a}	51.53^{a}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DAFB 45	8.94^{b}	8.13^{b}	34.94^{b}	23.02^{b}	13.59^{b}	13.26^{b}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DAFB 55	17.35^{a}	4.69^{c}	5.55^{c}	27.62^{b}	8.43^{c}	10.59^{b}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DAFB 65	5.98^{c}	4.45^{c}	9.77^{c}	13.75^{c}	3.87^{d}	4.22^{c}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LSD	0.4**	0.82**	8.64**	8.25**	1.39**	2.71**										

<LOD = Below the limit of detection, ND: No detected in any of the samples, LSD: Least significant difference, Statistical differences ($p<0.05$) between periods are indicated by different letters

Table 6. The concentration of gibberellins detected in leaf samples during different physiological periods in 'On' and 'Off' year trees of 'Uzun' Pistachio variety.

Bearing	Gibberellin (ng/g DW)											
	GA_8		GA_{19}		GA_{29}		GA_7		GA_{34}		GA_3	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
35 DAFB	11.99^{b}	32.95^{a}	88.65^{a}	78.24^{a}	14.26^{c}	23.36^{a}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
45 DAFB	12.43^{b}	4.45^{c}	28.04^{b}	26.57^{c}	17.49^{b}	5.98^{c}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
55 DAFB	14.28^{a}	7.17^{b}	9.24^{d}	20.41^{d}	10.25^{d}	12.83^{b}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
65 DAFB	9.62^{c}	5.80^{bc}	19.86^{c}	44.28^{b}	21.34a	13.73^{b}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LSD	0.47**	1.72**	4.76**	4.78**	1.63**	1.52**						

<LOD = Below the limit of detection, ND: No detected in any of the samples, LSD: Least significant difference, Statistical differences ($p<0.05$) between periods are indicated by different letters

Table 7. The content of gibberellins detected in flower bud samples during different physiological periods in 'On' and 'Off' year trees of 'Uzun' Pistachio variety.

Bearing	Gibberellin (ng/g DW)													
	GA_8		GA_{19}		GA_{29}		GA_3		GA_4		GA_7		GA_{34}	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
35 DAFB	14.16^{c}	18.34^{b}	126.62^{a}	177.14^{a}	28.54^{a}	24.92^{a}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
45 DAFB	22.59^{b}	24.32^{a}	19.72^{b}	29.05^{b}	20.14^{b}	16.52^{b}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
55 DAFB	26.74^{a}	14.14^{c}	11.31^{c}	15.18^{c}	9.68^{d}	13.24^{c}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
65 DAFB	11.13^{d}	7.00^{d}	8.03^{c}	13.23^{c}	11.85^{c}	11.13^{d}	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
LSD	0.76**	1.71**	6.42**	8.99**	61.91**	1.72**								

<LOD = Below the limit of detection, ND: No detected in any of the samples, LSD: Least significant difference, Statistical differences ($p<0.05$) between periods are indicated by different letters

Discussion

Alternate bearing or flower bud abscission is one of the most critical problems for irregular fruit yield in pistachios. For this reason, alternate bearing negatively affects the producer, consumer, and economy. It occurs in almost all tree-fruit crops, but severe in pistachio (Monselise & Goldschmidt, 1982). In contrast to other fruit species, pistachio produces a high number of flower buds every year which undergo abscission mainly on 'On' year trees during the period of seed development. There are many factors affecting alternate bearing according to Crane & Iwakiri (1987) and Koroglu & Koksal (1999). It has been suggested that the plant growth regulator that plays an essential role in regulating plant growth is responsible for this phenomenon. The function of ABA and GAs in abscission is still not well understood. Most reports studied the structures related to ABA and GA levels in different plants from the identification of endogenous and ABA and GA substances (Goldschmidt & Monselise, 1972; Gómez-Cadenas *et al.,* 2000; Okay *et al.*, 2011). However, they did not provide any information regarding the levels of ABA-metabolites and GAs-like compounds in different organs of pistachio. Also the various types of ABA-metabolites and GAs were not identified. Some plants are used more frequently for sensitive analysis of ABA-metabolites and GAs by UPLC-ESI–MS/MS (Abrams *et al.,* 2003; Turečková *et al.,* 2009; Lulsdorf *et al.,* 2013). Very important and interesting results were achieved in this research that analyzed the GAs and ABA-metabolite data in different tissues and periods. To the best of our knowledge, this is the first report that detects the ABA-metabolites and GAs in different tissues of pistachios at different physiological periods (Tables 2-7). In this study, the variations in ABA metabolites and GA content of different organs and during periods were significant in both 'On' year and 'Off' year samples by UPLC-ESI–MS/MS. Among the ABA-metabolites, DPA was the dominant metabolite. ABA levels were higher in the early period after full blooming (35 DAFB) in almost all organs. The ABA amount in the shoots and leaves gradually decreased and reached a minimum level at 65 DAFB (Tables 2 and 3). In contrast, ABA content in flower buds was higher in the early period after full blooming (35 DAFB) that decreased at 45 and 55 DAFB (intense bud abscission) and the lowest level was attained within 65 DAFB (embryo development) (Table 4). Compared with the qualitative research on ABA found in different organs of fruit varieties, it was determined that flowering stage showed higher levels and then decreased, which was consistent with the findings of present research (Rallo *et al.,*1994 Cetinkaya, 2004; Okay *et al.,* 2011). In another report by Gómez-Cadenas *et al*., (2000), it was clear that ABA rises after days 14 and 42 of flowering and results in fruitlet abscission in citrus. A comparison of ABA content among all the studied organs revealed that the 'On' year trees have higher levels than 'Off' year trees (Tables 2–6). ABA metabolites found in the flower bud had opposite effect compared to shoots and leaves. Moreover, ABA and its metabolites in flower buds were higher than shoots and leaves. Lovatt & Ferguson (2006) also reported that flower buds contained higher ABA than other organs. Contrastingly, Lovatt & Ferguson (1998, 2001) and Cetinkaya (2004) reported that ABA content in flower bud was lower. On the other hand, GAs are plant hormones that strongly inhibit flowering induction (Baktir *et al.,* 2004; Achard & Genschik, 2009). Although many studies have shown that GAs are effective at various stages of fruit development (Ulger *et al.,* 1999; Baktir *et al.,* 2004; Okay *et al.,* 2011; Kour *et al.,* 2018), but the concept about seasonal changes in these plant growth regulators and especially during alternate bearing is still not clear. Hence, the current study is different from the previous ones and it is the first report on the effect of GAs on alternate bearing in different tissues and periods of pistachio. According to the results, four dominant GAs (GA_8, GA_{19}, GA_{29} ve GA_{44}) were detected in different organs of pistachio. Compared with some recent reports (Hedden, 1993; Oyama *et al.,* 1996), lesser amount of specific GAs was detected in the samples of this research. Like GA_1 and GA_3, GA_7 is also a potent inhibitor of apple flower induction (Unrath and Whitworth, 1991; Tromp, 1982). The concentrations of GA_{19} in all tissues at 35 DAFB in both 'On' and 'Off' year trees were at the maximum levels. Also, GA_{19} and GA_{44} showed a sharp decrease in all tissues during the period of intense flower bud abscission (55 DAFB). The amount of GA_{19} and GA_{44} increased toward the fruit kernel development (Table 5–7). The contents of GAs-like substance in all samples were higher in the 'On' year trees in comparison to 'Off' year. In contrast, shoots of 'Off'' year trees have higher GAs content than 'On' year trees. Similarly, Cetinkaya (2004) and Okay *et al*., (2011) in pistachio, Ulger *et al*., (1999) and Baktır *et al*., (2004) in olive, Pal & Ram (1978) in mango, demonstrated higher GA level in the 'On' year. Looney *et al*., (1985) reported decreased GA_3 and increased GA_4 levels during the flower initiation periods in the 'On' year that suggest the role in flower bud formation in apple. Our findings are consistent with the studies performed in previous years during the growth periods of ABA and GA in the different pistachio organs (Cetinkaya, 2004; Ulger *et al.,* 2004; Okay *et al.,* 2011). According to the results, it is assumed that the levels of GA and ABA during pistachio flowering periods could affect alternate bearing, flower bud abscission, and embryo development.

Conclusion

In this study, the identification of endogenous ABA and GA showed only one aspect of the whole alternate bearing concept. More varieties, as well as ABA and GA, should be considered to ensure the results. To our knowledge, this is the first time that ABA metabolites and GAs have been detected in pistachios in different tissues during different periods. The study provides a basis for the role of two plant growth regulators (ABA-metabolites and GA_S) during flower bud abscission in pistachios. Seven ABA-metabolites and four GAs were identified in different tissues. Among the ABA-metabolites and GA_S, DPA and GA_{19} were dominant. They may play an important and controlling role in the regulation of flowering. The alternate bearing 'Uzun' pistachio variety

has higher levels of ABA and GA in fruit buds than other organs. However, the fact that it is higher in 'On'' year trees as compared to 'Off' year that reveals its relationship with flower bud abscission (alternate bearing). Especially in the early flowering period, it was found to be higher during fruit bud abscission (55 DAFB). The study of ABA and GAs in different organs of pistachio seems to be a promising way of examining bud abscission and embryo development in relation to alternate bearing. Future research should focus on the transport of ABA and GAs during the alternate bearing of fruit cultivars and their transport over time. However, the interaction with other hormones (auxins, cytokinins, ethylene, *etc*.) synthesized in plants may also indirectly affect alternate bearing. Efforts should be made to improve this method by looking for a way to better.

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