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Endogenous gibberellins and abscisic acid-metabolites: their role for during flower bud abscission and embryo development in pistachio

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Abstract: This study was carried out to determine the effect of different growth periods and possible role of gibberellins (GAs) and abscisic acid (ABA) metabolites on the alternate bearing of the pistachio (*Pistacia vera* L.). For this purpose, the levels of GAs and ABA in the panicles and nuts of the trees during flower bud abscission and embryo development were analyzed in the crop "on" year. The results showed significant differences and changes in ABA and GAs among the tissues and periods investigated. Dihydrophaseic acid (DPA) and GA_{19} were the dominant ABA and GAs in the pistachio concentrates analyzed in this work, respectively. The nut samples had higher values for almost all ABA metabolites and GAs than panicles. The ABA content of the panicles and nuts increased rapidly during flower development (35 DAFB) prior to flower bud abscission while the initial decrease in the ABA content remained constant at a relatively low level at the end of June (intense flower bud abscission), with the minimum levels being obtained during embryo development stage (65 DAFB). However, on day 65 after full flowering, GA_{19} and GA_{44} were found to have increased. The plant growth regulator profiles of the pistachio showed delayed spikes in GA and ABA groups indicating that there is a hormone requirement during flower bud abscission and embryo development in pistachio. As a results, GA and ABA metabolites produced in different organs play an important role in the control of pistachios during embryo development and flower bud abscission.

Keywords: Abscisic acid-metabolites, gibberellins, *Pistaciavera* L., alternate bearing (flower bud abscission)

1. Introduction

Turkey is one of the most important fruit producers in the world and the country not only has great diversity in the number of fruits grown, but also an astonishing quantity of total produce, about 18 million tons. The fruits grown in Turkey mainly consist of pome fruits, stone fruits, berries and citrus (Acar et al., 2006; Ercisli et al., 2008; Serce et al., 2010; Guney 2019).

Alternate bearing is a major economic problem in pistachio fruit production in the horticultural world. Pistachio-producing countries in the world are generally located in the Northern Hemisphere. Among these countries, Iran, the United States, and Turkey are important in terms of their share in the total volume of production. Turkey has rich plant diversity and genetic resources owing to its different climatic and soil conditions. There are also wild pistachio species distributed throughout Turkey (Ak and Acar, 2001; Kafkas, 2006). In particular, the Southeastern Anatolia Region is well suited for pistachio cultivation due to its suitable ecological conditions, covering 95% of the total pistachio growing area and accounting for 91.5% of pistachio production in Turkey (Yavuz, 2011; Gundesli et al., 2018, 2019). Alternate bearing is the tendency of a fruit tree to produce a heavy crop in one year ("on" year), followed by a light crop or no crop in the next year ("off" year). In contrast to other crop species, alternate bearing in pistachios, which occurs with the emergence of inflorescence buds in the abundant year, produces the next year's crop. Some authors have already reported that this is the main reason for alternate bearing, which is the growth period, the embryo development slows after rapidly growing from the last week of June through July. It was stated that the embryo developed rapidly after 50–55 days of the pollination. According to this, it is understandable that the flower bud abscission amount decreases intensely in June and July in which the development of the pistachio is continuous (Ayfer, 1990; Gunes et al., 2010; Okay et al., 2011). In previous studies, alternate bearing has been associated with genetic factors (Esmaeilpour and Khezri, 2006; Shalom et al., 2012), environmental and physiological factors, cultural management, (Mirsoleimaniet al., 2014), carbohydrates (Baninasab and Rahemi, 2006; Span et al., 2008), plant nutrients (Rosecrane et al., 1998; Vemmos, 2010), and

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plant growth regulators (PGRs) (Al-Shdiefat and Qrunfleh, 2008; Mirsoleimani and Shahsava 2018; Gundesli et al., 2019). The effects of PGRs (including auxin, cytokinins, gibberellins, abscisic acid, and ethylene) on different physiological stages in plants, such as shoot elongation, flower formation, flower bud abscission, embryo development, growth, and fruitinghave also been shown (Okuda, 2000; Okay et al., 2010; Mirsoleimani et al., 2014; Gundesli et al., 2019). Among PGRs, gibberellic acid (GAs) are plant hormones necessary for many developmental processes in plants, including seed germination, root elongation, leaf expansion, pollen ripening, and flowering induction (blooming). On the other hand, GAs were found to have a more direct and significant effect on the flower formation of many species than other PGRs. High GA levels showed an inhibitory effect on flower formation during induction and first periods (Dokoozlian and Peacock 2001; Baktir et al., 2004; Ulger et al., 2004; Achard and Genschik, 2009; Gunes et al., 2010; Giacomelli et al., 2013; Kouret al., 2018). In contrast with gibberellins, abscisic acid (ABA) acts as a stress signal to regulate plant growth in response to changes in water availability. ABA plays an important role in stress-responsive gene expression under seed maturation and germination, stoma closure, and osmotic stress conditions. ABA is required for embryo growth at the early stage of embryo and seed development (Frey et al. 2004), but higher levels of ABA at later stages inhibit embryo growth by suppressing gibberellin signaling (Yoshida et al., 2019).

Many studies have also applied PGRs externally to pistachios in order to prevent abscission, obtain regular yield, and increase flower bud formation (Acar et al., 2006; Lovatt et al., 2006; Askari et al., 2011; Oguz and Akkus, 2012; Padmalatha et al., 2017). However, some researchers did not report effective results (Ulger, 1997) due to insufficient data on the changes in PGRs and their relationship with alternate bearing in these species. Furthermore, the role of endogenous ABA and GAs in this process remains unclear. One of the challenges of internal ABA and GAs research is the lack of a precise and effective methodology to identify the type and amount of ABA and GAs in different plant organs. However, in recent years, the advances in technology have led to the development of high performance liquid chromatography–electrospray tandem mass spectrometry (UPLC-ESI–MS/MS) as a separation technique for the efficient quantification of specific ABA metabolites and GAs (Abrams et al., 2003; Chiwocha et al., 2003, 2005; Zaharia et al., 2005 and Lulsdorf et al., 2013), but there is a need to conduct further studies to investigate the efficacy of this method in demonstrating the role of GAs and ABA during (alternate bearing) flower bud abscission and embryo development. Thus, the main purpose of this study was to provide a better understanding of flower bud abscission and embryo development processes in relation to regulation by endogenous GAs (stimulant) and ABA-metabolites (inhibitory) using UPLC-ESI–MS/MS, and it will shed light on future studies. Investigation of endogenous GAs and ABA amounts of different plant species show alternate bearing fruit species especially on "on" year trees. Most of the existing studies on endogenous ABA and GAs in pistachios used bioassays and did not involve qualitative analyses. Furthermore, in a small number of studies that used UPLC-ESI–MS/MS, the relationship of the content of GAs and ABA metabolites with abscission or alternate bearing was not evaluated. This is what makes the current work significant.

2. Materials and methods

2.1. Plant material

In this study, in order to determine the level of changes in endogenous PGRs in cultivated pistachio, the Turkish "Uzun" cultivar developed at the Pistachio Research Institute was used. The experiment was conducted in the 2015 growing seasons at the research and experimental area of the institute in Gaziantep Province. Thirty-three-year-old trees grafted on the *Pistacia atlantica* Desf. rootstock and planted at 10×10 m space were used as the plant material. In order to ensure the same year alternate bearing in both "on" and "off" year pistachio trees, all the flowers of "off" trees were removed in April 2013. Subsequently, some flower removal in "off" year trees was essential in the early spring of each year to maintain the complete alternate bearing. The 2 groups of trees were maintained with their alternate bearing habit until the end of the study in 2015. In this study, the panicles and nuts were sampled from the "on" year trees.

2.1.1. Plant tissue sampling

The samples were collected between 8:00–10:00 am on 35, 45, 55, and 65 days after full blooming (DAFB) in 2015 (Table 1). The experiments were designed as a randomized complete block with 3 replications and 1

Table 1. Sampling dates of Gibberellins and Abscisic Acids-metabolites analyses.

	Panicle and nut
Physiological periods	35 DAFB
	45 DAFB
	55 DAFB
	65 DAFB

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

tree per replication. For the PGRs analysis, 1-year-old branches from different directions of the canopy (north, south, east and west) were excised (20 panicles and 30 shell nuts per replication), immediately placed on dry ice to be transferred to the laboratory to be separated into panicles and nuts, and frozen in liquid nitrogen. The samples were rinsed with sterile distilled water to remove the dust and soil and lyophilized using an ilShin freeze dryer (FD-8518, Ede, Netherlands), and then homogenized using a coffee grinder and stored at +4 °C. The nut samples were homogenized including the hull and embryo.

2.2. Method

2.2.1. PGR analysis

The quantification of ABA and GAs of pistachio samples was performed at the Plant Biotechnology Institute of the National Research Council of Canada using UPLC-ESI–MS/MS (http://www.nrc-cnrc.gc.ca/eng/solutions/advisory/plant_hormone.html). The following measurements were undertaken: (1) ABA and ABA metabolites [cis and transabscisic acid (ABA), phaseic acid (PA), dihydrophaseic acid (DPA), 7-0-hydroxy ABA, neophaseic acid (neoPA), and glucose ester (ABA-GE)] and (2) GA_1, GA_3, GA_4, GA_7, GA_8, GA_9, GA_{19}, GA_{20}, GA_{24}, GA_{29}, GA_{34}, GA_{44}, GA_{51}, and GA_{53} (Gibberellins 1-53: GA_{1-53}). In addition, the deuterated forms of GA and ABA were used as internal standards and synthesized according to either Abrams et al. (2003) or Zaharia et al. (2005). MassLynx TM and QuanLynx TM (Micromass UK Ltd., Manchester, UK) were used for data collection and analysis. The procedure for the quantification of multiple PGRs was performed as described in detail by Chiwocha et al. (2003, 2005) and Lulsdorf et al. (2013). The concentrations of PGRs were expressed as ng g^{-1} dry weight (DW).

2.3. Statistical analysis

The data were analyzed by JMP statistical software developed by SAS (V7) (SAS Institute Inc., Cary, NC, USA), and for eachanalytical value, the average of 3 replications was calculated. The significant differences were comparedby the least significant differences test at the 5% level of probability. The mean ± standard error (SE) values were calculated from 3 independent experiments.

4. Results

Using the latest available technology in PGRs can be relatively easily and sensitively analyzed. GAs and ABA metabolites in panicle and nut concentrates were identified and quantified only in the "on" year trees during flower bud abscission and embryo development. The concentration of ABA metabolites and GAs in the panicle and nut at different stages was significant at $P < 0.05$.

4.1. Identification and quantification of abscisic acid (ABA)-metabolites

4.1.1. ABA-metabolites content in panicle samples

The panicle concentration (ng g^{-1} DW) of the various ABA and metabolites for concentrates is shown in Table 2. Figure 1 shows the percentage of each ABA-metabolites expressed as a percentage of the total ABA-metabolites. The changes in ABA differed between the investigated periods of the 'on- year' trees. In total, 7 ABA-metabolites were identified in the panicle samples. Among the ABA metabolites, diphaseic acid (DPA: between 2435.12-18421.73 ng g^{-1} in "on" year) was the dominant metabolite and showed the highest value in 35 DAFB that over 76.87% of the total ABA-metabolites; hydroxy-ABA (7'OH-ABA: between 8.24–32.01 ng g^{-1}"on" year) was found to have the lowest value (Table 2; Figure 1). In the study, DPA, ABA (194.76–1449.41 ng g^{-1}) and PA (97.84–1952.49 ng g^{-1}) levels were high in the early stages of full blooming (35 DAFB) and decreased at the end of June (intense fruit abscission) and minimum levels 65 days after full blooming, followed by neophaseic acid (neoPA) and t-ABA levels with similar changes. (Table 2).

Table 2. The content of ABA and its metabolites detected in panicle samples in different physiological periods of the trees of, "on" year trees in 'Uzun' in Pistachio variety.

ABA-metabolites (ng/g DW)								
Periods	ABA	DPA	ABAGE	PA	7'OH-ABA	neo-PA	t-ABA	Total ABA
35 DAFB	$1449.41^{a} \pm 72.47$	$18421.73^{a} \pm 921.05$	$1911.71^{a} \pm 85.55$	$1952.49^{a} \pm 92.61$	$32.01^{a} \pm 1.60$	$72.54^{a} \pm 3.63$	$123.62^{a} \pm 6.18$	23.963,51
45 DAFB	$651.78^{b} \pm 32.53$	$6525.58^{b} \pm 320.28$	$1353.39^{b} \pm 57.66$	$311.37^{b} \pm 10.56$	$18.60^{c} \pm 0.93$	$33.77^{b} \pm 1.69$	$61.53^{b} \pm 3.08$	8956.02
55 DAFB	$422.74^{c} \pm 20.09$	$5431.86^{c} \pm 133.96$	$1152.02^{d} \pm 30.55$	$165.94^{c} \pm 4.78$	$23.68^{b} \pm 0.85$	$10.88^{d} \pm 0.30$	$15.11^{d} \pm 0.38$	7221.51
65 DAFB	$194.76^{d} \pm 9.73$	$2435.12^{d} \pm 101.74$	$1302.96^{c} \pm 50.14$	$97.84^{d} \pm 4.89$	$8.24^{d} \pm 0.41$	$12.58^{c} \pm 0.63$	$23.88^{c} \pm 0.75$	4074.90
Average	679.67	8203.57	1430.02	738.75	20.63	32.44	56.035	
D%5 $_{\text{"on"} \times \text{Periods}}$	1.96^{**}	1.25^{**}	1.60^{**}	1.29^{**}	1.03^{**}	0.56^{**}	0.52^{**}	

The results represent mean ± standard error. Means in columns with the same letter do not differ, *,**: Significant at $P < 0.05$, $P < 0.01$, respectively, by LSD test, other details are similar to Table 1. ABAGE: ABA-glucose ester, 7-OH-ABA: 7-Hydroxy-ABA. PA: Phaseic acid

4.1.2. ABA-metabolites content in nut samples

The nut concentration (ng g^{-1} DW) of various ABA metabolites for concentrates is shown in Table 3. Figure 2 shows the percentage of each ABA-metabolites expressed as a percentage of the total ABA-metabolites.The ABA showed different changes between periods in the "on" year. In total, 7 ABA metabolites were identified in the nut samples. Among the ABA-metabolites, diphaseic acid (DPA: between 32242.67–57855.23 ng g^{-1} in "on" year) was the dominant metabolite and showed the highest value in 35 DAFB that over 96.58% of the total ABA metabolites. ABA-alcohol (t-ABA: between 12.30–52.76 ng g^{-1}in "on" year) was found to have the lowest value (Table 3; Figure 2). In contrast to panicles,DPA and PA levels were low in the early stages of full blooming (35 DAFB), increased at the end of June (intense flower bud abscission), and reached maximum levels especially after 65 days of full blooming (Table 3); ABA and PA (202.97–1751.64 ng g^{-1} and 375.94–2068.07 ng g^{-1}, respectively) levels were high in the early stages of full blooming (35 DAFB), decreased at the end of June (intense fruit abscission), and showed minimum levels 65 days after full bloom, followed by neoPA (between 13.51–134.87 ng g^{-1}), 7OH-ABA (19.12–88.99 ng g^{-1})and t-ABA levels with similar changes (Table 3).

4.2. Identification and quantification of gibberellins

4.2.1. Gibberellin content in panicle samples

The panicle concentration (ng g^{-1} DW) of the various gibberellins (GAs) are shown in Table 4. Figure 3 shows the percentage of each GAs expressed as a percentage of the total gibberellin. Among the GAs showed different changes between periods in the "on" year trees. In total, 7 GAs were identified in the panicle samples. Among the GAs, GA_{19} (4.84–51.66 ng g^{-1} in "on" year) was the dominant metabolite and showed the highest value in 35 DAFB that over 59,63% of the total GAs (Figure 3);

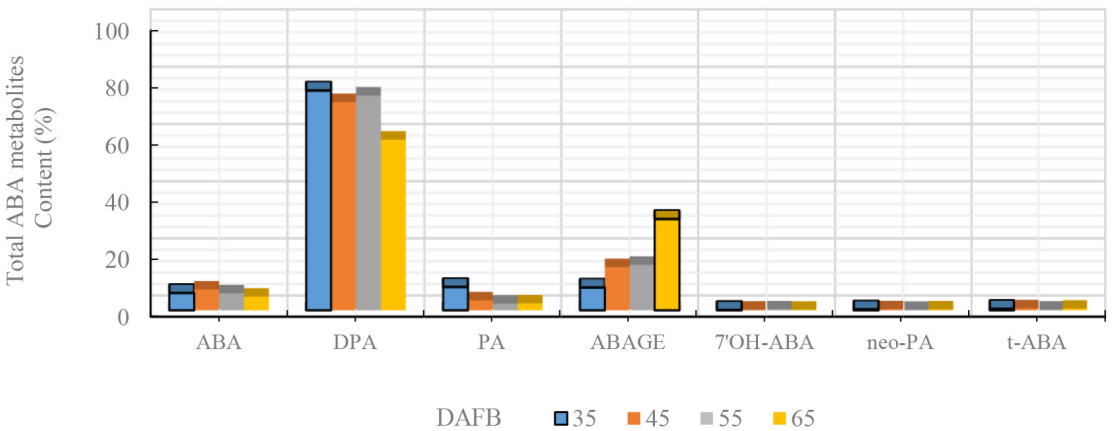

Figure 1. The ABA and its metabolites detected in pistachio panicle concentrates in "on" year derived from as a percentage of the total ABA content. DAFB, days after full blooming. Percentages were calculated based on each ABA values × 100/total ABA.

Table 3. The content of ABA and its metabolites detected in nut samples in different physiological periods of the trees of, "on" year trees in 'Uzun' in Pistachio variety.

ABA-metabolites (ng/g DW)								
Periods	ABA	DPA	ABAGE	PA	7'OH-ABA	neo-PA	t-ABA	Total ABA
35 DAFB	1751.64^{a} ± 80.58	57855.23^{a} ± 2582.76	1225.89^{a} ±60.29	2068.07^{a} ± 103.40	88.99^{a} ± 3.45	134.87^{a} ± 6.74	52.76^{a} ± 2.64	63177.46
45 DAFB	489.84^{b} ± 24.49	35497.60^{b} ± 1041.87	629.73^{c} ±18.66	943.75^{b} ± 27.84	30.29^{b} ± 1.13	61.01^{b} ± 1.66	12.30^{d} ± 0.42	37664.50
55 DAFB	202.97^{c} ± 9.15	35269.26^{c} ± 1063.46	456.41^{d} ±22.82	512.27^{c} ± 15.61	23.46^{c} ± 1.17	30.46^{c} ± 1.52	21.02^{b} ± 0.75	36515.85
65 DAFB	77.18^{d} ± 3.86	32242.67^{d} ± 1012.13	717.05^{b} ±30.85	375.94^{d} ± 16.80	19.12^{d} ± 0.96	13.51^{d} ± 0.68	14.38^{c} ± 0.72	33459.86
Average	630.41	27191.19	757.27	975.01	40.46	59.96	25.11	
D%5 $_{\text{"on"} \times \text{Periods}}$	1.61**	1.59**	1.28**	1.38**	0.80**	1.03**	0.96**	

The results represent mean ± standard error, Means in columns with the same letter do not differ, *,**: Significant at $P < 0.05$, $P < 0.01$, respectively, by LSD test, other details are similar to Table 1. ABAGE: ABA-glucose ester, 7-OH-ABA: 7-Hydroxy-ABA.

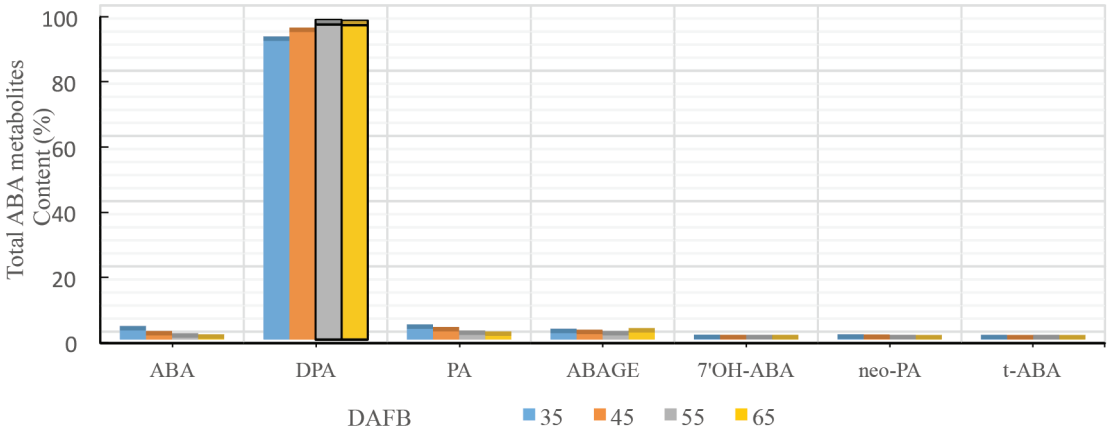

Figure 2. The ABA and its metabolites detected in pistachio nut concentrates derived from as a percentage of the total ABA content. Other details are similar to Figure 1.

Table 4. 'The content of gibberellins detected in panicle samples in different physiological periods of the trees of "on" year trees in 'Uzun' in Pistachio variety.

Gibberellins (ng/g DW)								
Periods	GA_8	GA_{19}	GA_{44}	GA_{29}	GA_3	GA_4	GA_{34}	Total gibberellins
35 DAFB	9.14^{c} ± 0.41	51.66^{a} ± 2.32	15.56^{a} ± 0.70	10.27 ± 0.46	<LOD	<LOD	<LOD	**86.63**
45 DAFB	11.11^{a} ± 0.44	21.92^{b} ± 0.88	14.42^{b} ± 0.58	9.17 ± 0.35	<LOD	<LOD	<LOD	**56.63**
55 DAFB	4.89^{d} ± 0.22	4.84^{d} ± 0.27	10.23^{c} ± 0.57	8.30 ± 0.46	<LOD	<LOD	<LOD	**33.36**
65 DAFB	9.33^{b} ± 0.42	7.73^{c} ± 0.35	5.15^{d} ± 0.23	5.94 ± 0.27	<LOD	<LOD	<LOD	**23.71**
Average	8.62	21.54	11.34	8.42				
$D\%5_{\text{"on" × Periods}}$	0.24**	0.14**	0.22**	0.32**				

No GA_1, GA_9, GA_{20}, GA_{24}, GA_{24}, GA_{51} and GA_{53} were detected in any of the samples, <LOD = below the limit of detection. The results represent mean ± standard error, means in columns with the same letter do not differ, *, **: Significant at $P < 0.05$, $P < 0.01$, respectively, by LSD test, other details are similar to Table 1.

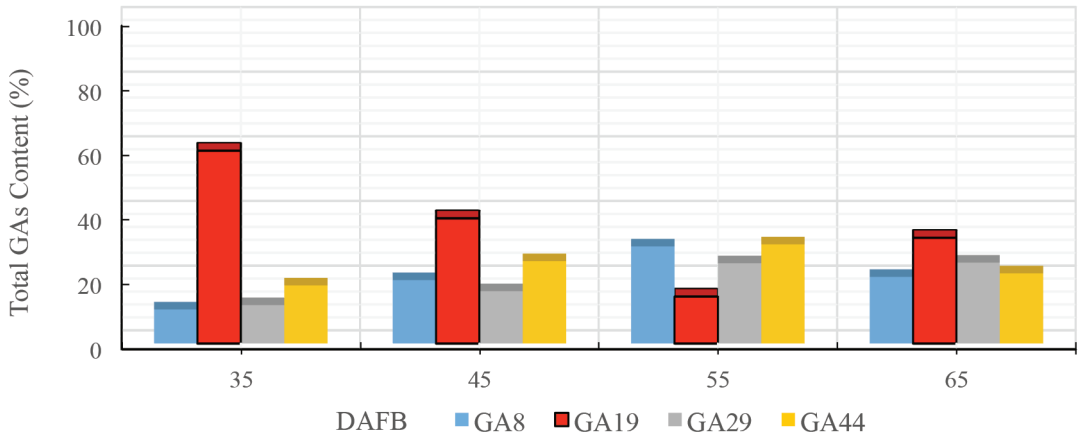

Figure 3. The gibberellins detected in pistachio panicle concentrates derived from as a percentage of the total gibberellins content. Percentages were calculated based on each gibberellin values × 100/total gibberellins.Other details are similar to Figure

GA_8(4.89–11.11 ng g^{-1}) was found to have the lowest value (Table 4). GA_{19} was the highest levels in the early periods of full flowering in May (35 DAFB), showed a regular decrease to the minimum levels after 55 DAFB and then increased 65 DAFB (Table 4).GA_8, GA_{29} and GA_{44},(4.89-11.11 ng g^{-1}, 5.94-10.27 ng g^{-1} and 5.15-15.56 ng g^{-1}, respectively) levels were high in the early stages of full blooming (35 DAFB) and decreased at the end of June (55 DAFB) and minimum decreased 65 days after full bloom (Table 4, Figure 3). It was determined that GAs in panicles were below GA_3, GA_4 and GA_{34} detection limit, GA_1, GA_9, GA_{20},GA_{24}, GA_{51} and GA_{53} could not be detected (Table 4).

4.2.2. Gibberellin content in nut samples

The nut concentration (ng g^{-1} DW) of various gibberellins (GAs) are shown in Table 5. Figure 4 shows the percentage of each GAs expressed as a percentage of the total gibberellin. Among the GAs showed different changes between periods in the "on" year trees. In total, 7 GAs were identified in the nut samples. Among the GAs, GA_{44}(13.52–69.71 ng g^{-1} in "on" year) was dominant and showed the highest value in 35 DAFB, over 69.97% of the total GAs (Table 5); GA_8(5.42–9.33 ng g^{-1}) was found to have the lowest value. GA_{19} and GA_{44} were the highest levels in the early periods of full flowering in May (35 DAFB), showed a regular decrease to minimum levels after 45 DAFB (Table 5), followed by an increase until 65 DAFB. GA_8 and GA_{29}(5.42–9.33 ng g^{-1}, 6.98–11.28 ng g^{-1}, respectively) levels were high in the early stages of full blooming (35 DAFB) and decreased at the end of June (-55 DAFB), followed by an increase 65 days after full bloom (Table 4, Figure 3). It was determined that GAs in flower buds were below GA_3, GA_4 and GA_{34} detection limit, GA_1, GA_9, GA_{19}, GA_{20}, GA_{24}, GA_{51} and GA_{53} could not be detected (Table 5).

5. Discussion

In the horticulture world, the alternate bearing of fruit is of great importance for a country's economy and producers. For almost all fruit trees showing alternate bearing, the

Table 5. The content of gibberellins detected in nut samples in different physiological periods of the trees of "on" year trees in 'Uzun' in Pistachio variety.

Gibberellins (ng/g DW)								
Periods	GA_8	GA_{19}	GA_{44}	GA_{29}	GA_3	GA_4	GA_{34}	Total gibberellins
35 DAFB	9.33^{a} ± 0.42	13.23^{a} ± 0.50	69.71^{a} ± 3.13	11.28^{a} ± 0.51	<LOD	<LOD	<LOD	99.63
45 DAFB	5.42^{d} ± 0.2	4.01 ± 0.20	32.92^{b} ± 1.65	10.71^{b} ± 0.54	<LOD	<LOD	<LOD	55.64
55 DAFB	7.28^{c} ± 0.36	5.56 ± 0.25	13.52^{d} ± 0.61	8.94^{c} ± 0.40	<LOD	<LOD	<LOD	37.29
65 DAFB	8.01^{b} ± 0.40	6.43 ± 0.33	31.76^{d} ± 0.10	6.98^{d} ± 0.35	<LOD	<LOD	<LOD	52.47
Average	7.51	7.31	36.98	9.48				
$D\%5_{\text{"on"} \times \text{Periods}}$	0.26^{**}	0.20^{**}	0.25^{**}	0.11^{**}				

No GA_1, GA_9, GA_{19}, GA_{20}, GA_{24}, GA_{24}, GA_{51} and GA_{53} were detected in any of the sample, <LOD = below the limit of detection. The results represent mean ± standard error, *,**: Significant at $P < 0.05$, $P < 0.01$, respectively, by LSD test, other details are similar to Table 1.

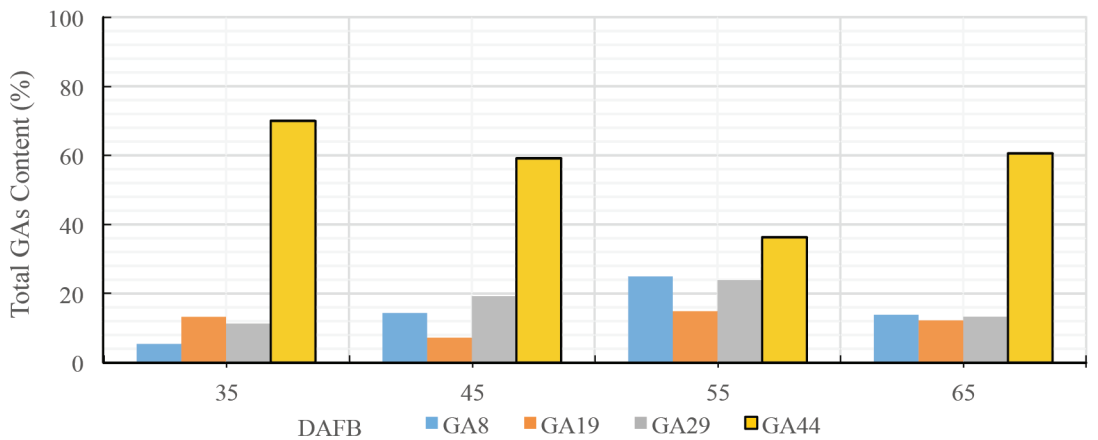

Figure 4. The gibberellins detected in pistachio nut concentrates derived from as a percentage of the total gibberellins content. Other details are similar to Figure 1.

yield depends on the flower buds formed. These histological studies revealed that abscission was initiated on May 22 (45 DAFB) and completed on July 2 (55–65 DAFB) in the "on" year samples, and bud abscission occurred in very few samples from the "off" year crop during this period (Gundesli, 2017). Many horticultural studies have been conducted on alternate bearing in general. However, the physiological causes of this condition have still not been determined. At the beginning, this problem was trying to be solved using several cultural applications, such as irrigation, pruning, and fertigation. Some researchers suggested that the competition between flower buds and food for fruit and assimilates is responsible for this phenomenon (Cetinkaya, 2004; Ulger et al., 2004; Baninasab et al., 2006; Gunes et al., 2010; Mirsoleimani et al., 2014) while others stated that unbalanced nutrition was not the main cause of the inhibition of flower bud formation, and more attention should be paid to the hypothesis of PGRs (Baktır et al., 2004; Gomez-Jimenez et al., 2010; Gunes et al., 2010; Vemmos, 2010; Gundesli et al., 2019). In higher plants, ABA and GA antagonistically regulate various physiological growth periods, including seed dormancy, seed germination, root growth, shoot elongation, leaf development, flowering, flower bud formation, abscission, and fruit development, as well as biotic and abiotic stresses (Vanstraelen and Benková, 2012; Liu and Hou, 2018). ABA was first isolated from the mature flowers of cotton, rose, peas, and fruit as an abscission accelerator (Lui and Carns 1961; Mayak et al., 1972; Ehuwens and Schwabe, 1975). Some researchers have suggested that alternate bearing was probably to be determined in abscission which effect separately and in combinations with ABA and GAs (ZacarõÂas et al. 1995; Mehouachi et al., 1996;Talon et al., 1997; GoÂmez-Cadenas et al., 2000; Baktır et al., 2004; Ulger et al., 2004; Okay et al., 2011). These reports described the determination of ABA and GAs activity in different plant organs (Tables 2–5; Figures 1–4). However, to our knowledge, no information is available on the active ABA-metabolites and GAs in pistachio and therefore this is the first-time study. In the present study, the results showed low and undetectable levels of ABA and GAs in panicles and nuts in many periods during flower bud abscission and embryo development. Among the ABA metabolites, DPA was dominant. These findings are in agreement with those of Lulsdorf et al. (2012). In a similar study by Lulsdorf et al., (2012), DPA content 1310.03 nM g^{-1}. The highest total DPA level was determined in panicle and nut (Tables 2–5; Figures 1–4). The ABA metabolite content of the panicles and nuts was high in the early period of May (at 35 DAFB) and decreased to minimum levels at 65 DAFB; i.e., during embryo development. Goldschmid (1976) reported that young fruitlets in citrus showed peak ABA concentration during the first few days after anthesis, which is in agreement with these findings. Other studies showed that the amount of ABA and similar components consistently decreased in the leaves, shoots, and flower buds from May to September (Lovatt and Ferguson, 1998, 2001; Cetinkaya, 2004; Okay et al., 2011). In contrast, Baktir et al., (2004) indicated that the ABA concentration in fruit constantly increased during embryo development and fruit set. Similar to these findings, many researchers reported that ABA was required for embryo growth during the early phase of flower and seed development (Goldschmidt 1980; Cheng et al., 2002; Frey et al., 2004), but high ABA levels in later developmental phases inhibited embryo growth by suppressing gibberellin signaling (White et al., 2000; Raz et al., 2001) and abscission was directly controlled by phytohormones (Addicott 1983). ABA has also been implicated in the abscission of young fruit trees, such as citrus (Sagee and Erner 1991; ZacarõÂas et al., 1995; GoÂmez-Cadenas et al., 2000) and apple (Vernieri et al. 1992). These findings are also consistent with previous studies in terms of plant growth regulating functions synthesized in plants and growth periods of fruit species (Talon et al., 1990; Westwood, 1993 Zacarias et al., 1995; Okay et al., 2011). GoÂmez-Cadenas et al., (2000) reported that the changes in ABA followed a biphasic pattern with transitory rises at 14 and 42 DAA (days after anthesis) and fruitlet abscission in citrus preceding both abscission waves. Thereafter, ABA decreased and returned to levels closer to those found in control fruitlets.This suggests that the amount of ABA decreases when flower bud abscission is intense (at 55–65 DAFB), during which the development and maturation in the plants continue. According to these results, ABA had a significant relationship with flower bud abscission (Tables 2 and 3, Figures 1 and 2). Previous studies investigating bioactive GAs concentrations in plants reported values within the range of 10^{-11} to 10^{-9} g g^{-1} fresh weight, depending on the organ and physiological period. Most GAs identified in the early years of GA research were shown to have the highest biological activity and act as an active hormone. GAs is found in the buds, embryos, roots, young leaves, flowers, and fruit of plants (Looney et al., 1985; Ward, 1993; Olszewski et al., 2002; Baktir et al., 2004). Although many studies have shown that GAs are effective at various stages of fruit development (Takahashi, 1974), we still do not know much about the seasonal changes of these hormones, especially in alternate bearing. This study differs from previous research in that it was the first to investigate the effect of GAs on alternate bearing in pistachio in different tissue samples and periods. We detected four dominant GAs in the different organs of pistachios, namely GA_8, GA_{19}, GA_{29} and GA_{44} (Tables 4 and 5).The literature contains studies that analyzed the

hormone amounts of the fruit, leaves and flower buds of pistachio (Cetinkaya, 2004; Gunes et al., 2010; Vemmos, 2010; Okay et al., 2011; Gundesli et al., 2019). Furthermore, Khalifah et al. (1965) and Goldschmidt (1976) reported that citrus samples contained three GA–like substances, two of which were tentatively identified as GA_1 and GA_9. Compared to recent qualitative studies of GAs in pistachio (Cetinkaya, 2004; Okay et al., 2011) and apple (Hedden 1990; Okamuro et al., 1996), more specific GAs was identified in these samples. In this research, GA_{19} and GA_{44} were the dominant metabolites with the highest value (Tables 4 and 5; Figures 3 and 4). These findings are in agreement with those of Rodrigo et al. (1997) and Lulsdorf et al., (2012).This is in agreement with Tromp (1982), who showed that GA_{19} was dominant in citrus which investigated the effects of GAs on the induction of the flower of citrus species during different physiological periods and stated that GA_3, GA_4 and GA_7 were effective. According to this study GA_{19} and GA_{44} showed to high in the early period of 35 DAFB (May) and after decreased to levels 45-55 DAFB during intense flower bud abscission. The amount of GA_{19} and GA_{44} increased toward the embryo development period (65 DAFB-July) (Tables 4 and 5). These findings are also in agreement with previous research demonstrating that GA-like substance functions increased in the fruit set and during the growth periods of different fruit trees (Baktır et al., 2004; Cetinkaya, 2004; Okay et al., 2011; Suman et al., 2017). Rodrigo et al. (1997) reported the highest content of GA_1 precursors (GA_{19} and GA_{20}) as well as the GA_{29} (catabolite of GA_{20}) shortly after anthesis in pea (*Pisum sativum* L.) which then rapidly declined until 12 DAA that these findings are in agreement with the findings. In the current research, the increase in GAs and the decrease in the ABA level show that both these plant growth substances control flower bud abscission and initial embryo development along with other essential components (Tables 2–5; Figures 1–4). Lui and Carns (1961) and Mayak et al., (1972) similarly reported that the ABA amount was in a positive relationship with abscission. In other studies, the GA and ABA amounts of the fruit, leaves and flower buds of different fruit trees showing alternate bearing also gradually decreased during embryo development [Cetinkaya 2004, and Okay et al., 2011 (pistachio); Shulman and Lavee 1980, Baktır et al., 2004 (olive)]. Swain et al., (1995) concluded that only GA_1 and/or GA_3 are associated with embryo and endosperm and are thus physiologically active in pea whereas GA_{20} and GA_{29} found in the testa of a seed had biological role. Therefore, the common finding of the previous studies indicated that are the amounts and proportions of PGRs carried by synthesis difference between various organs of the plant and their physiological growth stage (Ulger, 1977; Lavee, 1989; Baktır et al., 2004; Okay et al., 2011). Therefore, in some previous studies reported that tried to take control of abscission with external applications GAs and ABA both on- and off-crop years, but the results are not definitive (Lin et al., 1984; Ferguson and Maranto 1989; Lovat and Ferguson, 2006; Açar et al., 2006). These findings are consistent with those obtained from different plant growth regulating functions synthesized in pistachio during different growth periods (Takeda and Crane 1980; Salisbury and Ross, 1991; Westwood, 1993; Açar et al., 2006; Cetinkaya, 2004; Lovat and Ferguson, 2006;Gunes et al., 2010; Okay et al., 2011; Gundesli et al., 2019). It is important to monitor the changes of in PGRs, especially GA and ABA during flower bud abscission and embryo development in order to explain the physiological mechanisms in pistachio, such as the formation of flower bud abscission and embryo development (alternate bearing). In particular, the effect of external growth regulator applications during slow or low PGR synthesis should be investigated to determine the optimum times for such applications.

6. Conclusion

To this knowledge, this is the first study that detected ABA metabolites and GAs detected in different tissue samples obtained from pistachio during different growth periods. PGRs appear to regulate flower bud abscission interactively. This research provides a basis for the role of two PGR classes (ABA metabolites and GA_S) during flower bud abscission and embryo development in pistachio. In total, seven ABA metabolites and four GAs were identified in panicle and nut samples. As a result, DPA and GA_{19} were found to be the dominant ABA metabolite and GAs, respectively. These data confirm that ABA metabolite and GAs concentrations are related to the flower bud abscission process and embryo development; thus, ABA and GAs play a role in irregular or alternate bearing.It seems that in "on" year trees, ABA and GAs are translocated to the fruit, especially during embryo development. Through analyses aimed at explaining the physiological functions of the main ABA metabolites and GAs, we obtained useful data concerning how PGRs control flower bud abscission and embryo development.It is also concluded that GAs can control fruit development in various ways and at different stages of development. The data also show an important a general relationship between ABA metabolite and GAs levels during flower bud abscission (alternate bearing) and embryo development in pistachio.

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