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Changes in endogenous auxin level during flower bud abscission process in Pistachio (*Pistacia vera* L.)

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Abstract: The regulation of auxin (indole-3-acetic acid, IAA) level in plants is a complex mechanism and the concept is not completely understood. There are many lines of evidence suggesting that amino acid conjugates of IAA play a vital role in auxin homeostasis, but the involved plant enzymes are still under investigation. In the present study, the alteration in the levels of auxin and its conjugates in the different physiological stages and organs, as well as its role in flower bud abscission in *Pistacia vera* L., were investigated. The levels of the auxin and its conjugates, such as IAA, indole butyric acid (IBA), IAA-Ala, IAA-Leu, IAA-Asp, and IAA-Glu, were analyzed by ultraperformance liquid chromatography-electrospray ionization-mass spectrometry (UPLC/ESI-MS/MS) technique. Among the auxin conjugates, IAA was the main conjugate and dominant indole detected in pistachio extract observed in this work. It has also been determined that quite a few organs of 'Off-year' trees have higher auxin content than 'On-year' trees. It was observed that the auxin level is low in fruit-bearing trees in both leaves and shoots. It affects fruit yield and fruit bud abscission and plays some role in the alternate bearing. Decreased levels of auxin were observed in most of the organs of 'On-year' trees during kernel development resulting in bud abscission. This study shows that auxin conjugates play an important role in IAA metabolism, particularly in flower bud abscission and fruit kernel development.

Key words: Auxin, conjugate, *Pistacia vera* L., alternate bearing

1. Introduction

Flowering and abscission in plants have great economic importance in the horticultural world. In most fruit trees, the yield depends on the quantity and quality of the flower buds. Alternate bearing is a serious issue that occurs in both deciduous and evergreen fruits as well as in nut trees. Alternate bearing is a phenomenon wherein a tree has a higher yield in one year ('On-year') followed by a lower yield ('Off-year') in the succeeding year. The alternate bearing mechanism in pistachio is quite dissimilar from other tree species. Pistachio starts bearing the inflorescence buds in the preceding year and abscission commences during June and continues throughout July in 'On-year' trees, causing a yield loss in 'Off-year' season (Crane and Nelson, 1971; Crane et al., 1973; Porlingis, 1974; Monselise and Goldschmindt, 1982; Crane and Iwakiri, 1987; Stevenson and Shakel, 1998; Vemmos, 1999a, 1996b; Gundesli, 2017). Several researchers have tried to explain the physiological mechanism of flower bud abscission process in pistachio but the actual concept is still unknown (Monselise and Goldschmindt, 1982; Nzima et al., 1997). In the beginning, this problem was addressed by improving the conventional cultural applications, for instance irrigation, pruning, fertigation, etc. (Ferguson and Maranto, 1989; Ak and Kaska, 1992; Tekin, 1992; Weinbaum et al., 1994; Acar et al., 2006; Gunes et al., 2010; Cetinkaya et al., 2018). Subsequently, the effects of plant growth regulators, carbohydrates, polyamines, and phenolic compounds were also studied, and recently, molecular and biotechnology studies have been carried out to solve the issue of alternate bearing in pistachios (Crane and Nelson, 1971; Nzima et al., 1997; Vemmos, 1999a; Acar et al., 2006; Teale et al., 2006; Acar et al., 2011; Okay et al., 2011; Cetinkaya and Ozguven, 2016; Gundesli et al., 2019). Monselise and Goldschmidt (1982) described the possible endogenous (hormonal) factors that might influence the alternate bearing in fruit trees. Endogenous plant growth regulators, such as auxin, play a key role in controlling the developmental processes throughout the life cycle of a plant (Hoad, 1983; Bialek and Cohen, 1992; Lu et al., 2008; Davies, 2010). Indole-3-acetic acid (IAA), a naturally occurring plant growth regulator,

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controls a variety of physiological processes (Ribnicky et al., 1998; Teale et al., 2006; George et al., 2008; Abel and Theologis 2010; Ljung, 2013). The knowledge in this area has greatly evolved in recent years due to the advancement in modern biotechnological tools, genetic and biochemical processes, and precise methods for the identification and quantification of endogenous growth regulators. IAA may present as a free hormone or in-bound forms in which the carboxyl group is attached to the sugars via ester linkages or to amino acids via amide linkages (Ludwig-Muller, et al., 1996; Davies, 2004; George et al., 2008; Ludwig-Muller, 2011). IAA conjugates are considered to function in the storage, transportation, and segmentation of auxins. The formation and hydrolysis of conjugates are developmentally regulated and have been reported to vary significantly between the plant tissues (Piskornik and Bandurski, 1972; Bialek and Cohen, 1986; Ostin et al. 1992; Barlier et al. 2000; Ljung, 2013). The amide conjugates with Glu, Asp, Gly, Ala, Val, Leu, or other amino acids occur in a variety of plants such as IAA-myoinositol and IAA-glucose in maize (Piskornik and Bandurski, 1972), IAA-Asp and IAA-Glu in soybean (Epstein et al., 1986), and IAA-Ala in *Picea abies* (Ostin, et al., 1992). In *Arabidopsis*, IAA-glucose, IAA-Asp, IAA-Glu (Tam et al., 2000), IAA-Ala, IAA-Leu (Kowalczyk and Sandberg, 2001), and an IAA-peptide (Walz et al., 2002) have been reported. Although the pathway of auxin metabolism and transportation has been deduced, the mechanism of its effect on flower bud abscission and alternate bearing is not clearly known to date. Hence, this study was focused on the alterations in the levels of auxin and its conjugates in *Pistacia vera* during different physiological periods in order to address the issue of flower bud abscission.

2. Materials and methods

2.1. Plant material

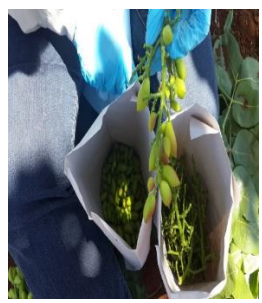
Twelve adjoining Uzun trees (*Pistacia vera* Desf. rootstock, 33-years-old, planted at 10 × 10 m intervals) were selected for the study in 2013 ('On-year' and 'Off-year') from the Research and Experimental farm of the Ministry of Agriculture's Pistachio Research Institute of Gaziantep. In order to ensure same year alternate bearing in both 'On-year' and 'Off-year' pistachio trees, all the flowers of 'Off-year' 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 two groups of trees were maintained with their alternate bearing habit until the end of the study in 2015. The shoots, leaves, panicles, and nuts were sampled from 'On-year' and 'Off-year' year trees for further analysis (Figure 1).



Leaf samples to 'On' year



Flower bud and shoot samples to 'On' year



Panicle and nut samples to 'On' year



'Panicle and nut samples to 'On' year



Flower bud and shoot samples to 'Off' year



Leaf samples to 'Off' year

Figure 1. Images of the materials used in the research.

2.1.1. Plant tissue sampling

The samples were collected between 08:00 am and 10:00 am after 35, 45, 55, and 65 days of full blooming (DAFB) in the year 2015 (Table 1). The sampling dates were selected based on previous studies (Gundesli, 2017) in which flower abscission layers initiated 45 DAFB and accomplished 55 DAFB from 'On-year' samples. This period coincided with the second phase of intense bud abscission that started 65 DAFB; increased kernel development of pistachio and bud abscission also occurred on a few samples of 'Off-year' samples during this period. Therefore, the time before 65 DAFB was considered in this paper. The experiments were designed as a randomized complete block with three replications and one tree per replication. For auxin analysis, one-year-old branches from different directions of the canopy (north, south, east, and west) were collected per replication; young leaves (50 in number), shoots (10 in number), panicles (10 in number), flower buds (30 in number), and shell nuts (30 in number) were excised and immediately transferred to dry ice, separated into leaves, shoots, buds, panicles, and nuts, and frozen in liquid nitrogen in the in laboratory (Figure 1). The samples were rinsed with sterile distilled water to remove dust and soil, lyophilized (ilShin Freeze Dryers, FD-8518, Ede, Netherlands) using a lyophilizer, homogenized using a coffee grinder, and stored at +4 °C. Nut samples were homogenized including hull and embryo.

2.2. Methods

2.2.1. Determination of auxin levels

The auxin content of pistachio samples, including IAA, IBA, IAA-Asp, IAA-Glu, IAA-Ala, and IAA-Leu was quantified at the National Research Council of Canada by means of ultraperformance liquid chromatography-electrospray tandem mass spectrometry (UPLC-ESI-MS/MS). The deuterated form of the auxin and its conjugates was used as internal standard and synthesized according to Abrams et al. (2003) and Zaharia et al. (2005). The extraction, purification, and quantification of the samples were performed according to the protocol reported by Chiwocha et al. (2003, 2005). UPLC/ESI-MS/MS was carried out using a Waters ACQUITY UPLC system equipped with a binary solvent delivery manager and a sample manager coupled to Waters Micromass Quattro Premier XE quadruple tandem mass spectrometer via Z-spray interface. MassLynxTM and QuanLynxTM (Micromass, Manchester, UK) were used for data acquisition and data analysis. The samples were injected into the ACQUITY UPLC HSS C18 SB column (2.1 9 100 mm, 1.8 μm) having an inline filter and separated by a gradient elution of water containing 0.02% formic acid in addition to a mixture of acetonitrile with methanol (volume ratio, 50:50). The quantification of auxin and its conjugates was performed following the procedure described by Lulsdorf et al. (2013).

Table 1. The dates on which auxin sampling was conducted in pistachio. *DAFB: Days after full blooming; * Full blooming date respectively in study years: 10.04.2015.

	Plant parts	
	Shoots, leaves, flower buds, panicles, and nuts	
Physiological periods	35 DAFB	15.05.2015
	45 DAFB	25.05.2015
	55 DAFB	04.06.2015
	65 DAFB	14.06.2015

2.3. Statistical analyses

The data were analyzed by JMP statistical software from SAS (V7) (SAS Institute Inc. Cary, NC, USA) and all the analytical values were an average of three replications. The significant differences were compared by the least significant differences (LSD) test executed at 5% level of probability. The means ± the standard error (SE) were calculated from three independent experiments and concentrations, which are reported in ng g^{-1} dry weight (DW).

3. Results

3.1. Identification and quantification of auxin

3.1.1. Auxin content in shoot samples

The significant differences ($P < 0.05$) in the levels of auxin and its conjugates (ng/g DW) in shoot samples were recorded from the analysis of 'On-year' and 'Off-year' Uzun pistachio trees during various periods (Table 2). Figure 2 depicts individual auxin conjugates expressed as a percentage of the total auxin content. Four auxin conjugates were identified from the shoot-derived accumulation. In total, 2 auxin conjugates were identified. There were irregular tendencies of auxin accumulation in the different physiological periods of 'On-year' and 'Off-year' trees. Among the auxin conjugates, IAA (19.12–51.13 ng/g DW in 'On-year'; 26.09–69.59 ng/g DW in 'Off-year' year) was the main conjugate and dominant indole detected and contributing over 80% of the total auxin content (Table 2, Figure 2). On the contrary, IAA-Asp (5.10–9.71 ng/g DW in 'On-year'; 10.48–42.65 ng/g DW in 'Off-year') was found to have the lowest value. Furthermore, it was noticed that IAA and IAA-Asp levels of 'On-year' were high during the early period of flowering in May (55 DAFB) and at a minimum level in July (65 DAFB). As mentioned in Table 2, the shoots of 'Off-year' trees showed higher auxin content than 'On-year' trees.

3.1.2. Auxin content in leaf samples

Significant differences ($P < 0.05$) were noticed among the four identified auxin conjugates in the leaf samples

Table 2. Auxin contents detected in shoot samples during different physiological periods from 'On-year' and 'Off-year' trees of pistachio. <LOD = below the limit of detection; ND: Not detected in any of the samples; the results represent mean ± standard error,*,**: Significant at P < 0.05, P < 0.01, respectively, by LSD test.

Auxin content (ng/g DW)								
Indole	35 DAFB		45 DAFB		55 DAFB		65 DAFB	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
IAA	69.59^{a} ± 3.13	51.13^{b} ± 2.32	27.90^{b} ± 0.22	44.09^{a} ± 0.33	26.09^{a} ± 0.23	23.40^{b} ± 0.18	36.62^{a} ± 0.22	19.12^{b} ± 0.23
D%5$_{\text{'On-year' × 'Off-year''}}$	0.86**		0.51**		0.61*		0.50**	
IBA	ND	ND	ND	ND	ND	ND	ND	ND
Amino acid conjugate								
IAA-Asp	42.65 ± 0.36	9.71 ± 0.16	31.45 ± 0.11	9.31 ± 0.15	14.37 ± 0.19	5.10 ± 0.12	10.48 ± 0.22	5.48 ± 0.24
D%5$_{\text{'On-year' × 'Off-year'}}$	0.50**		0.32**		0.38**		0.52**	
IAA-Ala	ND	ND	ND	<LOD	ND	ND	ND	<LOD
IAA-Glu	ND	ND	ND	<LOD	ND	ND	ND	ND
IAA-Leu	ND	ND	ND	ND	ND	ND	ND	ND
Total indoles	112.24	60.84	59.35	53.40	40.46	28.50	47.10	24.60

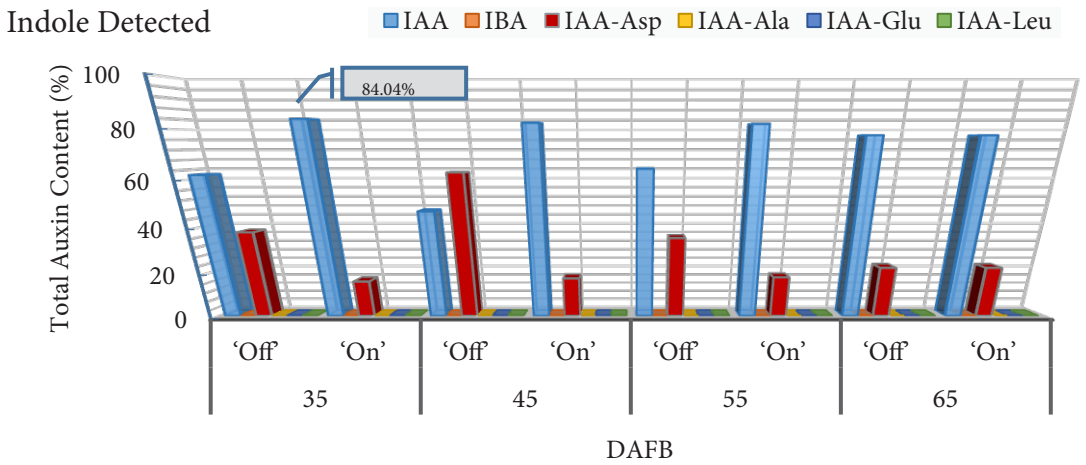


Figure 2. Indole conjugates detected in pistachio shoot concentrates as the percentage of total auxin content. DAFB, days after full blooming. Percentages were calculated based on each auxin values × 100/total auxin.

obtained from 'On-year' and 'Off-year' trees (Table 3). Figure 3 depicts individual auxin conjugates expressed as a percentage of the total auxin content. In total, 2 auxin conjugates were identified. Among the auxin conjugates, IAA (8.77–26.14 ng/g DW in 'On-year' and 9.18–31.48 ng/g DW in 'Off-year') was the main conjugate and dominant indole detected that was found at a maximum and contributing over 80% of the total auxin content (Figure 3). On the other hand, IAA-Asp (7.25–14.64 ng/g DW in 'On-year'; 7.59–23.26 ng/g DW in 'Off-year') was found to have the lowest value. In the early periods of full flowering during May, IAA levels were highest in the leaves at 45 DAFB and lowest at 35 DAFB. Subsequently, it started to increase again. It was determined that the leaves of 'Off-year' trees have higher auxin content than 'On-year' trees (Table 3).

3.1.3. Auxin content in flower bud samples

The flower buds obtained from 'On-year' and 'Off-year' Uzun pistachio trees exhibited significant variations in auxin conjugate accumulation (Table 4). As shown

Table 3. Auxin contents detected in leaf samples in different physiological periods from the 'On-year' and 'Off-year' trees of pistachio; other details are similar to Table 2.

Auxin content (ng/g DW)								
Indole	35 DAFB		45 DAFB		55 DAFB		65 DAFB	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
IAA	31.48[a] ± 0.31	26.14[b] ± 0.28	9.18 ± 0.20	8.77 ± 0.27	10.10 ± 0.22	10.19 ± 0.21	10.83[b] ± 0.13	12.16[a] ± 0.07
D%5 $_{\text{'On-year' × 'Off-year'}}$	0.66**		0.54		0.49		0.24**	
IBA	ND	ND	ND	ND	ND	ND	ND	ND
Amino acid conjugate								
IAA-Asp	7.59 ± 0.25	7.25 ± 0.09	18.92[a] ± 0.19	14.64[b] ± 0.46	23.26[a] ± 0.36	11.32[b] ± 0.35	21.07[a] ± 0.11	9.45[b] ± 0.09
D%5 $_{\text{'On-year' × 'Off-year'}}$	0.41		0.80**		0.81**		0.22**	
IAA-Ala	ND	ND	ND	ND	ND	ND	ND	<LOD
IAA-Glu	ND	ND	ND	ND	ND	<LOD	ND	ND
IAA-Leu	ND	ND	ND	ND	ND	ND	ND	ND
Total indoles	39.07	33.39	28.10	23.41	33.36	21.51	31.90	21.61

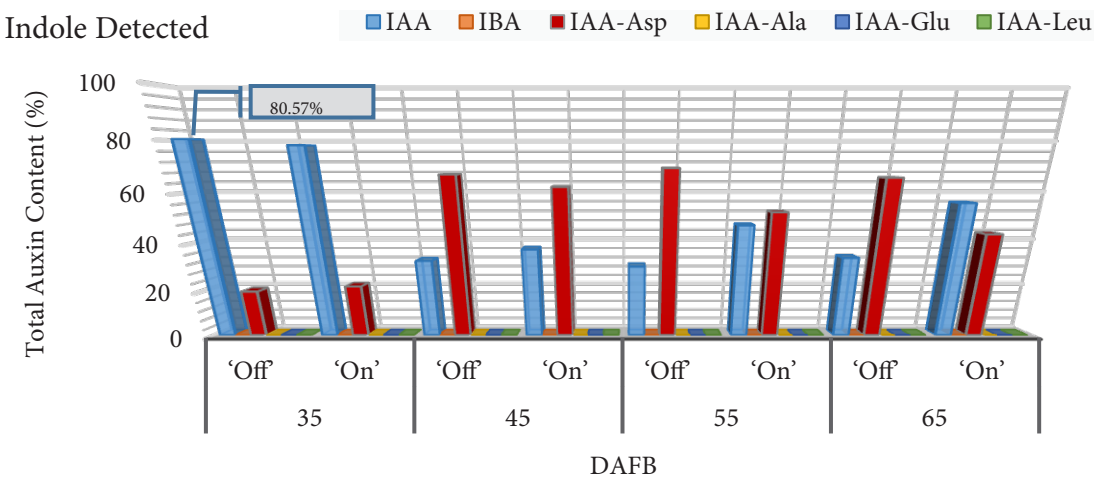


Figure 3. Indole conjugates detected in pistachio leaf concentrates as the percentage of total auxin content. DAFB, days after full blooming; other details are similar to Figure 3.

in Figure 4, IAA was the main conjugate and dominant indole detected that was found at the maximum (6.97–10.31 ng/g DW in 'On-year' and 7.68–19.23 ng/g DW in 'Off-year') and contributing over 19% of the total auxin content (Figure 4). The flower buds of 'Off-year' trees had higher auxin content than 'On-year' trees. The content of IAA was higher during the early stages of full blooming (35 DAFB) in the flower buds of 'On-year' and then decreased gradually in 65 DAFB. However, IAA content in the 'Off-year' trees was lower in 35 DAFB and then increased gradually. In this study, the opposite effect between the leaves and flower buds might have an effect on flower bud abscission.

3.1.4. Auxin content in panicle samples

Three auxin conjugates were identified in the panicles samples of 'On-year' Uzun pistachio trees at different periods that were significantly different (Table 5). In total, 3 auxin conjugates were identified. Among the auxins, IAA (32.81–46.92 ng/g DW in 'On-year') showed the highest value, i.e., 77.30% of the total auxin content (Figure 5). The lowest value was recorded by IAA-Glu (3.32–6.72 ng/g DW in 'On-year'). Although IAA-Glu was not found

Table 4. The content of auxins detected in flower bud samples in different physiological periods of 'On-year' and 'Off-year' trees of pistachio; other details are similar to Table 2.

Auxin content (ng/g DW)								
Indole	35 DAFB		45 DAFB		55 DAFB		65 DAFB	
	'Off'	'On'	'Off'	'On'	'Off'	'On'	'Off'	'On'
IAA	7.68 ± 0.25	10.31 ± 0.15	8.56 ± 0.30	8.78 ± 0.19	10.71 ± 0.31	7.94 ± 0.25	19.23 ± 0.28	6.97 ± 0.28
$D\%5_{\text{'On-year' × 'Off-year'}}$	0.47**		0.55		0.61**		0.63**	
IBA	ND	ND	ND	ND	ND	ND	ND	ND
Amino acid conjugate								
IAA-Asp	ND	ND	ND	<LOD	ND	ND	ND	ND
IAA-Ala	ND	ND	ND	ND	ND	ND	ND	ND
IAA-Glu	ND	ND	ND	ND	ND	ND	ND	ND
IAA-Leu	ND	ND	ND	ND	ND	ND	ND	ND
Total indoles	7.68	10.31	8.56	8.78	10.71	7.9	19.23	6.97

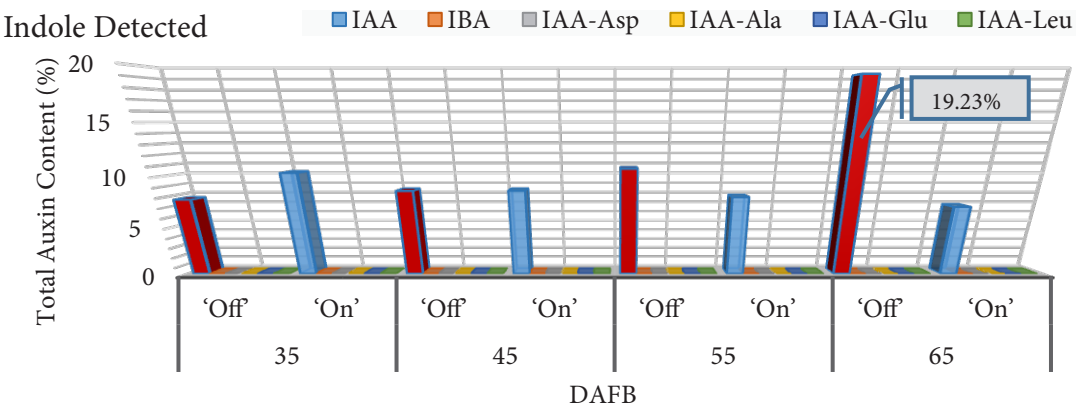


Figure 4. Indole conjugates detected in pistachio flower bud concentrates as the percentage of total auxin content. DAFB, days after full blooming; other details are similar to Figure 3.

in shoots, leaves, and nuts, it appeared in the panicles. IAA in the panicle was high at 35 DAFB and then decreased. However, IAA-Asp was found to have exactly the opposite effect in relation to IAA.

3.1.5. Auxin content in nut samples

Three auxin conjugates were identified in the nut samples that were significantly different between 'On-year' and 'Off-year' Uzun pistachio trees (Table 6). In total, 2 auxin conjugates were identified. Among them, IAA-Asp (5.16–160.73 ng/g DW in 'On-year') was the maximum and contributed 90.89% of the total auxin content (Figure 6). The IAA content in nuts was higher during the first period of full blooming, i.e., 65 DAFB, but it had an opposite effect on IAA-Asp in comparison to IAA. IAA-Glu was detected in the nuts, but not in shoots, leaves, buds, or panicles (Table 6).

4. Discussion

Alternate bearing is a very widespread phenomenon occurring in both deciduous and evergreen trees. Alternate bearing is one of the most important problems (to cause irregular fruit yield among the years) in pistachios. Alternate bearing occurs through shedding the inflorescences buds in the abundant year that would produce the next year's production. Flower bud initiation in pistachio begins in early April and May after 5 to 10 days of inflorescence, with flowers forming continuously until early July in both 'On-year' and 'Off-year' trees.

Flower bud abscission occurs in 'On-year' and 'Off-year' trees and is a two-phase process. The first phase (30%–38% bud-drop) occurs during the initial 5- to 6-week period of fruit growth in both 'Off-year' and 'On-

Table 5. Auxin contents detected in panicle samples in different physiological periods only from 'On-year' trees of pistachio; other details are similar to Table 2.

Auxin content (ng/g DW)						
Indole	35 DAFB	45 DAFB	55 DAFB	65 DAFB	Average	$D\%5_{\text{'On-year' × 'Off-year'}}$
IAA	46.92[a] ± 0.29	43.85[b] ± 0.13	32.81[d] ± 0.18	33.93[c] ± 0.19	39.38	0.47**
IBA	ND	ND	ND	ND		
Amino acid conjugate						
IAA-Asp	18.53[a] ± 0.26	8.27[c] ± 0.24	5.93[d] ± 0.15	15.67[b] ± 0.39	12.09	0.64**
IAA-Ala	ND	ND	ND	ND		
IAA-Glu	3.87[c] ± 0.19	4.61[b] ± 0.12	6.72[a] ± 0.18	3.32[d] ± 0.10	4.63	0.47**
IAA-Leu	ND	ND	ND	ND		
Total indoles	69.32	56.73	45.46	52.92		

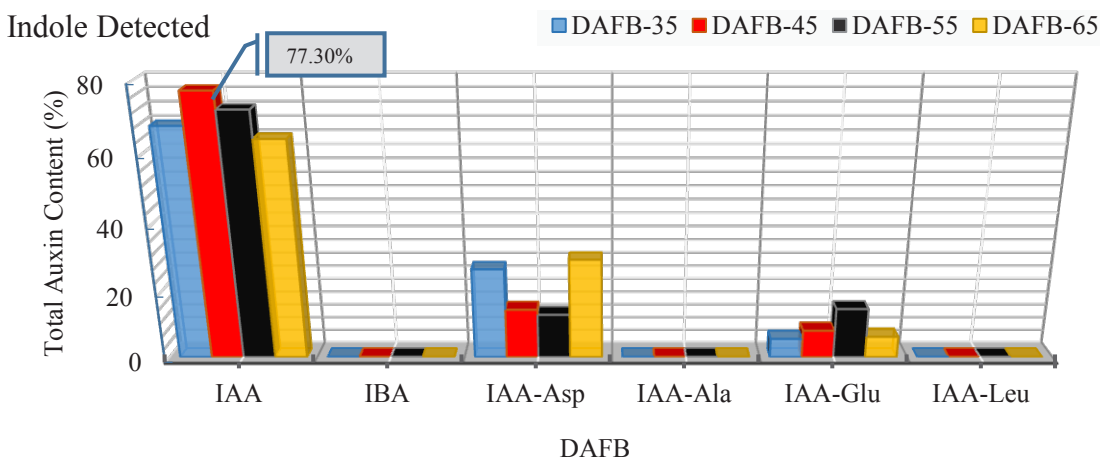


Figure 5. Indole conjugates detected in pistachio panicle concentrates as the percentage of total auxin content. DAFB, days after full blooming; other details are similar to Figure 3.

year' trees. This period coincides with the lignification of the endocarp and was attributed to a stimulus originating from the roots. The second and severe phase occurs only in the 'On-year' during the 10 to 12 week period of fruit growth. This period coincides with rapid seed growth. Four months after pollination, the fruits fill the shell and are harvested (Ayfer, 1967; Crane and Iwakiri, 1987; Ayfer et al., 1990; Ak and Kaşka, 1992; Tekin et al., 2001; Okay et al., 2011). Gundesli (2017) reported that pistachio showed flowering changes dependent on weather conditions that occurred in the first period in early April. At the end of this period, flower bud abscission started 55 days after full bloom (early June) and continued to increase rapidly during the period of fruit kernel development (75 DAFB). However, the microscopic examination performed by us showed that the rupture layer of pistachio flower buds started in the last week of May (Gundesli, 2017). Many papers were published previously attempting to solve the alternate bearing phenomenon. In the beginning, there was an attempt to solve this problem using several cultural applications such as irrigation, pruning, fertigation, etc. However, recently the effects of plant growth regulators, carbohydrates, polyamines, and phenolic compounds on alternate bearing of pistachios were also investigated; studies on molecular and biotechnology tools were also started recently. Yet, the mechanism of internal factors influence on bud abscission and alternate bearing mechanism were not entirely revealed. Many reviews have

Table 6. Auxin contents detected in nut samples in different physiological periods only from 'On-year' trees of Uzun pistachio variety; other details are similar to Table 2.

Auxin content (ng/g DW)						
Indole	35 DAFB	45 DAFB	55 DAFB	65 DAFB	Average	D%5 $_{\text{'On-year' × 'Off-year'}}$
IAA	20.55^{a} ± 0.25	18.65^{b} ± 0.17	11.49^{c} ± 0.20	10.05^{d} ± 0.21	15.19	0.47**
IBA	ND	ND	ND	ND		
Amino acid conjugate						
IAA-Asp	27.29^{b} ± .0.27	5.16^{c} ± 0.16	27.36^{b} ± 0.32	160.73^{a} ± 3.80	55.23	0.48**
IAA-Ala	ND	ND	ND	ND		
IAA-Glu	ND	ND	ND	ND		
IAA-Leu	ND	ND	ND	ND		
Total indoles	47.84	23.81	38.85	176.84		

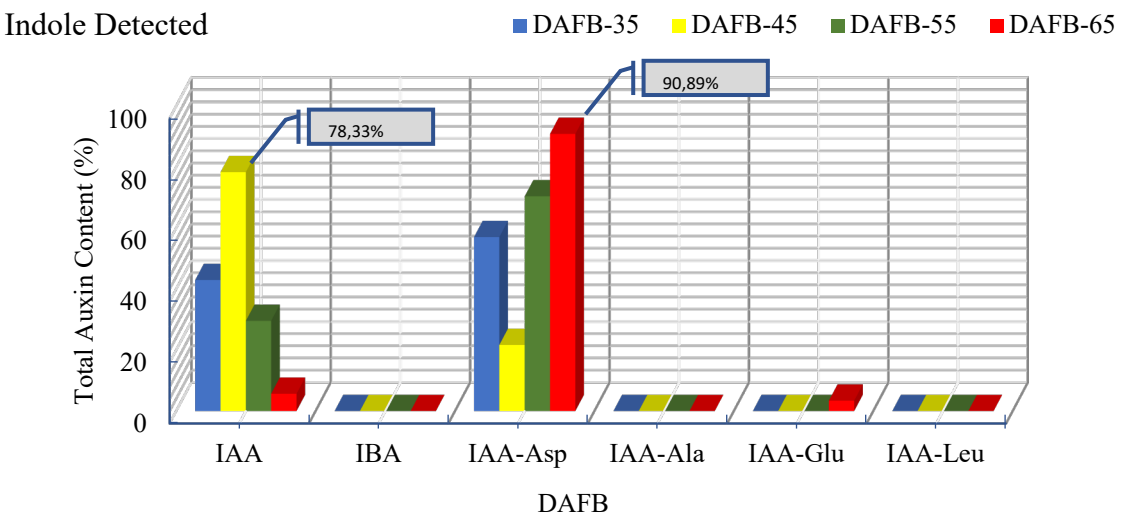


Figure 6. Indole conjugates detected in pistachio nut concentrates as the percentage of total auxin content; other details are similar to Figure 3.

been carried out about alternate bearing in general and on pistachio specifically. Recently, two hypotheses were proposed regarding the possible factors causing alternate bearing. In particular, internal factors corresponding to plant growth regulators compete among the buds, leaves, or fruits and cause bud abscission and fruit. However, the actual reason behind the inflorescence bud abscission in pistachio is still unknown (Porlingis, 1974; Takeda and Crane, 1980; Monselise and Goldschmidt, 1982; Rosecrance et al., 1998; Vemmos, 1999a, 1999b). Plant growth regulators have been used in pistachio fruit production for influencing flowering, fruit set, and flower bud abscission. These regulators have also been used to influence flowering, abscission, fruit quality factors like peel quality and color, fruit size, and juice quality, and to improve total soluble solids in different pistachio species (Takeda and Crane, 1980; Ferguson and Maranto, 1989; Vemmos et al., 1994; Lovatt and Ferguson, 1998, 2001; Cetinkaya, 2004; Acar et al., 2006; Okay et al., 2011). The primary natural auxin, indole-3-acetic acid, is produced mostly in subapical regions of actively growing shoots, young leaves, and developing embryos. It controls cell enlargement by affecting the extensibility of the cell wall and may induce or retard abscission of mature fruit (Bons et al., 2015). This research is the first report on auxin and IAA conjugates being found in different organs of pistachio

and assessing their role in alternate bearing or flower bud abscission. Significant auxin activity has been reported in different organs of many species and in later stages of growth. Further, there are reliable and precise measurement methods along with significant information with respect to the free plant growth regulator levels (Ribnicky et al., 1998; Prinsen et al., 2000; Tivendale and Cohen, 2015). However, it is not yet clear whether the auxins in pistachio have a direct biological function in flower bud abscission. The quantification of IAA and its conjugate metabolites in various organs of pistachio during flower bud abscission were assessed in this research (Tables 2–6). The auxin at the highest level in pistachio was found to be IAA and our findings are consistent with previous reports (Bartel, 1997; Normanly et al., 1997; Prinsen et al., 2000). The growth regulators, particularly IAA, play an important role during all stages of the life cycle of the plant, starting from embryogenesis to aging (Cohen and Bandurski, 1982; Davies, 1995). Some new IAA-amide conjugates were also found and they made an important contribution to the total IAA pool. Previously, individual IAA-amide conjugates were identified, such as IAA-Asp in Scots pine (Andersson and Sandberg, 1982) and Douglas fir (Chiwocha and von Aderkas, 2002), IAA-Glu and IAA-Asp in cucumber (Sonner and Purves, 1985) and soybean (Cohen, 1982; Epstein et al., 1986), and IAA-Ala in spruce (Ostin et al., 1992). A wide range of IAA derivatives conjugated to amino acids has also been studied and identified in *Arabidopsis* (Tam et al., 2000; Kowalczyk and Sandberg, 2001) and *Helleborus niger* (Pencik et al., 2009). In the current study, among the auxin conjugate IAA was the main conjugate and dominant indole detected in pistachio extract. Our findings are in agreement with IAA dominant in different plant species (Stirk et al., 2004; Ruzicka et al., 2007; Okay et al., 2011; Lulsdorf and Yuan, 2012). Many IAA conjugates with different levels of accumulation were identified in different organs of pistachio from both 'On-year' and 'Off-year' trees, and the results were significant during flower bud abscission. However, IAA-Glu conjugates have not been determined in most organs. IAA-Ala, IAA-Leu, IAA-Asp, and IAA-Glu were the only IAA-amino acid conjugates found in *Arabidopsis* seedlings (Tam et al., 2000). In addition, IAA-Asp and IAA-Glu were only 1% of the total IAA-amide conjugates in *Arabidopsis* (Tam et al., 2000), and even lower quantities of IAA-Ala and IAA-Leu were present (Kowalczyk and Sandberg, 2001; Rampey et al., 2004). In the current research, IAA-Ala and IAA-Leu were not determined. However, in a different report by Cetinkaya (2004), IAA levels in leaf and flower buds of pistachio were analyzed and it was found that IAA in leaves increased much more than in flower buds. Furthermore, IAA levels reached the highest level in June and the lowest in July. In contrast to our findings, the amount of IAA in leaves and shoots was higher in the 'On-year' trees and decreased after full bloom (65 DAFB). On the other hand, its level was higher in the flower buds of 'On-year' trees and decreased after full bloom (65 DAFB). Also, the IAA level was lower in flower buds in 'Off-year' trees and its transportation from shoots and leaves along with its accumulation in flower buds were decreased. The amount of IAA was gradually increasing in the flower buds of 'Off-year' trees after full blooming. It was especially low after 55 DAFB and 65 DAFB, which on 35 DAFB and 45 DAFB is high. Similar findings were also reported by Okay et al. (2011). It has been stated in another study reported by Baktir et al. (2004) that IAA content was highest in Memecik olive cultivar during July and June in 'On-year' trees. Bons et al. (2015) have reported that the auxin amount is in a positive relationship with abscission by causing a delay of abscission, resulting in improvement in fruit quality and yield in citrus. In our research, IAA and its conjugates (IAA-Asp) have a direct effect on bud abscission or alternate bearing. It is clear that auxin accumulation was less in both leaves and shoots of 'On-year' trees and the level increased, especially during flower bud abscission and alternate bearing. The evidence obtained shows that auxin conjugates play an important role in IAA metabolism, temporary storage reserves, bud abscission, and fruit kernel development. In fact, Crane et al. (1973) and Porlingis (1974) suggested that the growth regulators produced in the leaves or seeds are transferred to the inflorescence buds and induce bud abscission. According to this, it is understandable that the auxin content decreases 55 and 65 days after full bloom after fruit kernel development. Consequently, it is necessary to know the concept behind the seasonal effect of auxin to explain physiological changes like flower bud formation, abscission, fruit development, and alternate bearing.

In conclusion, thorough research was conducted to record the changes in the activity of auxin and its conjugates during flower bud abscission in the Uzun variety of *Pistacia vera* cv. In the previous studies, auxin and its conjugates have been identified in different organs and stages of growth. In the current study, it was found that between shoots and leaves, or between leaves and flower buds, auxin has the opposite effect. Among auxin conjugates, IAA was the main conjugate and dominant indole detected in pistachio extract observed in this work. Auxin content was low in 'On-year' trees in both leaves and shoots, associated with fruit yield, and especially increased during bud abscission and alternate bearing. Contrastingly, IAA declined during the flower bud abscission period in 'On-year' trees. In most cases, auxin concentration in shoots and leaves of

'On-year' trees was lower than that in 'Off-year' ones and it was reversed in case of flower buds. As a result, fruit kernel development started after 55 days of full blooming (in the middle of June) in Gaziantep conditions, embryos developed rapidly from mid-June to early August, and flower bud abscission decreased gradually from 55 to 115 days after full blooming. This study clearly demonstrated that auxin and its conjugates play an important role in bud abscission and cause alternate bearing in pistachio. The inverse relation between auxin and conjugates during bud abscission could be an indication of its activity in this unique phenomenon of Uzun pistachio variety. It was also thought that auxin could be an effective antiabscission agent for pistachio flower buds. Hence, external auxin applications, especially in periods of slow and low levels of auxin synthesis, are required to determine the effectiveness of its administration. In conclusion, the relative amount of auxin may differ not only from one form to another but with plant tissue and physiological conditions.

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