Turkish Journal of Agriculture and Forestry

Volume 42 | Number 2 Article 1

1-1-2018

The effects of GA3 treatment on yield, carbohydrate, and endogenous hormone changes in Memecik olive cultivar

SALİH ÜLGER

SABRİYE ATMACA

SARA DEMİRAL

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2018) 42: 75-81

doi:10.3906/tar-1705-76

The effects of GA_3 treatment on yield, carbohydrate, and endogenous hormone changes in Memecik olive cultivar

Salih ÜLGER[1,*], Sabriye ATMACA[2], Sara DEMİRAL[3]

[1]Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey
[2]Vocational School of Gazipaşa Rahmi Ballı, Alanya Alaaddin Keykubat University, Alanya, Antalya, Turkey
[3]Regional Agriculture Department, Antalya, Turkey

Received: 18.05.2017 • **Accepted/Published Online:** 29.12.2017 • **Final Version:** 26.04.2018

Abstract: In this experiment, 100 ppm gibberellic acid (GA_3) was applied to Memecik olive trees in the middle of June in the 'on' (bearing) year to research the effects on yield, reducing sugar amount, and endogenous hormones with the purpose of bearing fruit regularly. The yield was 19.33 kg/tree in control plants and 17.33 kg/tree in GA_3-treated plants in the 'on' year. The yield was 7.17 kg/tree in GA_3-treated plants and 4.00 kg/tree in control plants in the 'off' year. While GA_3 treatment was not very effective on the decrease of fruit amount in the 'on' year, it increased fruit amount in the 'off' year. Moreover, GA_3 treatment did not significantly affect total reducing sugar amount, but it affected endogenous hormones. Total reducing sugar amount was determined at the 4% level in both years ('on' and 'off'). Glucose content was determined to be higher than sucrose and fructose in all plant organs and during flower bud formation periods (induction, initiation, and differentiation). Furthermore, GA_3 treatment increased abscisic acid, GA_3, indole-3-acetic acid, and zeatin levels during flower bud formation periods and in all plant organs (leaf, node, and fruit) in both years.

Key words: Gibberellic acid, hormone, olive, sugar, yield

1. Introduction

Olive is one of the most important fruits for the Turkish economy. Due to excessive fruit set in 'on' year, fruits do not form in the following year. This situation influences olive growers negatively, and it decreases the preference of olive cultivation versus other fruit species.

Many experiments were conducted to solve alternate-bearing problems in olives. Lavee et al. (1983) determined that 25–100 ppm gibberellic acid (GA_3) treatment for olives in the flower period showed positive results on fruit set. Rotundo and Gioffree (1984) demonstrated that 500 ppm GA_3 treatment on Solana Tanera and S. Augustine olive varieties increased fruit amount in 'on' and 'off' years by preventing preharvest fruit drop.

Fernandez-Escobar et al. (1992) applied 100 ppm GA_3 on the Manzanillo olive variety between May and November. Shoot width increased when GA_3 was applied in May, June, and July; moreover, the flowering period extended when it was applied in November and February. Furthermore, when GA_3 was applied before endocarp hardening, the amount of flowering increased in the following years, a result of fruit thinning and seed damage. In addition, it was indicated that embryo sclerification by years occurred 7–8 weeks after the full flowering period (about 1 July), and induction occurred in the endocarp hardening period.

Boulouha et al. (1993) applied 30, 60, and 120 ppm GA_3; 500, 1500, and 2000 ppm Alar [daminozide]; and 1000, 1500, and 2000 ppm paclobutrazol (PP333) to olives in January 1985 and 1986. All GA_3 treatments increased annual vegetative growth in long and short fruit-bearing branches and increased fruit yield in the following year. There was no significant effect on flowering pattern, which varied between years, and 30 and 60 ppm GA_3 induced stabilization of fruit production over the 2-year period and reduced the degree of alternate bearing.

Proietti and Tombesi (1996) applied GA_3 treatment to the Maurino olive cultivar. In mid-June, 50% and 100% of the flowers were removed, and then 60 ppm GA_3 was applied to leaves in mid-July, at the end of August, and in mid-October. Moreover, paclobutrazol (PP333) at 1000 ppm was applied to leaves at the end of October. In addition, asparagine (10^{-5} M) and glutamine (10^{-5} M) were applied to the soil at the end of December. Thinning and complete removal of the flowers stimulated vegetative activity. GA_3 treatment in July slightly increased vegetative

* Correspondence: ulger@akdeniz.edu.tr

growth both in treatment and in following years, while asparagine and glutamine stimulated vegetative growth only in the following year. GA_3 and PP333 treatments did not have significant effects on flowering and fruit production in the following year. Therefore, GA_3 and other hormones did not have a direct influence on flower bud induction; however, these hormones affected it indirectly by providing use and transport of assimilates in plants.

Ulger et al. (2004) investigated endogenous sugar, mineral nutrition, and hormone levels in leaf, node, and fruit samples of Memecik olives during the induction, initiation, and differentiation periods in 'on' and 'off' years. The differences of the sugar concentrations, with the exception of fructose, were not significant between 'on' and 'off' years. However, hormone levels were significantly different between 'on' and 'off' years. Glucose had the highest concentrations in both years, followed by sucrose and fructose, respectively. Calcium (Ca) and iron (Fe) were found as the highest macro- and microelement concentrations, respectively. The results suggested that carbohydrates and mineral nutrients may not have a direct effect on inducing flower initiation. However, high GA_3 levels showed an inhibitory effect on floral formation during the induction and initiation periods. On the other hand, high concentrations of GA_4, ABA, and certain cytokinin levels may have a positive effect on flower formation in olive during the induction and initiation periods.

At the Kazerun Olive Research Station in Iran, Ramezani et al. (2010) conducted an experiment on the 10-year-old Shengeh olive variety to investigate the effect of GA_3 and zinc sulfate ($ZnSO_4$) on fruit yield and oil production. The plants were sprayed with 0, 15, 30, and 45 ppm GA_3 and 0%, 0.25%, 0.50%, and 0.75% $ZnSO_4$ in August, when they were about half way through the fruit growth period. The results showed that appropriate spraying of GA_3 in combination with $ZnSO_4$ increased the oil production by increasing both fruit oil and fruit retention.

El-Naby et al. (2012) carried out an experiment for three successive seasons on the 15-year-old Picual olive variety to reduce the degree of alternate bearing of olives through spraying GA_3 on the first and in the middle of December and on the first and in the middle of January at 0, 25, 50, and 75 ppm concentrations. It was found that 75 ppm GA_3 treatment on olive trees on the first of December reduced the alternate bearing in comparison with other GA_3 concentrations at different times. Furthermore, 75 ppm GA_3 treatment on the first of December increased vegetative growth, improved fruit characteristics, and increased the oil percentage.

Haberman et al. (2017) indicated that olive juvenility and seasonality of flowering were altered by overexpressing genes that encode flowering locus T (FT). OeFT1 and OeFT2 caused early flowering under short days when they were expressed in Arabidopsis. Expression of OeFT1/2 in olive leaves and OeFT2 in buds increased in winter, while initiation of inflorescence occurred in late winter. Trees that were exposed to an artificial warm winter had low levels of OeFT1/2 in the leaves and did not flower. Thus, flower induction in olive occurred by increased FT levels in cold winters.

In this experiment, the effects of GA_3 treatment in mid-June on yield, reducing sugar, and endogenous hormones in the Memecik olive variety were investigated with the purpose of decreasing fruit amount in the 'on' year and increasing it in the 'off' year.

2. Materials and methods

2.1. Plant materials

Fifteen-year-old trees of the Memecik olive cultivar were used in this experiment at the Research Station of the Faculty of Agriculture, Akdeniz University, Antalya, Turkey. The research station is located 3 km from the Mediterranean Sea with 50 m altitude. The soil type is a clay loam (lithic xerorthent) with a medium level of organic matter (2.69%) and pH 8.23.

Supplemental irrigation and standard fertilization practices were provided. GA_3 (100 mg L^{-1}) was applied to the trees in mid-June in the 'on' year, before endocarp hardening. Data were analyzed using a randomized complete block design with 3 replications. Each block had 3 trees.

2.2. Hormone analysis

Hormone analysis was done on leaf, node, and fruit samples. Samples were taken during the induction (15 July), initiation (15 November), and differentiation (28 February) periods of olives in 'on' and 'off' years. Collected samples were stored at –20 °C until analysis. Samples were taken from the periphery of the tree at 1.5 m height. Data were analyzed using a randomized complete block design with 3 replications and each replication comprised 3 trees.

The amounts of ABA, IAA, GA_3, and Z were determined according to Topçuoğlu and Ünyayar (1995). The samples of leaf, node, and fruit (5 g) were homogenized in a cold methanol : chloroform mixture (14:6 v/v) at room temperature and then were stored at –20 °C for 1 week. The extracts were filtered through Whatman No. 5 filter paper, the supernatants were rehomogenized with the same mixture, and the extracts were combined. The aqueous residue was adjusted to pH 8.5 with 1 N sodium hydroxide (NaOH) and transferred to a separating funnel to separate chloroform from the methanol, after which the chloroform phase was discarded. The methanol phase was reduced to an aqueous phase under reduced pressure on a rotary evaporator at 40 °C. It was then adjusted to pH

2.5 with 1 N hydrochloric acid (HCl) and extracted with ethyl acetate three times. The aqueous phase was adjusted to pH 7.0 with 1 N NaOH and extracted with ethyl acetate three times, and then the acidic and neutral ethyl acetate phases including free hormones were combined. In order to extract conjugated hormones, the aqueous phase was adjusted to pH 11.0 with 1 N NaOH and incubated in a water bath at 70 °C for 1 h. It was adjusted to pH 7.0 with 1 N HCl and extracted with ethyl acetate three times. The aqueous phase was then adjusted to pH 2.5 with 1 N HCl and extracted with ethyl acetate three times. Conjugated acidic and neutral ethyl acetate phases were combined, and then these combined conjugated extracts were combined with the previously combined free hormone extracts and dried under vacuum at 40 °C. The residue was dissolved in 1 mL of methanol and transferred to an Eppendorf tube. The methanol was reduced to 100 µL under vacuum and then line-loaded onto a 20 × 20 cm, 0.25 mm thick silica gel 60 F_{254} TLC plate (Merck, Darmstadt, Germany). Standard ABA, IAA, GA_3, and Z were also spot-loaded in scored strips at both edges of the plates. The plate was allowed to develop for 15 cm in the vertical direction using isopropyl alcohol : ammonia : water (84:8:8 v/v/v) as the solvent system. After development, the positions of ABA, IAA, GA_3, and Z were detected under ultraviolet (UV) light (254 nm wavelength) and marked. A band of silica corresponding to the Rf values of the standards was scraped off, dissolved in 0.5 mL of methanol in an Eppendorf tube, and then dried under vacuum. The purified samples were methylated with diazomethane (Schlenk and Gellerman, 1960) dissolved in ethyl ether and methanol (9:1 v/v). The derivatives were dried under vacuum and redissolved in 100 µL of ethyl acetate for gas chromatography analysis. The ABA, IAA, GA_3, and Z contents were determined with a Fisons 8560 HRGC Mega 2 series equipped with FID, using an SPB-1 (30 m × 0.32 mm I.D.) capillary column. Injection and detector temperatures were 200 °C and 300 °C, respectively. Samples (1 µL) were injected into the column at 80 °C. The temperature was programmed to 5 °C min^{-1} until the column was at 280 °C. The helium flow rate was 1 mL min^{-1} and inlet pressure was 22 psi. Amounts of ABA, IAA, GA_3, and Z were determined using peak areas. The ratio of response of the detector to putative ABA, IAA, GA_3, and Z peaks in the plant samples were compared to the response ratio of the detector for authentic ABA, IAA, GA_3, and Z standards (Sigma).

2.3. Sugar analysis

Sugar concentrations were determined according to Camara et al. (1996) with some modifications. Ten grams of homogenized sample were mixed with 40 mL of water. The mixture was homogenized using an Ultratorax at 24,000 rpm and centrifuged at 6000 rpm for 30 min at ambient temperature. The supernatant was filtered through Whatman No. 42 filter paper and then filtered through a Sep-Pack C_{18} (Waters) cartridge. An aliquot of 2.5 mL of filtrate was blended with 7.5 mL of acetonitrile; subsequently, the mixture was filtered through a 0.45 µm membrane filter before 20 µL injections.

High-performance liquid chromatography (HPLC) analysis of the sugars was performed by reversed phase HPLC on a Varian Model 9050 equipped with a Varian Model 9010 solvent delivery system and a Varian Model 9040 refractive index (RI) detector. Separation and determination were conducted at 25 °C using an Alltech Amino Bonded Carbohydrate Column (300 × 4.1 mm I.D.). The column was eluted with acetonitrile : water (75:25) mobile phase at a flow rate of 1.4 mL min^{-1}. The concentrations of glucose, fructose, sucrose, ribose, xylose, and maltose were quantified using a Varian Star Chromatography Workstation (Ver. 4.51) from the peak area ratios on the basis of the corresponding calibration parameter values.

2.4. Statistical analysis

Statistical analysis of the data was carried out using the SAS package program (SAS Inc., Raleigh, NC, USA) and data were subjected to analysis of variance with mean separation by Duncan's multiple range tests.

3. Results

3.1. Yield

While the yield was approximately 19.33 kg/tree in control plants in the 'on' year, it was 17.33 kg/tree in GA_3-treated plants. However, there was no statistically significant difference. In the 'off' year, the yield was 4.00 kg/tree in control plants and it was 7.17 kg/tree after GA_3 treatment, which was applied in the middle of June in the 'on' year. There was a statistically significant difference between these values ($P \leq 0.05$) (Table 1).

3.2. Sugar content

While fructose, glucose and sucrose were determined in the samples, maltose, ribose, and xylose were not found.

While the highest fructose (0.34%) was determined during the induction period in control and initiation in GA_3-treated plants, the highest sucrose (0.45%) was

Table 1. Mean of Memecik olive fruit amounts in 'on' and 'off' years as a result of GA_3 treatment (kg/tree).

	'On' year	'Off' year
Control	19.33 a[z]	4.00 b
GA_3	17.33 a	7.17 a

[z]: Mean separation within columns and main effects by Duncan's multiple range test, $P \leq 0.05$.

determined during the differentiation period in GA_3-treated plants. GA_3 treatment only increased the levels of fructose, glucose, and sucrose during the differentiation period. During the initiation period, GA_3 treatment had no effect on fructose, glucose, and sucrose levels. However, fructose, glucose, and sucrose levels were higher in control plants than in GA_3 treatments during the induction period. The total sugar contents did not change by GA_3 treatments during the initiation period, but they increased during the differentiation period and decreased during the induction period (Table 2).

While glucose (between 2.86% and 3.67%) was the highest determined sugar in all periods, fructose and sucrose levels were generally similar. After GA_3 treatment, in leaves and nodes, fructose, glucose, sucrose, and total sugar contents were not changed, but in fruit samples, glucose and total sugar levels were changed. GA_3 treatment increased glucose and total sugar levels in fruit. Glucose content was observed as the highest in all three organs and the values varied between 2.86% (in nodes of control plants) and 3.67% (in leaves of GA_3-treated plants). While the fructose level was higher in fruits than in leaves and nodes, glucose was found to be higher in leaves. In addition, sucrose level generally was lower in leaves than in nodes and fruits. The highest total sugar contents were determined in leaves (4.18% and 4.19%), followed by fruit (3.66%–4.06%) and node (3.44%–3.45%) samples (Table 3).

After GA_3 treatment, there was no significant difference among fructose, glucose, sucrose, and total sugars in 'on' and 'off' years (Table 4).

In 'on' and 'off' years, GA_3 treatment did not have a significant effect on fructose, glucose, sucrose, and total sugar levels in comparison with the control treatment (Table 5).

3.3. Endogenous hormones

GA_3 treatment affected endogenous hormone contents during flower bud formation periods (induction, initiation, and differentiation) and in plant organs (leaf, node, fruit), and the differences were found to be statistically significant ($P \leq 0.05$). GA_3 treatment increased ABA, GA_3, IAA, and Z during induction, initiation, and differentiation periods (Table 2). ABA levels decreased from the induction (4.84 μg g^{-1}) to the differentiation (1.38 μg g^{-1}) period in GA_3-treated plants. However, in control plants, ABA levels decreased between the induction (0.21 μg g^{-1}) and initiation (0.15 μg g^{-1}) periods and then increased during the differentiation (0.47 μg g^{-1}) period. IAA level regularly increased from the induction (6.81 μg g^{-1}) to the differentiation (15.90 μg g^{-1}) period in GA_3-treated plants; however, in control plants, the IAA level was approximately the same during the induction (0.70 μg g^{-1}) and differentiation (0.72 μg g^{-1}) periods, but it was higher during the initiation period (1.75 μg g^{-1}). After GA_3 treatment, the GA_3 level fluctuated and the highest GA_3 level was determined during the induction period (43.34 μg g^{-1}), followed by the differentiation (34.08 μg g^{-1}) and initiation (16.25 μg g^{-1}) periods, respectively. However, in control plants, the GA_3 level slightly tended to increase from the induction (1.50 μg g^{-1}) to the differentiation period (4.12 μg g^{-1}). Z level regularly increased from the induction (5.42 μg g^{-1}) to the differentiation (18.09 μg g^{-1}) period in control plants; however, in GA_3-treated plants, the Z level was higher during induction (190.40 μg g^{-1}) and lower during the initiation period (60.40 μg g^{-1}) (Table 2).

After GA_3 treatment, ABA, IAA, GA_3, and Z levels in leaf, node, and fruit samples were found statistically

Table 2. Mean of fructose, glucose, sucrose, total sugar, ABA, IAA, GA_3, and Z amounts determined in flower formation stages of Memecik olive plants exposed to GA_3 treatment in the middle of June in 'on' year.

Floral developing stages	Induction		Initiation		Differentiation	
	Control	GA_3	Control	GA_3	Control	GA_3
Fructose (%)	0.34 a[z]	0.18 b[z]	0.26 a	0.342 a	0.18 b	0.30 a
Glucose (%)	3.26 a	2.99 b	3.12 a	3.342 a	3.08 b	3.39 a
Sucrose (%)	0.36 a	0.22 b	0.37 a	0.32 a	0.23 b	0.45 a
Total sugar (%)	3.99 a	3.39 b	3.81 a	4.01 a	3.49 b	4.14 a
ABA (μg g^{-1})	0,21 b	4.84 a	0.15 b	2.94 a	0.47 b	1.38 a
IAA (μg g^{-1})	0.70 b	6.81 a	1.75 b	8.52 a	0.72 b	15.90 a
GA_3 (μg g^{-1})	1.50 b	43.34 a	3.88 b	16.25 a	4.12 b	34.08 a
Z (μg g^{-1})	5.42 b	190.40 a	8.63 b	60.40 a	18.09 b	86.35 a

[z]: Control and GA_3 are compared for each induction, initiation, and differentiation separately within each row using Duncan's multiple range test, $P \leq 0.05$.

Table 3. Mean of fructose, glucose, sucrose, total sugar, ABA, IAA, GA_3, and Z amounts determined in leaf, node, and fruit samples of Memecik olive plants exposed to GA_3 treatment in the middle of June in 'on' year.

Sample	Leaf		Node		Fruit	
	Control	GA_3	Control	GA_3	Control	GA_3
Fructose (%)	0.19 a[z]	0.23 a[z]	0.21 a	0.20 a	0.48 a	0.54 a
Glucose (%)	3.66 a	3.67 a	2.86 a	2.93 a	2.87 b	3.13 a
Sucrose (%)	0.29 a	0.28 a	0.37 a	0.36 a	0.33 a	0.41 a
Total sugar (%)	4.18 a	4.19 a	3.44 a	3.45 a	3.66 b	4.06 a
ABA (μg g^{-1})	0.31 b	2.51 a	0.17 b	3.10 a	0.28 b	3.21 a
IAA (μg g^{-1})	1.29 b	14.83 a	0.72 b	7.03 a	1.37 b	9.02 a
GA_3 (μg g^{-1})	3.27 b	32.73 a	2.61 b	29.72 a	3.41 b	23.54 a
Z (μg g^{-1})	11.76 b	117.21 a	9.90 b	108.94 a	6.68 b	72.24 a

[z]: Control and GA_3 are compared for leaves, nodes, and fruit separately within each row using Duncan's multiple range test, $P \leq 0.05$.

significant ($P \leq 0.05$) (Table 3). GA_3 treatment increased ABA, GA_3, IAA, and Z in leaf, node, and fruit samples. In control plants, the highest ABA level was approximately equal in leaf (0.31 μg g^{-1}) and fruit (0.28 μg g^{-1}) samples, but it was lower in node (0.17 μg g^{-1}) samples. However, the ABA level was higher in fruit (3.21 μg g^{-1}) and lower in leaves (2.51 μg g^{-1}) in control plants. While the highest IAA in control and GA_3-treated plants was found in fruit (1.37 μg g^{-1}) and leaf (14.83 μg g^{-1}) samples, the lowest IAA in control and GA_3-treated plants was in node samples (0.72 and 7.03 μg g^{-1}, respectively). After GA_3 treatment, the highest GA_3 level was determined in leaf (32.73 μg g^{-1}) samples, followed by node (29.72 μg g^{-1}) and fruit (23.54 μg g^{-1}) samples. However, in control plants, the highest GA_3 was in fruit (3.41 μg g^{-1}) and the lowest was in node (2.61 μg g^{-1}) samples. While the highest Z level was in leaves in control and GA_3-treated plants (11.76 and 117.21 μg g^{-1}, respectively), the lowest Z level was in fruit in control and GA_3-treated plants (6.68 and 72.24 μg g^{-1}, respectively) (Table 3).

GA_3 treatment increased ABA, GA_3, IAA, and Z levels in 'on' and 'off' years (Table 4). ABA, IAA, and Z levels were higher in control and GA_3-treated plants in the 'off' year than in the 'on' year; however, only the GA_3 level was higher in the 'on' year (35.90 μg g^{-1}) than the 'off' year (22.63 μg g^{-1}).

In the 'on' year, GA_3 treatment did not affect IAA level significantly in comparison with the control treatment; however, it raised the ABA, GA_3, and Z levels significantly ($P \leq 0.05$) (Table 5).

Table 4. Fructose, glucose, sucrose, total sugar, ABA, IAA, GA_3, and Z amounts determined in 'on' and 'off' years after GA_3 treatment in the middle of June in 'on' year.

Years	'On' year		'Off' year	
	Control	GA_3	Control	GA_3
Fructose (%)	0.31 a[z]	0.31 a	0.23 a	0.26 a
Glucose (%)	3.05 a	3.26 a	3.28 a	3.27 a
Sucrose (%)	0.33 a	0.35 a	0.34 a	0.34 a
Total sugar (%)	3.65 a	3,92 a	3.85 a	3.87 a
ABA (μg g^{-1})	0.15 b	1.81 a	0.35 b	4.08 a
IAA (μg g^{-1})	0.28 b	3.08 a	1.92 b	18,63 a
GA_3 (μg g^{-1})	3.62 b	35.90 a	2.50 b	22.63 a
Z (μg g^{-1})	8.71 b	98.42 a	10.88 b	110.5 a

[z]: Control and GA_3 are compared for each 'on' year and 'off' year separately within each row using Duncan's multiple range test, $P \leq 0.05$.

4. Discussion

GA_3 at 100 ppm was applied in the middle of June in the 'on' year with the purpose of increasing the yield in the 'off' year and decreasing the yield in the 'on' year. Although GA_3 treatment did not decrease the yield in the 'on' year, it increased it in the 'off' year. While the average yield was 4.00 kg/tree in the control plants, it was 7.17 kg/tree in the plants exposed to GA_3. These results showed that the alternate bearing can be decreased by GA_3 treatment even a little. El-Naby et al. (2012) found similar results in the Picual olive variety. They applied 0.25, 50, and 75 ppm GA_3 in the beginning and middle of December and January. GA_3 treatment at 75 ppm in the beginning of

Table 5. Mean of fructose, glucose, sucrose, total sugar, ABA, IAA, GA_3, and Z amounts after GA_3 treatment in the middle of June in 'on' year.

Application	Fructose (%)	Glucose (%)	Sucrose (%)	Total (%)	IAA (μg g^{-1})	Z (μg g^{-1})	ABA (μg g^{-1})	GA_3 (μg g^{-1})
Control	0.27 a[z]	3.22 a	0.33 a	3.82 a	10.10 a	9.97 b	2.52 b	28.97 b
GA_3	0.29 a	3.15 a	0.39 a	3.83 a	9.50 a	110.25 a	3.25 a	32.19 a

[z]: Mean separation within columns and main effects by Duncan's multiple range test, $P \leq 0.05$.

December reduced the alternate bearing in comparison with other GA_3 concentrations and increased vegetative growth. Table 3 shows that final fruit set was significantly affected by different times and concentrations of GA_3 in the three seasons. In addition, spraying olive trees with GA_3 at 75 ppm on the first of December significantly decreased the final fruit set in the three seasons of the study. However, the control gave the highest final fruit set in the three seasons. This was on par with GA_3 at 25 ppm at mid-December and the first and middle of January in the 2010 season, and 25 ppm at mid-January in the 2011 season (El-Naby et al., 2012). These results revealed that GA_3 treatments during the induction period in the beginning of July and during the initiation period after November can be effective on adjusting fruit amount in 'on' and 'off' years. Similar results about increased yield in 'off' years by GA_3 treatment in 'on' years were obtained by Rotundo and Gioffrea (1984), Boulouha et al. (1993), and Fernandez-Escobar et al. (1992). However, contrary to these results, Proietti and Tombesi (1996) indicated that 60 ppm GA_3 treatment on the Maurino olive variety in the middle of June, July, August, and November did not have a significant effect on flower bud formation and fruit set. They asserted that GA_3 and other hormones do not affect flower bud formation directly; however, these hormones affect it indirectly by providing use and transport of assimilates in plants. This result could have been obtained due to using low-dose GA_3; thus, like in the experiment of El-Naby et al. (2012), the effects of GA_3 changed with different doses. On the other hand, Haberman et al. (2017) indicated that when potted trees were exposed to artificial cold treatments in early summer (June) or fall (September), inflorescence formation could occur before the winter season. They identified two FT-encoding genes in the olive genome and provided evidence of a dramatic increase in both genes during the winter or by artificial cold-temperature treatment in other seasons. These results indicate that many factors affect floral development in olive.

In this experiment, GA_3 treatment did not change the total sugar amount significantly, and there was no significant difference between total reducing sugar amounts in 'on' and 'off' years. Thus, these results showed that sugars do not have a significant effect on flower bud formation. Stutte and Martin (1986) determined that temperature and light treatments in greenhouse conditions in the winter affected mannitol, fructose, and sucrose contents a little; moreover, a high level of starch did not have a positive effect on flowering. However, GA_3 treatment had no effect on fructose, glucose, and sucrose in the flower formation periods. GA_3 treatment increased the levels of fructose, glucose, and sucrose during the differentiation period. However, GA_3 treatment did not increase fructose, glucose, and sucrose levels during the induction period and their levels were found to be lower in GA_3-treated plants. GA_3 treatments also did not change fructose, glucose, sucrose, and total sugar contents in leaves, nodes, and fruit (except glucose and total sugar levels in fruit). Ulger et al. (2004) reported that glucose did not show a significant change in all flower formation periods, but fructose and sucrose levels changed. In the experiment, GA_3 treatment was only effective during the differentiation period and it increased glucose, sucrose, fructose, and total sugar levels. The results revealed that GA_3 treatment did not change total sugar levels during flower initiation periods; however, it changed them during the induction and differentiation periods.

Ulger et al. (2004) found the highest total sugar and glucose in the leaves and fructose in fruit samples; however, they did not determine a significant change in sucrose levels. In the experiment, GA_3 treatment did not change glucose, fructose, sucrose, and total sugar in leaf, node, and fruit samples. The results revealed that GA_3 treatment did not affect sugar contents in leaves, nodes, and fruit.

GA_3 treatment increased ABA, GA_3, IAA, and Z levels during flower bud formation periods (induction, initiation, and differentiation) and in organs (leaves, nodes, and fruit) in both years ('on' and 'off') in comparison to the control treatment; however, GA_3 treatment did not increase the IAA level, which was higher in the control treatment.

Ulger et al. (2004) determined the highest ABA, GA_3, and Z levels during the morphological differentiation period and IAA level during the initiation period. However, in this experiment, ABA, IAA, GA_3, and Z levels were found to be higher than those previously reported by

Ulger et al. (2004). This showed that GA_3 treatment before the flower formation period may increase endogenous hormone levels.

Acknowledgment
This work was financially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK).

References

Boulouha B, Wallali LD, Loussert R, Lamhamedi M, Sikaoui L (1993). Effects of growth regulators on growth and fruiting of olive (*Olea europaea* L.). Al Awamia 70: 74-96.

Camara MM, Diez C, Tarija ME (1996). Free sugar determination by HPLC in pineapple products. Z Lebensm Unters Forsch 202: 233-237.

El-Naby SKMA, El-Sonbaty MR, Hegazi ES, Samira MM, El-Sharony TF (2012). Effect of gibberellic acid spraying on alternate bearing of olive trees. J Appl Sci Res 8: 5114-5123.

Fernandez-Escobar R, Benlloch M, Navarro C, Martin GC (1992). The time of floral induction in the olive. J Am Soc Hortic Sci 117: 304-307.

Haberman A, Bakhshian O, Cerezo-Medina S, Paltiel J, Adler C, Ben-Ari G, Mercado JA, Pliego-Alfaro F, Lavee S, Samach A (2017). A possible role for flowering locus T-encoding genes in interpreting environmental and internal cues affecting olive (*Olea europaea* L.) flower induction. Plant Cell Environ 2017; 40: 1263-1280.

Lavee S, Ben-Tal Y, Klein I, Epstein E (1983). Regulation of Fruiting in Olives. Bet-Dagan, Israel: Institute of Horticulture, Agricultural Research Organization, Volcani Center.

Proietti P, Tombesi A (1996). Effects of gibberellic acid, asparagine and glutamine on flower bud induction in olive. J Hortic Sci 71: 383-388.

Ramezani S, Shekafandeh A, Taslimpour MR (2010). Effect of GA_3 and zinc sulfate on fruit yield and oil percentage of 'Shengeh' olive trees. International Journal of Fruit Science 10: 228-234.

Rotundo A, Gioffree D (1984). The effects of GA_3 on the productivity of two olive cultivars. Tecnica Agricola 34: 187-202.

Schlenk H, Gellerman JL (1960). Esterification of fatty acids with diazomethane on a small scale. Anal Chem 32: 1412-1414.

Stutte GW, Martin GC (1986). Effect of light intensity and carbohydrate reserves on flowering in olive. J Am Soc Hortic Sci 11:27-31.

Topçuoğlu F, Ünyayar S (1995). Determination of growth substances (auxin, gibberellic acid, abscisic acid and cytokinin) production and biological activity in white rot fungus *Phanerochate chrysosporium* ME 446. Malatya, Turkey: İnönü University Araştırma Fonu (in Turkish with English abstract).

Ulger S, Sonmez S, Karkacier M, Ertoy N, Akdesir O, Aksu M (2004). Determination of endogenous hormones, sugars and mineral nutrition levels during the induction, initiation and differentiation stage and their effects on flower formation in olive. Plant Growth Regul 42: 89-95.