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Study of anatomical and Acc (1-aminocyclopropane-1-carboxylate) during flower bud abscission indicate an important role of alternate bearing

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Abstract: This study investigates whether, and how, 1-aminocyclopropane-1-carboxylic acid (ACC) is concerned in flower bud abscission of pistachio (*Pistacia vera* L.). Abscission was studied about the activation of the abscission zone, located between shoot and flower bud, from Uzun pistachio cultivar where the abscission profiles and the scanning electron micrographs pictorial differences in the abscission program, under natural conditions of flower bud. ACC content was investigated in the flower bud abscission zone during growth and abscission using liquid chromatography coupled with electrospray tandem mass spectrometry (UPLC/ESI-MS/MS). Results showed that abscission layers were started at first 45 days after full blooming (DAFB) and accomplished on 55 and 65 DAFB from 'On' year sampling. The activities of ACC in the flower bud abscission zone were significantly increased and decreased by flower bud abscission. A strong rise of ACC synthase in leaf and panicle was observed at the beginning of abscission, corresponding to stage 45 DAFB. High levels of ACC activity were found in whole abscission period indicating a possible role of ACC in pistachio flower bud abscission. At the end of the period (65 DAFB), the ACC levels gradually decreased and reached to minimum. Consequently, abscission-promoting effect of ACC depends on the phenological periods. These results provide strong evidence of flower abscission zone and the role of ACC in flower bud abscission possibly for the first time.

Key words: Flower bud abscission, 1-aminocyclopropane-1-carboxylat, *Pistacia vera*, fluorescence and scanning electron microscopy (SEM)

1. Introduction

Abscission occurs in an anatomically distinct cell layer known as the abscission zone. Abscission is the controlled shedding of different plant organs such as leaves, flower petals, flowers, bud and fruits (Lewis et al., 2006; Gomez-Jimenez et al., 2010). The presence of different organs abscission is an important feature in the life cycle of many species of plants. Pistachio (*Pistacia vera* L.) is the most economically important fruit crop worldwide**.** Alternate bearing is a widely spread phenomenon that occurs in both deciduous and evergreen trees. Unlike other fruit species with alternate bearing, abundant flower buds are produced each year in pistachios. However, alternate bearing in pistachios occurs as a result of flower bud abscission instead of the absence of flower bud in 'On' year trees. Extreme flower bud abscission casting in pistachio started towards the end of June in 'On' year and the time of fruit kernel development increased throughout July and caused the next year to be 'Off' (Monselise and Goldschmidt, 1982; Gundesli et al., 2019; Gundesli et al., 2020a,b; Khezri et al., 2020; Gundesli et al., 2021). Many aspects of the abscission process have been reviewed in the past. Generally, this zone, known as the abscission zone (AZ), occurs in an anatomically different cell layer (Estornell et al., 2013; Jahromi et al., 2015). Abscission includes a sequence of coordinated events that lead to wall digestion in well-defined cell layers (abscission zones), which the target for abscission-stimulating. (Patterson and Bleecker, 2000; Roberts et al., 2000; Merelo et al., 2017). Abscission, growth and development processes in the plant are mainly specified as genetically programmed processes. Much information is available about the physiological and molecular process of alternate bearing and abscission (Aziz et al., 2001; Dal Cin et al., 2007; Heerema et al. 2008; Rosenstock et al. 2010; Khezri 2013; Ferguson and Kallsen 2016). But, much less is known about the formation of abscission fruit and the regulation of flower bud abscission.

Plant growth regulators (PGs) are involved in several hormones and its concentration is determined by plant developmental processes such as flower formation, fruit ripening, senescence, abscission of plant parts, etc. Clues for abscission occurring in the life cycle of plants

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support this abscission by decreasing abscission-inhibiting hormones such as auxin and cytokinin and increasing abscission-promoting hormones such as ethylene and abscisic acid (Chavaux et al. 1997; Taylor and Whitelaw, 2001; Aziz, 2003; Roussos et al., 2004; Spann et al., 2008; Vemmos et al., 2012). For a common precursor use abscission and ACC (1-aminocyclopropane-1-carboxylic acid) biosynthesis has been proposed to explain some of their relationships in developmental processes. Although there are many studies on the relationship of ethylene in abscission in plant developmental processes, the role of ACC, which affects ethylene formation, in plant abscission has been less studied. It has been reported in many studies that ethylene triggers shedding in many different organs (Bonghi et al., 2000; Hilt and Besis, 2003). Although it has been hypothesized that the abscision of different organs in many plants can be regulated by the primary metabolite ethylene, the role of ACC in abscission is unknown.

ACC is the main precursor molecule of ethylene. Methionine forms S-adenosylmethionine (SAM) using S-adenosylmethionine (Adomet, SAM) by centrifugation of enzyme ATP and it forms ACC using the ACC synthase enzyme. ACC has been shown to form ethylene using the enzyme ACC oxidase (Doorn et al., 2002; Patterson et al., 2004; Arc et al., 2013) (Figure 1). In plants, ACC is small molecules and known to participate in a wide range of growth processes ACC content and activity in different plant tissues, particularly in relation to abscission. As a result, ACC is the rate-limiting enzyme in ethylene biosynthesis (Arc et al., 2013). Despite many technical researches for plant physiologists to analyze ACC, it is difficult to direct hormone analysis in plants, especially to identify ethylene synthesis and to learn about ACC as a possible rate limiting factor in the ethylene biosynthetic pathway. Therefore, reliable techniques are essential for the analysis of this molecule in biological samples. With direct analysis of ethylene, some (separated) plant parts are difficult to synthesize with ethylene because the synthesis is stimulated by injury and drying. The level of ACC, which is the immediate precursor of ethylene (Chauvaux et al., 1997), is still considered a rate limiter for hormone production and its concentration is determined by the activity of the ACC-synthase enzyme. In many previous studies, different enzymatic and structural patterns of response to ethylene have been observed in the abscission zone of different plant species (Jahromi et al., 2015; Merelo et al., 2017).

The results demonstrate that, due to the possible relationship between ACC and ethylene biosynthesis in plant tissues, ACC levels are complexly controlled during abscission, possibly for the benefit of ethylene synthesis in abscission involving ACC biosynthesis. Despite this interest, little is known about the role played by ACC

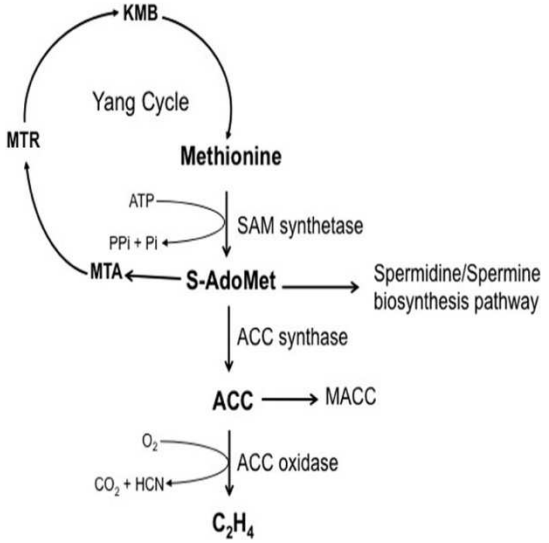


Figure 1. Pathways of plant ACC biosynthesis *(*Arc et al., 2013).

in the onset of alternate bearing in fruits and by ACC and abscission zone in the onset of alternate bearing in pistachio. This detailed temporal profile of ACC, which is rare in fruit in the profile of ACC metabolism in abscission and specific activities provides new information to better understand the physiological role of ACC in flower bud abscission and the formation of the flower bud abscission zone. This is the first report available describing the effect of ACC metabolism during flower bud abscission.

2. Materials and methods

2.1. Plant material

Twelve adjacent, thirty-three-year-old 'Uzun' trees (on *Pistachio vera* Desf. rootstock, planted at 10 ′ 10 m intervals) were selected for research in 2013 ('On' and 'Off' year). The trees were located at the Research and Experimental field of the Ministry of Agriculture's Pistachio Research Institute in Gaziantep. To ensure alternate bearing with 'On' and 'Off' year trees in the same year, 6 of the trees remained flowering while the flowers of 6 trees were plucked in April 2013. So that, in the early spring of the following year, plucking all of the flowers was provided with the 'Off' year trees with full crop alternation and the other trees as 'On' year trees which will drop all of the flowers.

In the study, while flower buds of next year were used for histological analysis in 25, 35, 55, 65, 75 and 85 days after full blooming (DAFB); shoots, leaves, panicles and nuts were used for ACC analysis in 35, 45, 55 and 65 DAFB (Table). So that, in critical periods of flower bud abscission, the alteration of ACC synthesis in shoots leaves, panicles and nuts, which are already found on the tree with flower buds, were demonstrated.

Table. Sampling dates of histological and ACC synthase analyses in 2015.

	Sampling dates			
	Histological		**ACC synthase**	
Physiological periods	**25 DAFB***	05.05.2015	**35 DAFB**	15.05.2015
	35 DAFB	15.05.2015	**45 DAFB**	25.05.2015
	45 DAFB	25.05.2015	**55 DAFB**	04.06.2015
	55 DAFB	04.06.2015	**65 DAFB**	14.06.2015
	65 DAFB	14.06.2015		
	75 DAFB	24.06.2015		
	85 DAFB	05.07.2015		

* Days after full blooming (DAFB). Full flowering date of study year: 10.04.2015.

2.2. Preparing and viewing histological sections using fluorescence microscope

For histological analysis, flower buds from 'On' and 'Off' year trees were sampled separately with their woody parts at 10 days intervals starting at 25 DAFB till 75 DAFB (Table). Samples were fixed in FPA 70 (formaldehyde-propionic acid-alcohol) solution immediately after cutting the end of the flower buds to facilitate fast penetration of the fixative and paraffin. For paraffin embedding, all flower buds were dehydrated for 4–5 h in ethyl and tertiary butyl alcohol series. Then the samples were embedded in paraffin and sectioned (10–12 μ) longitudinally with a rotary microtome (Leica RM2134, Leica Microsystems, Wetzlar, Germany), then stained with 0.125% hematoxyline and mounted in Entellan (Johansen, 1940). The abscission layer within the section was viewed using a fluorescence microscope (Olympus System, BX51, Hamburg, Germany) and photographed with a DP72 camera.

2.3. Preparing and viewing tissues using scanning electron microscope

For scanning electron microscopy (SEM) of the abscission zone, flower buds were removed from the tree at the same periods with fluorescent microscopy. Samples were fixed immediately with a 5% glutaraldehyde and 100 mM sodium phosphate solution at 4 °C for 24 h. Then, for dehydration process, the samples were moved into a series of increasing ethanol concentrations, each for 24 h: 25%, 50%, 75%, 95% and 100% ethanol. Then samples were dried using a critical point dryer (Emitech-K580, Quorum Technologies, UK). Following the drying process, samples were coated with gold-palladium (Emitech-SC7620, Quorum Technologies, UK), and observed using a JSM-6390LV (JEOL, USA, Inc., USA) scanning electron microscope (property of Gaziantep University) (Foster et al., 2003).

2.4. ACC quantification by UPLC/ESI-MS/MS

2.4.1. Extraction, measurement and identification of ACC

Isolation of ACC from plant material was done as described by Chauvaux et al., 1997. ACC was extracted from in pistachio which by briefly rinsing approximately between 0.1 and 1 mg of different tissues and frozen material was homogenised in 80% methanol, after addition of 1000 pmol of [H]ACC as an internal standard and kept at –20 °C for 1 h. The slurry was centrifuged at 24,000 g for 20 min and half of the supernatant was brought in glass tubes and dried (Speedvac) for future analysis of ACC conjugates. Samples were injected into the HPLC system, connected to a Quatro II mass spectrometer (Micromass) equipped with an electrospray interface on a UPLC/ESI-MS/MS utilizing a Waters ACQUITY UPLC system, equipped with a binary solvent delivery manager and a sample manager coupled to a Waters Micromass Quattro Premier XE quadrupole tandem mass spectrometer via a Z-spray interface. MassLynx and QuanLynx (Micromass, Manchester, UK) were used for data acquisition and data analysis by Masslynx software. The quantification of ACC 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 procedure for the quantification of multiple ACC was performed as described in detail by Chavaux et al. (1997). Results are expressed in nanograms per gram of as ng g^{-1} dry weight (DW).

2.5. Statistical analysis

Descriptive analysis for the present data was given in means and standard deviations, due to the nature of the data (continuous variables). The normality tests suggested

that the data is not parametric and nonparametric tests were applied for the comparison of the independent variables. Mann–Whitney U test was used for 2 variables and Kruskal–Wallis test was performed for 2+ variables. Then, the Bonferroni test was applied to determine the significances among the 2+ variables after Kruskal–Wallis test. Pearson correlation was then performed to evaluate the correlation among the variables. The significance level of $p = 0.05$ was applied in all statistical analysis. SPSS 24 was used for the statistical comparison tests and R 3.6.1 was used for the correlation statistics.

3. Results

3.1. Histological changes during abscission

Figure 2 shows the fluorescence and SEM photographs of 'Uzun' pistachio flower buds taken in 'On' and 'Off' years. The abscission layer zone or any cell differentiation was not observed on the samples taken on 25, 35 days after full bloom (DAFB) and at this time the flower cluster is starting to form (Figures 2a–2d). The first visual evidence of abscission zone or cell differentiation was observed and obtained in 45 DAFB in 'On' year trees, while in the 'Off' year trees there was no sign of any abscission layer in the buds examined in this period (Figures 2e–2h). In 'Off' year, the first signs of cellular dissociation were observed in the 55 DAFB (Figures 2g–2h).

It was found that the cells in the woody part below the bud were damaged and disintegrated causing the degeneration of cells and vertical cleavages (Figure 3). According to our observations, visible abscissions coincided with the period when fruit begins to fill its pod, but the abscission layer approximately begins one month before this date.

3.2. Identification and quantification of ACC

Results indicate that the possible relationship between the ethylene precursor ACC content and the flower bud abscission. We analysed the ACC profiles in the different tissues which especially flower bud abscission zone during development and abscission of Uzun pistachio cultivar. Nowadays, technological developments used in research are increasing rapidly, so it is necessary to use different techniques and new devices, especially in alternative bearing. Therefore, using the recent available technology (UPLC/ESI-MS/MS), the ACC in the different tissues of pistachio were identified and significant differences in ACC content between "Off" and "On" trees throughout the experiment ($p < 0.01$) (Figures 4–9). ACC content in different tissues of pistachio showed differences during flower bud abscission and fruit kernel development.

3.3. ACC content in shoot samples

In a study on the shoot of pistachio, the ACC content values of pistachio shoot ranged from 546.82 to 4662.23 ng/g DW in 'Off' year and 651.24 to 3075.75 ng/g DW in 'On' year (Figure 4). The time course of the levels of ACC was showed different trends from those observed. Shoot ACC content gradually decreased in both "On" and 'Off' year trees during the season and the value at the last sampling date (65 DAFB) and lower from the first sampling date (35 DAFB). When the amount of ACC synthase is examined in the trees of the ‚On' year, the highest value is 3076 ng/g 35 DAFB, the lowest is 651 ng/g 65 DAFB, and the highest value in the year ‚Off' value was determined as 4.673 ng/g in 35 DAFB period, and the lowest in 547 ng/g 65 DAFB period. In contrast, the content of ACC in the leaf, flower bud and panicle of "On" year trees were significantly increased reaching the maximum level at 45 DAFB (Figures 5 and 6). The pattern of seasonal changes in the ACC content of leaf, flower bud and panicle in 'On' trees were in similar dates but higher than the changes of 'Off' year trees during early development leaf, flower bud and panicles of 'On' tree contained a high content of ACC, which peaked in 45 DAFB and sharply declined to a low level until 65 DAFB. When the results were compared with flower abscission dates, it was seen that the increasing ACC content affected the onset of abscission (Figures 4, 9, and 11).

3.4. ACC content in leaf samples

When the ACC Synthase amounts were examined in 'On' year trees, the highest amount was found as 1061 ng/g 45 in DAFB and the lowest amount was in 90 ng/g 65 DAFB period. The highest amount in 'Off' year was found to be 510 ng/g in 45 DAFB, and the lowest amount was detected in 89 ng/g periods (Figure 5). While the amount of ACC increased up to 45 DAFB periods after full flowering in 'On' year and 'Off' year leaf samples, it gradually decreased after this period and became minimum at 65 DAFB periods. Although the ACC amount in the leaves of 'Off' year trees were found to be higher compared to 'On' year trees, it was also found that the 'On' year and 'Off' year trees showed different changes in terms of ACC changes. It was determined that the ACC amounts were at minimum levels 65 DAFB both in 'On' year and 'Off' year trees (Figures 5, 9, and 11).

3.5. ACC content in flower bud samples

When the ACC synthase amounts were examined in 'On' year trees, the highest value was found as 2715 ng/g 45 days after full flowering and as 2896 ng/g in the 35 DAFB period in 'Off' year (Figure 6). The amounts increased in 'On' year flower bud samples as of the early periods of full flowering until 45 DAFB period, and following this, decreased gradually; and was minimum in 65 DAFB period. It was also found that these amounts decreased sharply 45 days after full flowering in 65 DAFB period. It was also found that the ACC amounts were at minimum levels in June when flower bud abscissions were intense. The ACC amount in flower buds in 'On' year trees were

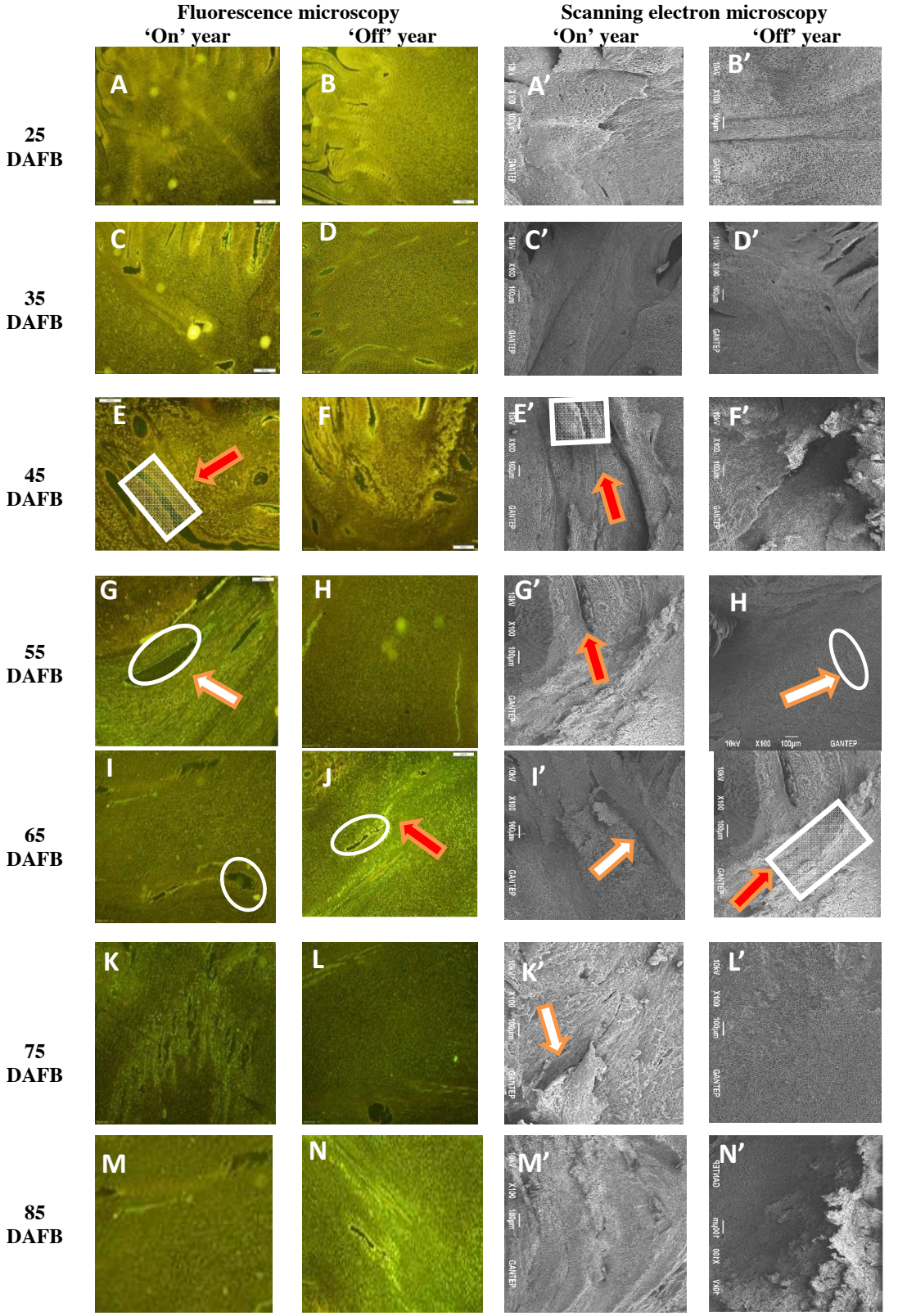


Figure 2. Morphology of flower bud abscission zone (AZ) in pistachio. Fluorescence (left) and SEM (right) microscope views of flower buds in different days after full blooming (DAFB) of cv. 'Uzun' pistachio. Scale bars: 100 µm.

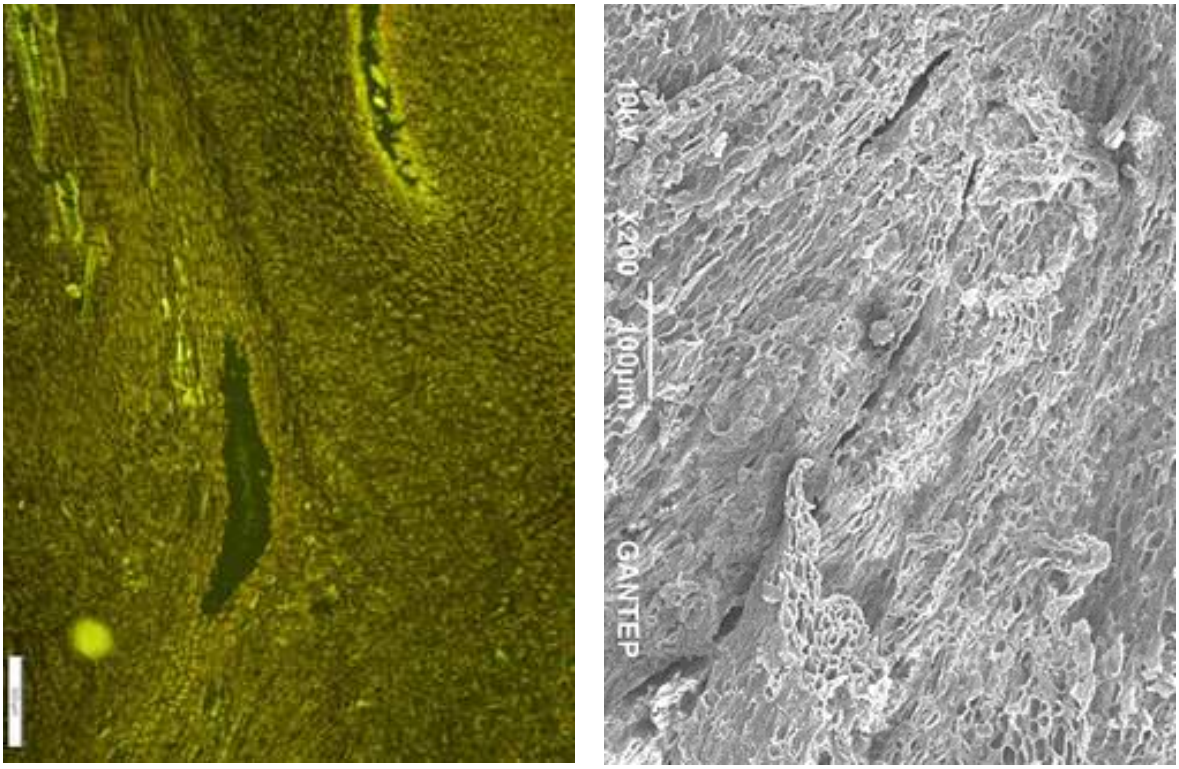


Figure 3. Detailed fluorescence microscope (left) and SEM (right) views of abscission zone (AZ) in pistachio. Scale bars: 100 µm.

found to be higher compared to 'Off' year trees (Figures 6, 9, and 11).

3.6. ACC content in panicle and nut samples

When the ACC synthase amounts were examined in 'On' year trees, the highest ACC synthase value was found as 1703,46 ng/g in 45 DAFB period, and the lowest (446,94 ng/g) amounts were detected in 65 DAFB period. Full flowering showed a sharp increase in panicle samples 65 DAFB (Figure 7). As seen from these results, it was found that the values increased rapidly especially in the period when the flower bud split layer started to form (45 DFAB). When the ACC synthase amount was examined in the nuts in 'On' year trees, the highest ACC synthase value was found to be 565 ng/g in 35 DAFB, and the lowest value was in the 65 DAFB (Figure 8). The ACC amount in nut samples in 'On' year trees was found to be high in the early period of flowering, abscissions were intense 35 DAFB, and decreased until embryo formation period, and was at minimum level (Figures 8, 9, and 11).

3.7. Principal component analysis of results

PCA is a two-linear modeling method that gives an interpretable result, an overview of the main information in a multidimensional data table. The information carried by the original variables is reflected in a smaller number of underlying variables called principal components. By plotting the main components, we can view the relationships between different variables and identify and interpret sample patterns, groups, similarities, or differences (CAMO Software AS, 1998; Kara, 2009). Principal component analysis (PCA) was performed to statistically reveal the effect of individual phenol compounds content on 'Uzun' pistachio variety on bud abscission level (alternate bearing). PCA was used to see the correlation between the various parameters and their relationship with different sample data collection dates (Figures 9–11).

Correlation analysis was also conducted to statistically reveal the effect of different tissues and ACC on alternate bearing of pistachio (Figure 9). Results showed that correlations detected between the tissues based on ACC content. There is a positive and negative relationship between of 'On'–'Off' year leaf and 'On' year fruit cluster among ACC contents. A high positive significant correlation was also found between 'Off' shoot and 'On' fruit ($p < 0.05$). A high positive significant correlation was found between fruit and shoot. Besides, a significant positive correlation was detected between leaf 'On' and leaf 'Off' with $r = 0.98$. Principal component analysis (PCA) was performed to statistically reveal the effect of ACC content on 'Uzun' pistachio variety on bud abscission level

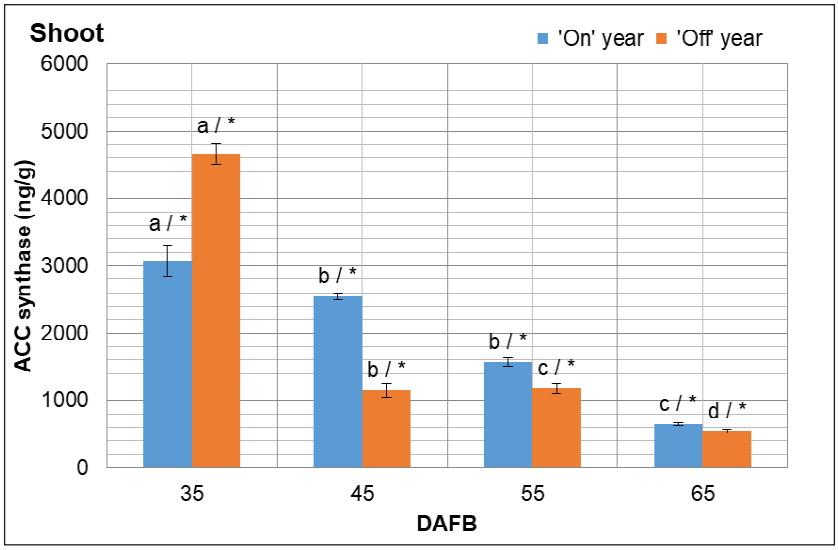


Figure 4. ACC contents detected in shoot samples during different physiological periods from 'On' year and 'Off' year trees of pistachio. DAFB, days after full blooming. *Different letters were used on the columns to show significant differences among the periods. Moreover, to compare the 'On' and 'Off' years, * was used for significant differences and ns was used for the nonsignificance.

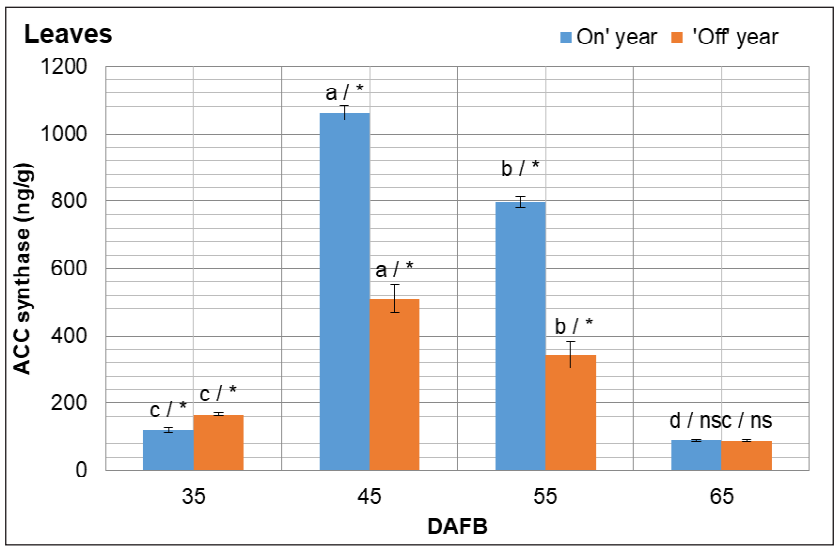


Figure 5. ACC contents detected in leaf samples during different physiological periods from 'On' year and 'Off' year trees of pistachio; other details are similar to Figure 4.

(alternate bearing). PCA was used to see the correlation between the various parameters and their relationship with different sample data collection dates (Figures 10 and 11). According to the results obtained, the effect of important that define the 'Uzun' cultivar was found to be the ACC in leaves both 'On' and' Off' and panicle. All these figures are a summary of the variables studied in this experiment. The result of the PCA analysis confirmed some important differences between ACC content and alternate bearing in different tissues of the 'Uzun' pistachio variety. DM1 and DM2 explained 49.02% and 38.52% of the total variance in nut samples. The variance of the two factors was 87.58% of the total variance. In general, the variability of PCA results in all tissues was found to be quite high, with the total variation 87.58%. At the same time, it showed that all tissues analysed generally had higher ACC compound content in 'On' year trees than 'Off' year trees. Besides, it was observed that the ACC levels were higher in the

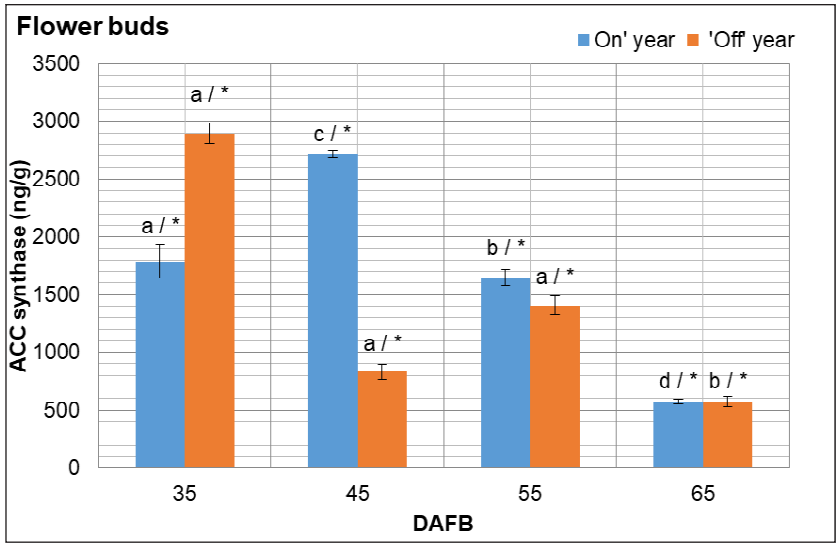


Figure 6. ACC contents detected in flower bud samples during different physiological periods from 'On' year (blue column) and 'Off' year (orange column) trees of pistachio. Other details are similar to Figure 4.

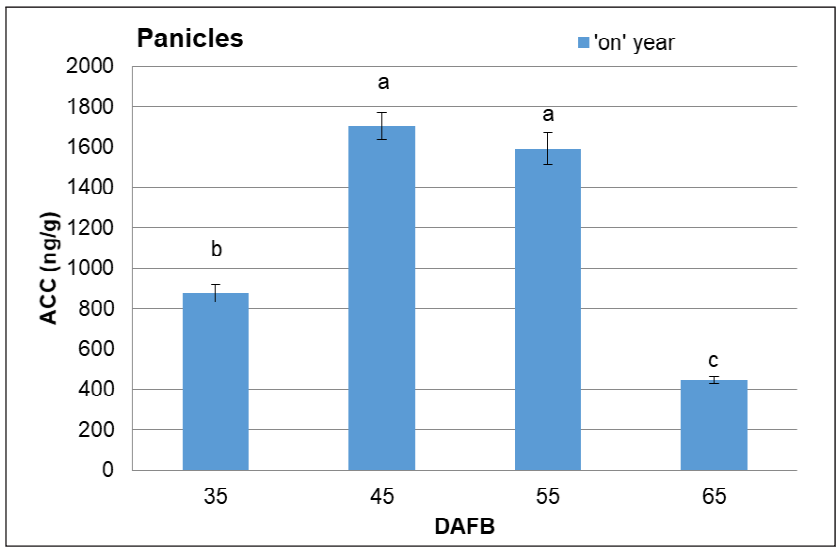


Figure 7. ACC contents detected in panicle samples in different physiological periods only from 'On' year (blue column) trees of pistachio. *Different letters were used on the columns to show significant differences among the periods.

first days and decreased especially during the fruit filling period. All these figures are a summary of the variables studied in this experiment. The result of the PCA analysis confirmed some important differences ACC compound and alternate bearing in different tissues of the 'Uzun' pistachio variety. In general, the variability of PCA results in all tissues was found to be quite high (except the 65 DAFB). Finally, the 'Uzun' pistachio cultivar is considered to be an interesting raw material with important ACC compound, and this compound is particularly effective in flower bud abscission (alternate bearing), which are all problematic from pistachio.

4. Discussion

The anatomy of abscission attracted the caution of researchers in plant science for long years. Abscission of different plant organs are caused by dissolving cell walls, therefore it is an active physiological process (Figure 2). Abscission which causes alternate bearing is the most important problem because it restricts fruit set of many

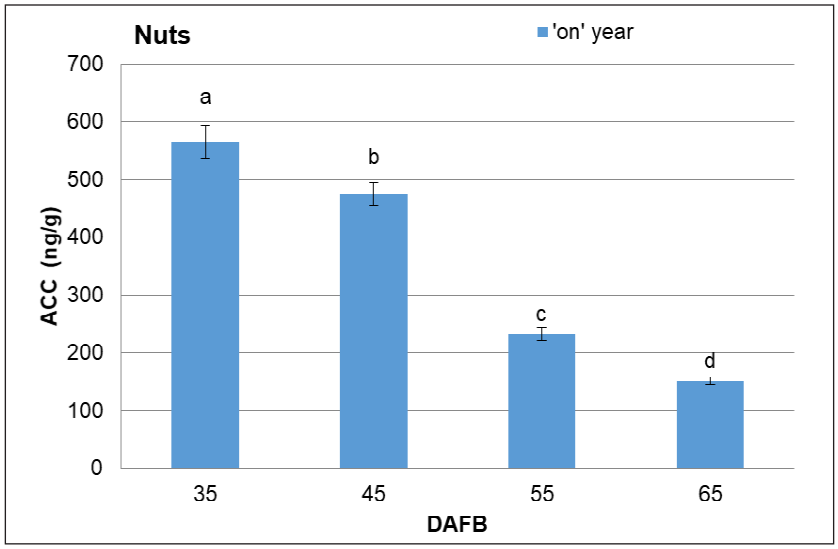


Figure 8. ACC contents detected in nut samples in different physiological periods only from 'On' year (blue column) trees of pistachio. *Different letters were used on the columns to show significant differences among the periods.

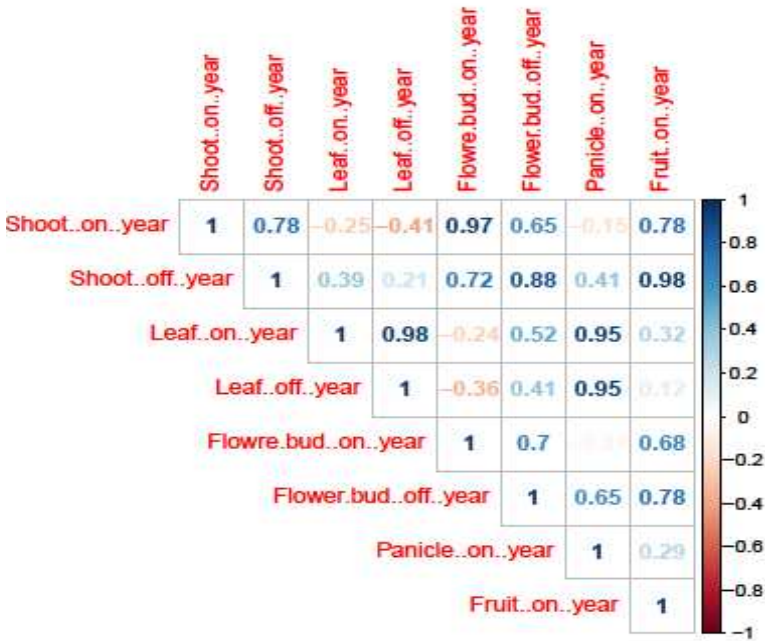


Figure 9. Pearson's correlations among tissues.

species such as pistachio (Esmaeilpour and Khezri, 2006; Talaie et al., 2010 Vemmos, 2010), olive (Gomez-Jimenez et al., 2010), walnut (Catlin and Olsson, 1990), citrus (Jahromi et al., 2015; Merelo et al., 2017), mango (Malik et al., 2003) and grapevine (Aziz et al., 2001). Pistachio is a deciduous tree with a very strong alternate bearing character. Pistachio trees produce heavy crops in alternate year due to the abscission of up to 90% of the flower buds during summer between late May and June in 'On' year. Although a great number of studies have been made on pistachio tree alternate bearing, its physiological mechanism is still not clearly understood. Most studies are emphasizing on the role of many internal factors in alternate bearing and flower bud abscission such as nutrients, carbohydrates and plant growth regulators (Monselise and Goldschmidt, 1982; Stevenson and Shackel, 1998; Gundesli et al., 2020a,b; Gundesli et al., 2021). However, there is not enough information about

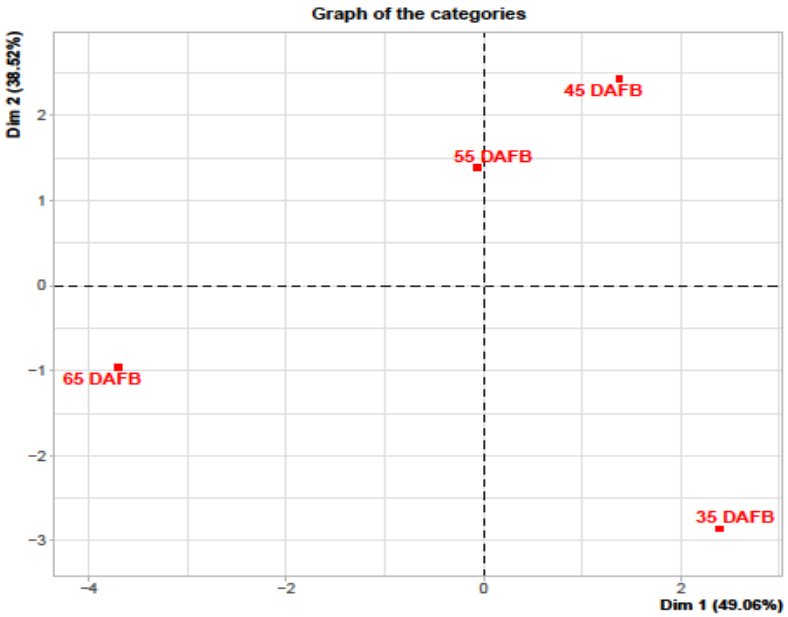


Figure 10. PCA for histochemical properties.

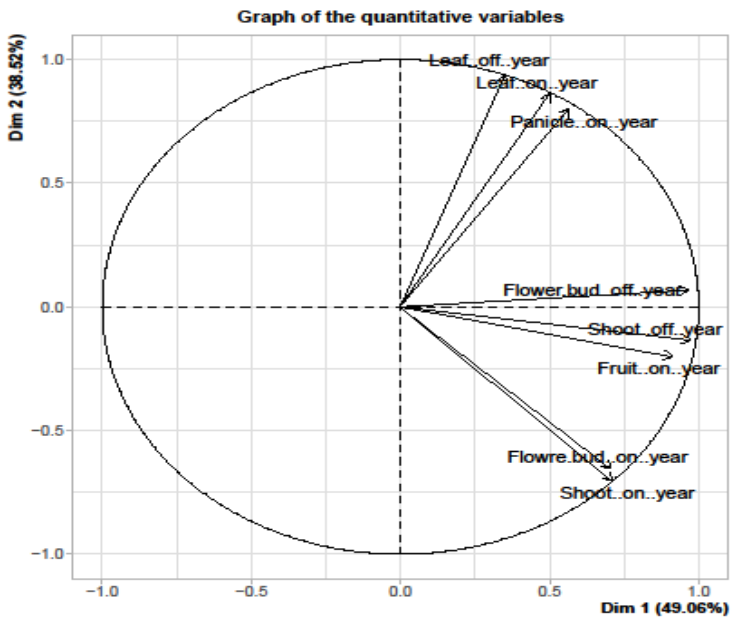


Figure 11. PCA graph for ACC.

time of flower bud abscission zone formation in pistachio and the ACC synthase activity in this period. In this research, some directions of the regulatory roles of ACC synthase and its metabolism have been explored with the aid of histological studies showing cell degeneration dates in flower buds. (Monselise and Goldschmidt, 1982; Stevenson and Shackel, 1998; Gundesli et al., 2020a,b; Gundesli et al., 2021).

Generally, differences were observed in cell structures of flower buds of different dates and between 'On' and 'Off' year tissues. As it seems in the fluorescence and SEM microscopy, the anatomical evidence provided that cells were degenerated causing vertical cleavages as abscission layers (Figures 2 and 3). Abscission has attached the attention of many researchers for many years (Iglesias et al., 2007; Zhang and Zhang, 2009). Previous studies have shown that fruit drop in peaches occurs by the activation of abscission zone (Rascio et al., 1985; Tirlapur et al., 1995). Following the detailed anatomical studies of Rascio et al. (1987), Ruperti et al., (1998) and Taheri et al., (2012)

reported some of the structural aspects related to peach fruit abscission. Structural our microscopic observations showed that the cells in the abscission zone are very compact. Our results specified that abscission zone or cell differentiation were observed firstly from 'On' year samples on 25 May, 2015 (45 DAFB) and accomplished on 4, 14 July, 2015 (55, 65 DAFB). Flower bud abscission also occurred on some samples of 'Off' year during this period. In our study no specific abscission layers in zone were detected because these cells were spread increasingly into flower bud during fruit kernel development and lead to abscission (Figure 2). Some researchers as opposed to our results and have stated that the timing of this bud loss is closely related to the growth of pistachio seed development (Crane and Iwakiri, 1986), starting of flower bud abscission at the end of June or beginning of July (90 days after full bloom) and abscission of ending mid-August (Crane and Nelson, 1971). However, it is thought that our results reveal quite new information about flower bud abscission in terms of the literature. Many researchers have found similar results about abscission in different varieties that fruit of *Olea europae* (Gomez-Jimenez et al., 2010), fruitlet of *Vitis vinifera* (Bonghi et al., 2000; Hilt and Besis 2003), fruit of *Rubus spp*. (Burdon and Sexton, 1993) fruit of *Prunus cerasus* (Stösser et al., 1969) and fruit of *Citrus reticulata* (Jahromi et al., 2015; Merelo et al., 2017). Abscission of different organs of plants might be caused by few factors, such as nutrient deficiency, disturbances in embryogenesis and/or embryo abortion, sink competition between fruits, and abiotic and biotic stressors (Talaie et al., 2010; Gomez-Jimenez et al., 2010; Jahromi et al., 2015). The amount of available carbohydrates in fruit set time, environmental, hormonal and nutritional factors can affect abscission (Aziz et al., 2001; Aziz, 2003; Zhang and Zhang, 2009; Patharkar and Walker, 2018). However, some researchers suggest that some PGs are used in leaves or seeds that move on the flower buds and trigger the abscission (Vemmos, 2010; Gomez-Cadenas et al., 2000; Okay et al., 2011; Gundesli et al., 2019; Gundesli et al., 2020 a, b). But, the definitive role of ACC metabolism in flower bud abscission is still unknown. Ethylene has long been recognized as a strong deprivation accelerator (Nakatsuka et al., 1998; Bregoli et al., 2002; Arc et al., 2013) and recent studies support the opinion held by previous researchers (Hilt and Bessis, 2003) that it may also process in different tissues native to abscission. Studies with abscission explants clearly show that ethylene plays a role in inducing the production of hydrolytic enzymes in the abscission zone. For example, the effects of ethylene include stimulation of flower, leaf, fruit abscission, fruit ripening, senescence, seedling extension inhibition and stimulation of root induction (Vemmos et al., 1994; Nakatsuka et al., 1998; Llop-Tous et al., 2000; Barry and Giovannoni, 2007). ACC synthase is the main regulatory enzyme in ethylene synthesis in high plants (Arc et al., 2013). We investigated the content of ACC in various plant organs during flower bud abscission. According to our results, during the early development of leaf, flower bud and panicle in 'On' year trees contained a high content of ACC, which peaked 45 days after full blooming (45 DAFB) and sharply declined to a low level until 65 DAFB (Figures 5–7). While the results were compared with flower bud abscission zone analysis, it shows that the increasing ACC content affects the abscission time. Many researchers have reported similar results that the ACC production rate usually increases before flower bud abscission and fruit kernel development, which plays a decisive role in the regulation of abscission. Our results show that an inverse relationship between ACC content and abscission and that many researchers suggesting that high levels may increase ethylene production in different tissues of pistachio (Figures 4 and 11) (Mansour et al., 1986; Paksasorn et al., 1995; Nakatsuka et al., 1998; Hilt and Bessis, 2003; Merelo et al., 2017). ACC synthesis in regulating flower bud abscission may be possible. These reports and our data support the assumption that ACC in regulating flower bud abscission. If so, the ACC will primarily stimulate the synthesis of hydrolases, increase ACC production and coordinate the destruction process itself.

5. Conclusion

Abscission is an important development process of the plant throughout its life, involving the natural separation of organs from the parent plant. Ethylene is known as the major plant hormone responsible for the initiation and progression of abscission. However, the interaction of other hormones such as auxin and abscisic acid has also been reported to play important roles. However, there is insufficient evidence regarding the repturing layer of fruit bud abscission, especially in pistachios, and that important plant growth regulator such as ACC can cause abscission. In this study, histochemical demonstrations of abscission layer formation in *Pistacia vera* L. was studied. The histological studies showed that the abscission layers showed their first signals long before flower bud abscission and this period has coincided with an acceleration of ACC synthase in flower bud and leaves. This knowledge ultimately will lead to improved control of abscission and show alternate bearing in many crops.

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