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Effect of the exogenous application of abscisic acid (ABA) at fruit set and at veraison on cell ripeness of olives *Olea europaea* L. and the extractability of phenolic compounds in virgin olive oil

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Abstract

Background: The effect of the exogenous application of abscisic acid (ABA) at the fruit set and veraison on the cell maturity of the olive *Olea europaea* L. and on the extractability of phenolic compounds (PC) in the virgin oil olive was studied.

Methods: The ABA was sprayed on olive trees of the Moroccan Picholine variety at a concentration of 10^{-3} mg/l, some olive trees are treated at fruit set stage and other olive trees are treated at veraison stage. The effects of these treatments were evaluated by fruit yield and determination of the date of veraison and ripening period of the olives. The extractability of olive oil and diffusion of PC in the latter as well as the weakening of the parietal structures are also estimated.

Results and discussion: The application of ABA at fruit set causes a decrease in the production of fruit about 50% and precocity of ripening estimated 45 days. At this stage, comparing with the control in the same period, there was a significant accumulation of fat in olives, an increase in oil extractability and a significant improvement in the diffusion of PC in oils. The treatment of the olives by the ABA at veraison has no effect on yield. However, we observe physiological and biochemical changes to be identical during the treatment by ABA at veraison but smaller than that at fruit set.

Keywords: Abscisic acid, Olive oil, Phenolic compounds, Ripening

Background

The improvement of the agronomic characteristics of fruit is essential to mitigate the problems associated with low consumption. Plant hormones (phytohormones or growth regulators) have long been known to be closely associated with the development of fruit and maturation [1, 2]. The results on the regulation of many genes related to the biosynthesis and signaling of several phytohormones (ethylene, ABA, cytokinin, the brassinosteroid, auxin, and gibberellic acid) indicate the involvement of the latter in the regulation of fruit set and maturation [3] and suggest that a precise balance between their biosynthesis and their responses is of fundamental importance.

The abscisic acid (ABA), which is a growth inhibitor, is involved in fruit development, but knowledge of its precise role and mode of action remains poorly detailed [4, 5]. The ABA levels show a reduction at fruit set and this decrease was associated with downregulation of ABA biosynthesis genes and regulation of the gene encoding the enzymes involved in degradation of ABA after pollination [6]. The ABA also abolishes changes induced by gibberellic acid (GA) during fruit set in pea [7]. However,

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several studies show that the biosynthesis and accumulation of phenolic compounds are under dependence of hormonal factors: generally the plant growth stimulator (auxins, cytokinins, …) have a negative effect on these processes whereas growth inhibitors promote.

This work aims to study the influence of exogenous ABA treatment on the maturation of olives and the extractability of phenolic compounds in virgin olive oil. The effects of this treatment was assessed by determining changes in physical (weight, diameter, humidity) and biochemical parameters during the ripening of olives (anthocyanin content, oil accumulation, percentage of oil flow, and its content of phenolic compounds). The embrittlement of the parietal structures of fruit estimated by changes in levels of protopectins and the endogenous enzyme activities during the ripening of olives was also determined.

Methods

Plant material

This study was conducted on non-irrigated olive trees of the Moroccan Picholine located in the botanical garden of the Faculty of Science and Technology of Fez-Morocco. These olive trees are of the same age, and have the same exposure to the sun and the same type of soil. For these olive trees, fruit setting stage was estimated at the 3rd week of June 2014.

Two treatments by ABA (10^{-3} mg/l) were made during the development and growth of the olives: olive trees are treated at fruit set, other trees are treated at veraison, and other trees are left without treatment as control. Periodic samples were taken from the veraison to the total maturity of the olives to follow the evolution of the parameters studied.

Moisture determination olives

The moisture content of olive drupes was determined gravimetrically according to the UNE standard Spanish method [8].

Determination of the total oil content

The extraction of the total oil content is carried out using hexane in a Soxhlet extractor for 4 h from the dryer used for the determination of humidity content material [9]. After that, the traces of solvent are removed by evaporation. The determination of the oil content is determined by the weight of dry matter (% dry weight), and the weight of wet matter (% wet weight).

Extraction and determination of phenolic compounds (PC)

The procedure of phenolic compounds extraction from olive fruit is based on the method of Brenes [10], with some changes. Olive pulp (10 g) is mixed with 30 ml of methanol: water 80/20 (v/v). The mixture was centrifuged for 5 min at 3500 rpm and then filtered. This extraction was repeated three times. The extracts are collected after evaporation of the organic solvent. Phenolic compounds are extracted with ethyl acetate (5 × 20 ml). The extraction of oil polyphenols is performed with methanol according to the method of Vázquez-Roncero [11]. The assay of total polyphenols is based on the reduction of phosphomolybdic acid of Folin Ciocalteu agent by polyphenols in alkaline medium [12].

The assay of anthocyanins is based on the method used by Ribereau-Gayon and Stonestreet [13] implementing discoloration of cations flavylium by sulfur dioxide.

Isolation and determination of total polysaccharides

The isolation of pectins is achieved from 50 g-olive pulp according to the procedure of Saulnier and Thibault [14] which implements the precipitation of insoluble material in alcohol (MIA) by several washings with ethanol 95°. From this MIA, successive extractions with water, sodium oxalate, hydrochloric acid, and soda are done.

The assay principle of the pectic substances is based on the colorimetric determination of the galacturonic acid content of the pectic chains hydrolysed in hot acidic medium of different fractions isolated in the presence of 3-hydroxydiphenyl [15].

Extraction and measurement of endogenous enzyme activities

1 g of olive pulp homogenized for 30 s in 25 ml potassium phosphate buffer (0.05 M, pH 6.6) with 0.2 g of Triton X-100 using a Polytron homogenizer. 25 mg polyvinylpyrrolidone (PVPP) were added and the suspension was centrifuged at 4 °C for 15 min at 13,000 rpm. The supernatant is filtered through glass wool and used as a source of crude enzyme [16]. The various activities are determined as follows:

The polygalacturonase (PG) is assayed by the method of Somogyi-Nelson [17].

The pectinestérases enzymes (PE) are assayed by the method of Baron [18].

Statistical analyses

An analysis of variance was performed for each parameter studied. The multiple comparison test averages Tukey post hoc is used to test for significant differences between treatments (at 5%). Univariate analysis was used to test for significant differences in treatment, their interaction for a single parameter. All statistical analyses were performed with IBM. SPSS statistics, version19. The results of every experiment are obtained from triplicates.

Table 1 Influence of ABA applied at fruit set and at veraison on the yield of olive fruit

Treatment	Control	ABA (N)	ABA (V)
Number of fruits per branch	94.7 ± 7.4^{a}	49.7 ± 5.7^{b}	96.33 ± 15^{a}
% Of fruit per branch	100 ± 0^{a}	52.8 ± 8.8^{b}	101.4 ± 8.5^{a}

[a,b] Values followed by different letters are significantly different ($P = 0.05$)

ABA (N) treatment at fruit set, *ABA (V)* treatment at veraison

Results and discussion

Determination of the period of the growth, maturation, and agronomic indexes during the ripening of the olives treated with ABA

Influence of ABA on the number of fruit per olive branch

Table 1 shows the influence of ABA on fruit yield by olive branch of the Moroccan Picholine. We found that exogenous application of ABA at fruit set on trees leads to a significant decrease in the number of fruit on the branches. This reduction can reach 50% compared to control, while the application of ABA at veraison has no influence on the number of fruits.

Influence of ABA on the period of growth and maturation of the olives

Table 2 shows the influence of exogenous application of ABA on the olive trees of the Moroccan Picholine at fruit set and at veraison. We found that the olives treated at fruit set stage reached veraison 2 weeks earlier than the control and reach full maturity early towards the end of October, about one and a half months before the controls.

However, the treatment of olive trees at veraison leads to earlier ripening than control fruit. The full maturity is reached around the third week of November, 2 weeks before the full maturity of the control olives.

Study of the effect of ABA on the extractability of oil and its PC content during the ripening of the olives

During the ripening of the olives, the fat content increases in a regular manner from ripening until full maturity (Table 3). This increase follows the increase in weight and diameter and the decrease in olive moisture. Furthermore, fluctuations in the total polyphenols were observed during the ripening of olives, with their contents being increased and decreased at veraison and during the ripening, respectively, and then increased again at maturity.

However, the application of exogenous ABA treatment to fruit set accelerates the accumulation of fat in the oil-bearing cells with higher contents in total fruit maturity compared to the same stage in the control olives (Table 4). This accumulation is accompanied by an increase of the weight and diameter of the olives. Nevertheless, these two parameters are too low compared to the control: the weight of full maturity olives does not even reach the values recorded in control olives at veraison, while the treated fruits have a lower moisturecontent at full maturity compared to the control. However, the concentration of the olives in these compounds is high compared to the control along the ripening period except the last sample.

Table 2 Influence of ABA on the period of fruit set and ripening of the olives

Treatment	Period of growth and ripening						
	Juin	Juillet	Aout	Sept.	Oct.	Nov.	Déc.
Control	▪▪▪▪	▪▪▪▪	▪▪▪▪	▪▪▪▪	———	———	
ABA (N)	▪▪▪▪	▪▪▪▪	▪▪▪▪	———	———		
ABA (V)	▪▪▪▪	▪▪▪▪	▪▪▪▪	▪▪▪▪	———	———	

ABA (N) treatment at fruit set, *ABA (V)* treatment at veraison, (▪▪▪▪) period of growth, (———) period of ripening

Table 3 Agronomic indices of control olives during ripening

Parameter	Samples (control)						
	S1 (21/09)	S2 (03/10)	S3 (14/10)	S4 (25/10)	S5 (07/11)	S6 (19/11)	S7 (05/12)
Weight of 100 olives (g)	394.1 ± 7.6	401.4 ± 5.4	433.8 ± 7.1	443.4 ± 3.5	445.5 ± 2.2	451.12 ± 2.33	455.9 ± 3.4
Diameters (mm)	17.25 ± 1.32	17.25 ± 1.04	17.68 ± 1.3	17.7 ± 2.12	17.9 ± 1.9	17.93 ± 1.78	18 ± 2.7
Moisture content (%)	64.4 ± 0.13	63.6 ± 0.2	61.9 ± 0.16	58.7 ± 0.15	57 ± 0.11	56.42 ± 0.46	56 ± 0.14
Oil content of olives (g/100 g F.O.P)	2.56 ± 0.35	3.64 ± 0.19	4.51 ± 0.33	7.72 ± 0.39	14.31 ± 0.43	18.03 ± 0.43	20.8 ± 0.51
T.P content of olives (mg/g F.O.P)	58.8 ± 1.95	69.1 ± 2.1	50.7 ± 2.42	52.4 ± 1.51	54.2 ± 1.25	66.9 ± 1.7	69.9 ± 1.4

T.P total polyphenols, *F.O.P* fresh olive pulp

Table 4 Influence of the application of ABA at fruit set on agronomic indices of olives during ripening

Parameter	Samples (ABA_N)				
	S1 (09/09)	S2 (21/09)	S3 (03/10)	S4 (14/10)	S5 (25/10)
Weight of 100 olives (g)	314.45 ± 6.25	318.2 ± 4.7	348.4 ± 3.2	360.3 ± 4.9	376.1 ± 3.2
Diameters (mm)	15.71 ± 0.67	15.75 ± 1.02	16.73 ± 1.28	16.84 ± 0.72	17.22 ± 0.84
Moisture content (%)	60.35 ± 1.1	56.8 ± 0.1	55.83 ± 0.2	53.8 ± 0.23	47.9 ± 0.23
Oil content of olives (g/100 g F.O.P)	4.11 ± 0.12	4.66 ± 0.41	7.26 ± 0.12	14.19 ± 0.4	26.26 ± 0.37
T.P content of olives (mg/g F.O.P)	77.9 ± 1	83.8 ± 2.6	83.5 ± 1.9	70.6 ± 1.8	64.8 ± 1.95

ABA_N treatment at fruit set, *T.P* total polyphenols, *F.O.P* fresh olive pulp

Table 5 Influence of the application of ABA at veraison on agronomic indices of olives during ripening

Parameter	Samples (ABA_V)					
	S1 (21/09)	S2 (03/10)	S3 (14/10)	S4 (25/10)	S5 (07/11)	S6 (19/11)
Weight of 100 olives (g)	392 ± 2.03	396.5 ± 4.7	403.5 ± 3.4	414.3 ± 4.3	425.5 ± 2.3	434.2 ± 3.6
Diameters (mm)	16.92 ± 1.14	17.05 ± 0.98	17.15 ± 1.33	17.42 ± 1.75	17.56 ± 1.72	17.64 ± 1.48
Moisture content (%)	63.9 ± 0.1	61.73 ± 0.1	58.1 ± 0.16	55.2 ± 0.11	53.5 ± 0.1	49.04 ± 0.04
Oil content of olives (g/100 g F.O.P)	2.86 ± 0.12	3.86 ± 0.3	4.94 ± 0.23	7.91 ± 0.2	14.68 ± 0.33	24.45 ± 0.53
T.P content of olives (mg/g F.O.P)	61.7 ± 2.6	90.8 ± 1.7	77.4 ± 1.4	69.5 ± 1.1	61.1 ± 1.7	67.6 ± 1.5

ABA_V treatment at veraison, *T.P* total polyphenols, *F.O.P* fresh olive pulp

Conversely, spraying olives by ABA at veraison causes a lower acceleration of the accumulation of fat than that recorded by the treatment at fruit set with contents of less than full maturity and higher compared to the control at the same stage (Table 5). In parallel, the diameter and weight of olives increased during ripening but their total maturity values remain lower than the values recorded in olives controls at veraison. As for total polyphenols in olives, their contents are experiencing fluctuations during ripening, they increase at color change after applying of treatment, and then decrease during ripening before increasing again at full maturity. We further observe that these levels are higher than in control olives at the beginning of the ripening but they tend to stabilize at full maturity.

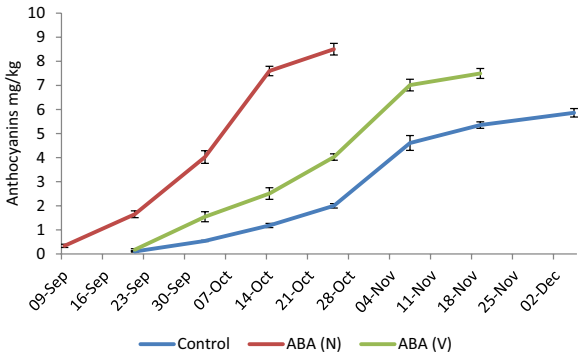

Fig. 1 Influence of ABA on the contents of anthocyanins during the maturation of the olives. *ABA (N)* treatment at fruit set, *ABA (V)* treatment at veraison

Influence of ABA on the levels of anthocyanins

Figure 1 shows the evolution of anthocyanins during the ripening of olives treated by the ABA at fruit set and at veraison. The accumulation of anthocyanins starts just after veraison of olive fruits and their levels increase gradually to reach their maxima at full maturity. In olives treated by the ABA at fruit set, the appearance of anthocyanins begins 2 weeks earlier than in the controls and their contents are increasing rapidly and in a very important way to reach their maxima at total maturity with significantly high levels. This causes early maturity of the olives. In olives treated by the ABA at veraison, the accumulation of anthocyanins is increasing immediately after application of the treatment and their levels increase exponentially to reach their maxima at full maturity with significantly high levels compared to control. Note in this case that the levels of anthocyanins at maturity are less important than when treating at fruit set.

Influence of ABA on the olive oil extraction during of ripening of the olives

For all treatments, olives gradually accumulate fat in their cells during ripening (Table 6). This accumulation begins before veraison and reaches its maximum at full maturity of the fruit. The levels of oils extracted from olives are

Table 6 Influence of ABA on the total fat in the olives, the quantities of oil extracted, and their flow percentages during the ripening of the olives

Samples	Total fat (g/100 g olive pulp)			Quantities of oil extracted (g/100 g olive pulp)			% of flow		
	Control	ABA (N)	ABA (V)	Control	ABA (N)	ABA (V)	Control	ABA (N)	ABA (V)
09-Sept	–	4.11 ± 0.13	–	–	1.28 ± 0.1	–	–	31.1 ± 1.7	–
21-Sept	2.56 ± 0.35^{a}	4.66 ± 0.41^{b}	2.86 ± 0.12^{a}	0.65 ± 0.05^{a}	1.59 ± 1.2^{b}	0.71 ± 0.03^{a}	25.84 ± 4.17^{a}	34 ± 1^{b}	24.8 ± 0.41^{a}
03-Oct	3.64 ± 0.2^{a}	7.26 ± 0.12^{b}	3.86 ± 0.3^{a}	1.28 ± 0.03^{a}	4.12 ± 0.11^{b}	1.4 ± 0.03^{a}	35.23 ± 1.22^{a}	56.71 ± 0.62^{b}	36.25 ± 2^{a}
14-Oct	4.51 ± 0.33^{a}	14.2 ± 0.4^{b}	4.94 ± 0.23^{a}	2.55 ± 0.3^{a}	12.2 ± 0.92^{b}	2.75 ± 0.1^{a}	56.52 ± 3.26^{a}	86.08 ± 8.72^{b}	55.58 ± 0.83^{a}
25-Oct	7.72 ± 0.4^{a}	26.26 ± 0.37^{b}	7.91 ± 0.2^{a}	5.43 ± 0.25^{a}	21.82 ± 0.33^{b}	6.11 ± 0.4^{a}	70.36 ± 0.49^{a}	83.06 ± 1^{b}	77.43 ± 6.64^{a}
07-Nov	14.31 ± 0.43^{a}	–	14.68 ± 0.33^{a}	11 ± 0.2^{a}	–	11.35 ± 0.4^{a}	76.86 ± 1.2^{a}	–	77.28 ± 1.14^{a}
19-Nov	18.03 ± 0.43^{a}	–	24.45 ± 0.53^{b}	14.2 ± 0.45^{a}	–	19.94 ± 0.4^{b}	78.8 ± 4.2^{a}	–	81.58 ± 2.9^{a}
05-Dec	23.5 ± 0.51	–	–	19.46 ± 0.3	–	–	82.2 ± 1.12	–	–

^{a,b} Values followed by different letters are significantly different ($P = 0.05$)

ABA (N) treatment at fruit set, *ABA (V)* treatment at veraison

low at veraison and subsequently increase during ripening and reach their maximum at full maturity.

Treatment with ABA at fruit set entrains a very rapid and significant accumulation of lipids in the olives. This fast accumulation causes a early ripening of fruits since the maximum accumulation is recorded 6 weeks before the control with higher levels (26/100 g against 23.5/100 g FW for the control). Furthermore, there is an increase of extracted oil quantities and this from veraison until total maturity to reach higher values compared to the control at the same stage (respectively 22/100 g FW and 19.5/100 g FW). Under these conditions, the oil extractability represented by the flow percentage is maximum at maturity and can even exceed that of the control. It should be noted that on the date of maturity of the olives treated at fruit set, the latter have already accumulated 22/100 g of oil and have a flow rate of 83% whereas control olives that have accumulated 5.5/100 g of oil and have a flow rate of 70%.

Conversely, olives treated at veraison show no significant difference in the accumulation of the fat or the oil extractability at stage maturity compared to the control. Nevertheless, there is an early maturity since these olives ripened 2 weeks before control olives.

Influence of ABA on total polyphenol content of the extracted oils and their diffusion percentages

Table 7 shows the evolution of total polyphenol contents during ripening in both olives than in their extracted oils. It is observed that the contents of these phenolic compounds in extracted oils are extremely very small compared to their total content in the olives.

However, the total polyphenol content of the extracted oils is quite high at veraison, then decreases gradually during the ripening independently of the treatment applied. Moreover, the treatment of olives by ABA at fruit set causes a significant increase of total polyphenols

Table 7 Influence of exogenous treatment with ABA on the total polyphenol contents of the extracted oils during ripening

Samples	Total polyphenol content of the olives (mg/g olive pulp)			Diffusible total polyphenol content in extracted oils (mg/kg olive pulp)		
	Control	ABA (N)	ABA (V)	Control	ABA (N)	ABA (V)
09/09	–	77.9 ± 1	–	–	1284.4 ± 20.4	–
21/09	58.8 ± 1.95^{a}	83.8 ± 2.6^{b}	61.7 ± 2.6^{a}	804.6 ± 47.9^{a}	1214.8 ± 43.8^{b}	826.8 ± 39.4^{c}
03/10	69.1 ± 2.1^{a}	83.5 ± 1.9^{b}	90.8 ± 1.7^{c}	685.5 ± 64^{a}	988.8 ± 82.4^{b}	920.9 ± 65.6^{b}
14/10	50.7 ± 2.42^{a}	70.6 ± 1.8^{b}	77.4 ± 1.4^{c}	578.7 ± 54.1^{a}	677.2 ± 46.9^{b}	647.8 ± 56^{b}
25/10	52.4 ± 1.51^{a}	64.8 ± 1.95^{b}	69.5 ± 1.1^{b}	446.5 ± 91.1^{a}	533.1 ± 83.9^{b}	502.4 ± 62.2^{b}
07/11	54.2 ± 1.25^{a}	–	61.1 ± 1.7^{b}	366.3 ± 73.3^{a}	–	456 ± 86.8^{b}
19/11	66.9 ± 1.7^{a}	–	67.6 ± 1.5^{a}	306.2 ± 12.9^{a}	–	354.5 ± 76.8^{a}
05/12	69.9 ± 1.4	–	–	216.1 ± 74.9	–	–

^{a,b} Values followed by different letters are significantly different ($P = 0.05$)

ABA (N) treatment at fruit set, *ABA (V)* treatment at veraison

in olives and in oils obtained throughout ripening. At maturity, this oil is much richer in polyphenols than the control oil (respectively 533 and 216 mg/kg) or a gain of 146%.

The treatment with ABA at veraison shows no significant differences in the levels of polyphenols in olives. Nevertheless, there is a significant increase in the levels of total polyphenol extracted oils. This increase is smaller than in the case of treating at fruit set.

Effect of ABA on cell embrittlement during ripening of the olives

Evolution of pectins content and enzyme activities endogenous in olives during ripening

At the ripening, the component pectins of the cell walls of olive consist mainly by insoluble protopectins. The soluble pectins extracted by water and Na oxalate represent a small part compared to total pectins (Table 8). During the ripening of the drupe, the contents of protopectins declined steadily and very significantly to represent at maturity about 25% of their initial contents. At the same time, there has been a steady increase in soluble pectin contents so that at maturity, the difference between these two pectin fractions becomes very low.

Furthermore, Fig. 2 shows the effect of ABA on the development of enzymatic activities PE and PG of olives during ripening. It is found that both activities generally increase during ripening: the PE activities increases gradually to a maximum recorded in mid-ripening then drops to a lower content than observed at veraison. The PG activities decreased during veraison then slowly increasing during the ripening to reach its maximum at full maturity. The increase in these activities is accompanied by lower contents of protopectins and an increased content of soluble pectins (Table 8).

However, the treatment of olives by the ABA at fruit set causes a drop in contents PE from veraison, the decrease was observed in olives controls towards the end of ripening. Similarly, PG activities are increasing rapidly at the beginning of ripening, something found in olives controls at the end of ripening. In addition, the treatment of olives

Table 8 Influence of ABA on the contents of various fractions of pectin (µg galacturonic acids/g dry matter) of olives during ripening

Treatment	Soluble pectins			Protopectins		
Harvest date	Control	ABA (N)	ABA (V)	Control	ABA (N)	ABA (V)
09-Sept	–	1922	–	–	15,246	–
21-Sept	1846	2035	1701	18,604	10,334	17,867
03-Oct	1928	2202	1967	12,888	9597	13,639
14-Oct	1771	2365	2089	11,613	5696	11,389
25-Oct	2525	2875	2147	9288	2974	8690
07-Nov	2934	–	2410	7150	–	6312
19-Nov	3011	–	2550	6239	–	3794
05-Dec	3198	–	–	4572	–	–

ABA (N) treatment at fruit set, *ABA (V)* treatment at veraison

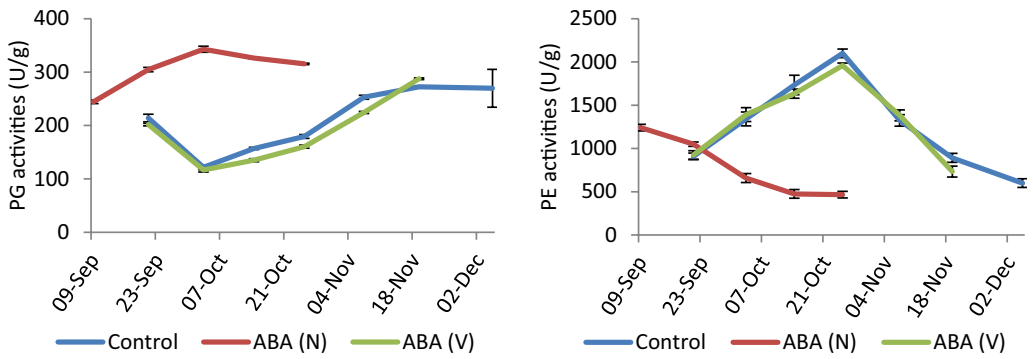

Fig. 2 Influence of ABA on the evolution of the contents of endogenous enzyme activities, polygalacturonase (PG) and pectinestérases activities (PE) during the ripening of olives. *ABA (N)* treatment at fruit set, *ABA (V)* treatment at veraison

at veraison contained no significant difference in these enzyme activities during the ripening of the olives.

Influence of ABA on the degradation of pectic substances constituting the cell wall during ripening olives

At the ripening, fruit cell walls consist mainly of protopectins which represent about 90% of all pectins (Table 8). During ripening, these protopectins decrease while soluble pectins increase so that at maturity, the difference between these two types of pectins decreases and this regardless of the treatment used. The application of exogenous ABA at fruit set leads to a very rapid and intense degradation protopectins (over 80%) from veraison so that after a month and a half, the difference observed between the protopectins and soluble pectins disappears. In these conditions, protopectins reach precociously low contents compared to the control and even content lower than that recorded at total maturity of the control. Furthermore, the treating performed at veraison contained no significant difference in the degradation of pectin compared to the control but with a slight decrease at maturity.

Discussion

The application of ABA had a different impact on both growth and ripening of the drupes depending on whether the treatment was applied at fruit set or at ripening:

The application of ABA at fruit set plays a very important role on the growth and ripening of the olives and on the biochemical characteristics of the oil: it is first of all a fruit yield decline of about 50% resulting in a chemical thinning of the olive tree and confirming other work done on other fruit trees such as apple [19] and pear [20]. Very significant morphological changes as well as physiological and biochemical accompanies this yield reduction: there is a decrease of the diameter and weight of fruit observed from veraison to so that at maturity, these two parameters do not reach even to those of the control at veraison. This weight reduction is mainly due to a reduction of water content during ripening which has the effect of increasing the fat content in olives treated compared with olives controls (respectively 26 and 20%). Furthermore, precocity is observed in veraison of fruit materialized by a synthesis of anthocyanins which starts about 2 weeks before the control olives and their contents are increasing rapidly and in a very important way to achieve their maximum at full maturity with values significantly higher. This accumulation of anthocyanins is found in grapes treated with ABA at fruit set [21].

The location of fat in cells of the olives has been the subject of numerous works: according to B. Rangel, the fat accumulates in the cytoplasm of cells of oil-bearing mesocarp, endocarp, and epicarp [22]. According Ranalli, this fat is localized in the cytoplasm in bound form but also in the vacuoles in free form [23]. In any case, the extraction of the oil is in close relation with the state of cellular maturity, itself linked to the degree of brittleness of cell membranes and walls which constitute a physical barrier to its flow. The brittleness of plant cells is estimated by the contents of protopectins that ensure the rigidity of the cell walls. In our case, regardless of the treatment performed, these protopectins decrease during ripening of olives but in a very intense and quickly in olives treated at fruit set and less than those treated at veraison. This promotes the softening of the parietal and membrane structures of the fruit thus facilitating the flow of the fat. These results are in disagreement with the work of Isabel Mínguez-Mosquera on olives cv. Hojiblanca who observed that the protopectins content remains stable during the ripening of olives [24]. Furthermore, pectin degradation is related to the contents of olives of endogenous pectolytic activities. These enzymes convert the protopectins to soluble substances firstly by demethylation using pectinesterases activities, then hydrolysis leading to small chains of galacturonic acids using polygalacturonase. These physiological events that are influenced by the period of the application of ABA, result in more or less intense embrittlement of the parietal structures and release of cell contents causing a flow of fat that exceeds 83% at full maturity of olives when they are treated by the ABA at fruit set. At that stage, control drupes have not reached yet their maturity and they present a flowing percentage estimated at 70%. When olives are treated at veraison, a similar but less important phenomenon is observed, leading to a slight acceleration of ripening and diffusion of fat.

Nevertheless, cell brittleness does not allow an important diffusion of phenolic compounds in the fat during extraction, a small fraction is found in oil, and this proportion decreases during the ripening of olives. The passage of these compounds is related to the concentration of oleuropein which is the major phenolic compound of the fruit. At veraison, the concentration of this precursor compound can reach 14% of the dry weight [25]. During ripening, the appearance of anthocyanins leads to a very significant reduction in oleuropein [26–28] through the β-glucosidase activity which causes its conversion into aglycone and oleuropein 3,4-DHPEA-EDA during extraction. This decrease may be due also to the activation of the polyphenoloxidase (PPO) and peroxidase (POD) during mixing [28, 29]. However, olives treated by ABA at fruit set have a very important extractability of phenolic compounds. ABA considerably reduces the extensibility of the pectocellulosic walls by inhibiting any deposition of substances in the cell wall which facilitates the extractability of the phenolic compounds; unlike phytohormones that

stimulate growth, which lead to an increase in the plasticity of the cell wall according to the principle of "embrittlement-deposit." This decrease of wall rigidity promotes the release of the phenolic compounds. Then there is the stimulation of anthocyanins synthesis whose content reaches far higher values than the control. Hale and his collaborators showed that the exogenous application of growth inhibitors on clusters during the growth phase accelerates biosynthesis of anthocyanins [30], confirming the work of Amrani Joutei on the grapes [21]. Several studies show the importance of hormonal factors in the biosynthesis and accumulation of phenolic compounds; generally growth inhibitors such as ABA promotes these processes and activate enzymes phenolic biosynthesis in general, and in particular anthocyanic, including the phenylalanine ammonia-lyase (PAL) [31]. Thus, we have seen a dramatic increase in contents of phenolic compounds in olives, specifically the total polyphenols and anthocyanins, and also in their oils extracted when treatment is applied at fruit set.

The application of ABA at the veraison follows an pattern of ripening dentical to that observed for the application of the phytohormone at fruit set. However, no influence is observed on the fruit yield. These results are in agreement with the work of Hartmann who showed that concentrations 1000 and 2000 mg. l-1 of ABA have no effect on the fall of the Manzanilla variety olives [32]. Contrariwise, Américo and Thomas found that olives of Arbequina variety were treated at veraison by ABA at concentrations of 300 or 400 mg. l-1 causes a fall of fruit estimated at over 11% [33]. Precocity of harvest is also observed by the treatment at veraison but less than that observed in the case of treatment at fruit set that is estimated about a fortnight. This advancement the date of the harvest is due to the stimulatory effect of exogenous ABA on the accumulation of anthocyanins [34]. This phenomenon is observed in other fruits such as grapes [35–39] and in rambutan [40]. This result does not confirm those of Américo and Thomas who observed that the application of exogenous ABA at veraison causes a delay in the accumulation of anthocyanins in the Arbequina variety olives [33].

On the other hand, the application of exogenous ABA at veraison has no significant effect on the fat content of olives and on the quantities of oil extracted and in their contents of phenolic compounds. This results is in agreement with that of Americo C. and Thomas FL who have noticed no effect on the oil content in olives from the Arbequina variety [33].

Conclusion

The influence of the exogenous application of ABA was studied on cell maturity and extractability of the fat and phenolic compounds during the ripening of the olives from the Moroccan Picholine variety. In this study, we have shown that the application of ABA causes precocity of ripening of olives especially when the treatment is applied at fruit set. This precocity is less important when the application is carried out at veraison. This precocity is accompanied by a very important and rapid accumulation of fat and a significant increase in contents of these phenolic compounds olives during olive oil extraction. We are also seeing an precocity of cellular maturity after degradation of protopectins through endogenous enzyme activities of type pectolytic causing very significant oil yields with good phenolic quality.

Abbreviations

ABA: abscisic acid; GA: gibberellic acid; MIA: insoluble material in alcohol; PAL: phenylalanine ammonia-lyase; PC: phenolic compound; PE: pectinestérases enzymes; PG: polygalacturonase; POD: peroxidase; PPO: polyphenoloxidase.

Authors' contributions

YM assured the application of the treatments and the samples during the period of ripening of olives and the analyses in the laboratory. In addition, he contributed to the writing of the manuscript. ZH assured the processing of the samples (extraction of olive oil, preparations of phenolic and enzymatic extracts) and contributed to the writing of the manuscript. KAJ assured statistical treatment of the results obtained and also contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have are no competing interests.

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