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Effect of exogenous application of indole-3-acetic acid (IAA) on cell maturity of *Olea europaea* L. and extractability of phenolic compounds in virgin olive oil

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ABSTRACT

The effect of exogenous application of indole-3-acetic acid (IAA) at fruit set and at veraison on the cell maturity of the olive and on extractability of phenolic compounds (PC) in virgin olive oil was studied. The IAA was sprayed on the olive trees at fruit set and on other olive trees at veraison at a concentration of 10-3 mg / l. The effect of these treatments was evaluated by fruit yield and the period of growth and maturation of olives. The extractability of olive oil and diffusion of PC in this latest as well as the embrittlement of the parietal structures are also tracked. The IAA applied at fruit set leads to inhibition of fruit drop but without changing the period of growth and maturation. At full maturity, a significant increase in total lipids was observed and the quantity of oil extracted but without changing their phenolic quality. The treatment at veraison leads to precocity of maturation estimated a month and a gain of 11% fruit. At this stage, a significant increase in the accumulation of fat and the amounts of extracted oils and a significant improvement in the extractability of PC diffusible in these oils at maturity.

INTRODUCTION

Plant hormones (phytohormones or growth regulators) have long been known for being closely involved in the fruit development and ripening (Klee and Giovannoni, 2011; Seymour *et al.*, 2013). The most studied phytohormone in relation to fruit ripening is ethylene. However, few studies on the influence of other growth regulators on the fruits development and ripening are carried. Generally, the complex series of fruit ripening reactions is controlled by an interaction of several classes of these plant hormones (Bangerth, 1983). It has been proposed that endogenous factors in fruits resist the action of ethylene in the progression of ripening (Hanson, 1966). The auxins are among the natural constituents of fruits that can function as resistance factors for maturation (Mapson, 1970). However, several studies have shown that the tissues of the fruit undergoing changes of the levels of IAA throughout their development and maturation (Buta and Spaulding, 1994; Miller *et al.*, 1987; Miller, 1990).

Therefore, Frenkel suggested that the lower level of auxins concomitant with the ripening is necessary to sensitize the tissues of the fruit to ethylene action (Frenkel, 1972). Nevertheless, the contents of IAA seem to be related to maturation in the various fruits in two different ways: the first model is represented by stone fruits like peach, where levels of the IAA increase during the fruit growing period, reduce to a lower level during ripening, and finally increase dramatically during the period before the rise of the levels of ethylene at maturity (Miller *et al.*., 1987). A second model is represented by some pip fruit: in tomato, the contents of IAA fall throughout the expansion of fruit at very low levels and these tissues in deficiency to auxin who realized the program for terminals events of fruit ripening (Buta and Spaulding, 1994). On the other hand, several growth regulating substances have also shown their effect in preventing the fruit drop.

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There are many growth regulators such as Indole Acetic Acid (IAA), Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA), 2, 4-Dichlorophenoxyacetic Acid (2,4-D), 2,4,5-Trichlorophenoxy and Propionic Acid (2,4,5-TP) which have been used to prevent the fruit drop (Saeed *et al.*, 2013).

This work aims to study the effect of exogenous treatment with IAA on the ripening of olives and extractability of phenolic compounds in virgin olive oil. The effects of this treatment were evaluated by the fruit yield at harvest and the evolution of the physical and biochemical parameters (weight, diameter, humidity) during the ripening of olives (anthocyanins content, accumulation of oil, its percentage of flow and its phenolic content).

MATERIAL AND 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- during 2014. These olive trees are the same age, exposure to the sun and type of soil. For these trees, The fruit setting stage was estimated in this study at the 3rd week of June 2014 while the veraison stage was estimated at the 4th week of September 2014.

Two treatments by IAA (10^{-3} mg / l) using a spray just before sunrise were made during the development and growth of the olives: olive trees are treated at fruit set, other trees are treated at veraison. For control trees, no treatment was applied. Periodic samples were taken from the veraison to the total maturity of the olives to follow the evolution of the studied parameters. All analyzes and assays were made in three repetitions.

Moisture determination olives

Sample moisture was determined by drying olive fruits in an olive stove at 105° C according to the Standard Spanish Method UNE 55-020-73. A 40 g sample is placed into a porcelain capsule and dried in a stove at 105° C for six hours. Subsequently, the sample was cooled in a desiccator, weighted and reintroduced into the oven. This operation was repeated until variations in moisture weight loss and volatile loss were <0.02g.

Determination of the total oil content

The extraction of the total oil content was carried out using hexane in a Soxhlet extractor for four hours from the dryer used for the determination of humidity content material (UNE 55030). After that, the traces of solvent were removed by evaporation. The oil contents was 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 *et al.*, (1995), with some changes. Olive pulp (10 g) was mixed with30 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 were collected after evaporation of the organic solvent. Phenolic compounds were extracted with ethyl acetate (5×20 ml). The extraction of oil polyphenols was performed with methanol according to the method of Vázquez-Roncero *et al.* (1973).

The assay of total polyphenols is based on the reduction of acid phoshomolybdique of Folin-Ciocalteu agent by polyphenols in alkaline medium (Catalano *et al.*, 1999).

The assay of anthocyanins is based on the method used by Ribereau-Gayon and Stonestreet implementing discoloration of cations flavylium by sulfur dioxide (Ribereau-Gayon and Stonestreet, 1965).

Isolation and determination of total polysaccharides

The isolation of pectins was achieved from 50 g olive pulp according to the procedure of Saulnier and Thibault (1987) 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 (Robertson, 1979).

Extraction and measurement of endogenous enzyme activities

1 g of olive pulp homogenized for 30 seconds 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 13000 rpm. The supernatant was filtered through glass wool and used as a source of crude enzyme (Jesús Tovar *et al.*, 2002).

The polygalacturonase (PG) activity was by the method of Somogyi-Nelson (Somogyi, 1952).

The pectinesterases enzymes (PE) were assayed by the method of Baron (Baron, 1984).

Statistical Analyses

Analysis of variance was performed for each parameter studied. The multiple comparison test averages Tukey post-hoc was used to test for significant differences between treatments (at 5%). Univariate analysis was used to test for significant differences in treatment and their interaction for a single parameter. All statistical analyzes were performed with IBM.SPSS statistics, version19. The results obtained for each tested parameters are the average of three repetitions.

RESULTS AND DISCUSSION

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

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

Table 1 shows the influence of the exogenous application of the IAA on the Moroccan Picholine olive trees at fruit set and at veraison. Is noticed that the fruits of olive trees treated at fruit set do not differ from controls, i.e. their veraison and full maturity are at the same period. While, the fruits of olive trees treated with IAA at veraison have ripened earlier than controls and reached full maturity around the first week of November, thus one month before the full maturity of the control olives.

Table 1: Influence of IAA on the period of fruit set and ripening of the olives. IAA (N) : Treatment at fruit set; IAA (V) : Treatment at veraison; (▪▪▪▪) : Period of growth; (▬▬) : Period of ripening.

Treatment	Period of growth and ripening						
	June	July	August	September	October	November	December
Control	▪▪▪▪	▪▪▪▪	▪▪▪▪	▪▪▪▪	▬▬	▬▬	
IAA (N)	▪▪▪▪	▪▪▪▪	▪▪▪▪	▪▪▪▪	▬▬	▬▬	
IAA (V)	▪▪▪▪	▪▪▪▪	▪▪▪▪	▪▪▪▪	▬▬		

Table 2: Influence of IAA applied at fruit set and at veraison on the yield of olive fruit. IAA (N) : Treatment at fruit set; IAA (V) : Treatment at veraison. Values followed by different letters are significantly different (P = 0.05).

Treatment	Control	IAA (N)	IAA (V)
Number of fruits per branch	94.7±7.4^{a}	122.3±4^{b}	104.7±3.5^{c}
% of gain compared to control	-	29,8±11,2^{b}	11±9,8^{c}

Influence of IAA on the number of fruit per olive branch

The exogenous application of the IAA at fruit set on the Moroccan Picholine olive trees leads to a significant increase in the number of fruit on the branches (Table 2), i.e. a significant increase in the number of flowers that could develop into drupes and returnees in period of growth and maturation. This parameter directly influences the production of plant matter, and consequently influences the quantity of oil produced. The IAA applied at fruit set results in estimated gain of over 29%. Rather, applying the same treatment at veraison, the influence on this parameter is less significant and the number of fruit on the branches of trees treated at this stage shows a significant difference compared to the control with a gain estimated at 11%.

Study of the effect of IAA 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 is the consequence of the increase in weight, diameter and decrease of moisture olive. Furthermore, the total polyphenol content show fluctuations during the ripening of olives, these contents increase at veraison, and decrease considerably during the ripening and then increases again at maturity. The treatment of olive trees by the IAA at fruit set (Table 4) leads to increased accumulation of olives into total fat so that at full maturity of fruits, they have a higher content than that recorded in control olives. However, the weight and diameter increased during ripening of olives while their water contents decrease: the weight of olives recorded higher values than the control and this from veraison until full maturity of fruit. The moisture level at full maturity of the olives is lower than that of controls. The application of treatment with IAA at fruit set will not affect the contents of total polyphenols in olives that are experiencing fluctuations during the ripening thus following the same trend as control olives. However, the treatment of olives by the IAA at veraison (Table 5) accelerates the accumulation of fat olives in comparison to the results obtained with treatment at fruit set, but no remarkable differences in their contents at full maturity. Nevertheless these are higher than the control at the same stage. Jointly, the diameter and weight of olives increased during ripening and their total maturity values are lower than the values recorded in treated olives at fruit set and higher than controls. In addition, total polyphenols olives experience fluctuations, they increase at veraison then decrease during ripening before increasing again at total maturity of fruit and recording smaller values than those observed in the control and in the case of the treatment at fruit set.

Table 3: Agronomic indices of control olives during ripening. T.P: total polyphenols; O.P: olive pulp.

	Samples **(control)**						
Parameter	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
Moisiture 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/100g 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 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

Table 4: Influence of the application of IAA at fruit set on agronomic indices of olives during ripening. $(IAA)_N$: Treatment at fruit set; T.P: total polyphenols; O.P: olive pulp.

	Samples $(IAA)_N$						
Parameter	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)	418.2±2.7	435.1±3.1	453.1±3.7	460.2±7.9	472.7±5.3	502.2±5	521.3±3.8
Diameters (mm)	17.1±1.2	17.3±0.9	17.8±1.1	18.1±1.7	18.3±1.5	18.9±1.7	19.1±1.3
Moisiture content (%)	59.7±0.2	58.5±0.1	53.1±0.1	53.1±0.1	52.9±0.1	52.8±0.1	52.7±0.1
Oil content of olives (g/100g O.P)	2.8±0.2	3.6±0.4	5.2±0.2	9±0.4	15.7±0.4	19.2±0.4	24.1±0.2
T.P content of olives (mg/g O.P)	57.2±1.5	66.7±2.8	49.3±1.8	51.6±1.3	54.8±0.6	66.6±1.3	70.4±0.9

Table 5: Influence of the application of IAA at veraison on agronomic indices of olives during ripening. $(IAA)_V$: Treatment at veraison; T.P: total polyphenols; O.P: olive pulp.

	Samples $(IAA)_V$				
Parameter	S1 (21/09)	S2 (03/10)	S3 (14/10)	S4 (25/10)	S5 (07/11)
Weight of 100 olives (g)	393.3±3.7	404.1±3.3	419.3±2.5	455.8±4.9	481.4±2.9
Diameters (mm)	17.5±0.9	17.7±1.4	17.9±1.2	18.1±1.3	18.4±1.7
Moisiture content (%)	63.2±0.2	59.3±0.1	56±0.1	54.4±0.2	53.4±0.1
Oil content of olives (g/100g O.P)	3±0.1	5.7±0.4	8.8±0.3	15.4±1.2	24.6±0.4
T.P content of olives (mg/g O.P)	57.4±2	70.3±2.5	56.6±6.6	46.4±8.5	55.9±1.3

Table 6: Influence of IAA on the total fat in the olives, the quantities of oil extracted and their flow percentages during the ripening of the olives. IAA (N) : Treatment at fruit set; IAA (V) : Treatment at veraison. Values followed by different letters are significantly different (P = 0.05).

Samples	Total fat (g/100g olive pulp)			Quantities of oil extracted (g/100g olive pulp)			% of flow		
	Control	IAA (N)	IAA (V)	Control	IAA (N)	IAA (V)	Control	IAA (N)	IAA (V)
21-Sept.	2.56±0.35^a	2.8±0.2^a	3±0.1^a	0.65±0.05^a	0.69±0.03^a	0.74±0.04^a	25.84±4.17^a	24.6±0.8^a	24.9±0.9^a
03-Oct.	3.64±0.2^a	3.6±0.4^a	5.7±0.4^b	1.28±0.03^a	1.35±0.08^a	2.58±0.17^b	35.23±1.22^a	38±5.5^a	45.5±0.9^b
14-Oct.	4.51±0.33^a	5.2±0.2^a	8.8±0.3^b	2.55±0.3^a	2.65±0.2^a	6.03±0.15^b	56.52±3.26^a	51.3±4.5^a	68.9±1.3^b
25-Oct.	7.72±0.4^a	9±0.4^b	15.4±1.2^c	5.43±0.25^a	6.47±0.24^a	11.11±0.32^b	70.36±0.49^a	71.8±1.4^a	72.6±3.6^a
07-Nov.	14.31±0.43^a	15.7±0.4^a	24.6±0.4^b	11±0.2^a	11.82±0.2^a	20.59±0.18^b	76.86±1.2^a	75.1±0.9^a	83.8±0.7^b
19-Nov.	18.03±0.43^a	19.2±0.4^a	-	14.2±0.45^a	15.25±0.26^a	-	78.8±4.2^a	79.3±3^a	-
05-Déc.	20.8±0.51^a	24.1±0.2^b	-	17.12±0.4^a	20.13±0.1^b	-	82.2±2.8^a	83.4±0.4^a	-

Influence of IAA on the levels of anthocyanins

The accumulation of anthocyanins is one of agronomic indices used by olive growers to determine the stage of fruit ripening. Their accumulation in olives (Figure 1) begins at the time of ripening fruits and their levels increase gradually to reach their maximum at total maturity of the olives. The treatment of olives at the fruit set shows no difference in accumulation compared to the control. Contrariwise, olives treated by IAA at veraison show significant and exponentially accumulation of anthocyanins and this just after applying the treatment, reaching maximum at full maturity with significantly high levels compared to control.

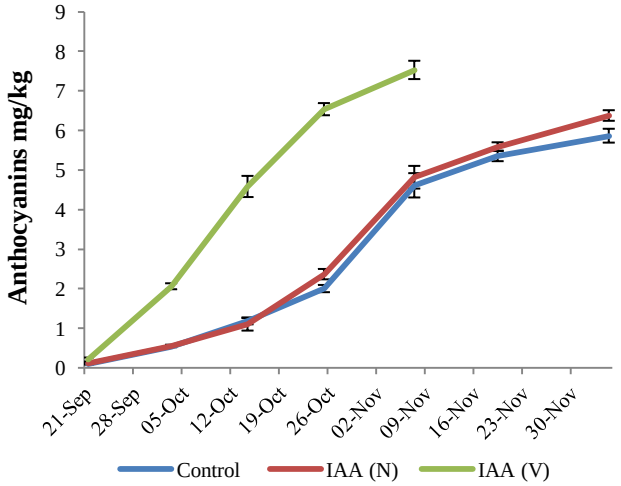

Fig. 1: Influence of IAA on the contents of anthocyanins during the maturation of the olives. IAA (N) : Treatment at fruit set; IAA (V) : Treatment at veraison.

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

The olives progressively accumulate fat in their cells during maturation (Table 6). This accumulation begins before veraison and reaches its maximum at full maturity of the fruit. The olives treated at fruit set by the IAA do not show a significant difference in lipid accumulation except at full maturity of fruits compared to the control. Contrariwise, the treatment of olives by the IAA at veraison result a very rapid and significant accumulation of fat in parallel to the precocity of the maturation. From the application of the treatment, there has been a doubling of these contents of a sample to another, to get a rate of about 25 g / 100 g W.F at full maturity which was reached very early compared to control. These latter are in need of another month to reach full maturity and fruit accumulate only 21 g / 100 g W.F.

Regardless of the treatment, the contents of oils extracted from olives are low at veraison and subsequently increase during ripening and reach their maximum at full maturity (Table 6). However, there is among olives treated at fruit set a slight, insignificant increase, oil contents extracted gradually as the fruits become ripe whose increase becomes significant at full maturity. Furthermore, applying the treatment at veraison leads to a significant increase in extracted oil contents, and since the application of treatment until full maturity of the fruits so that at this stage the quantities of oil extracted reaching almost 21 g / 100 g of olive pulp while the control olives allowed to extract oil estimated quantities just 11g / 100g of olive pulp.

These results are confirmed by the flow percentages of fat (Table 6). After 6 weeks of maturation of the olives processed at veraison, the flow rate increases from 25% to almost 84% at full maturity. At the same time, the control recorded barely 77%. For the latter, the maximum flow rate of 82% was obtained after four additional weeks.

Influence of IAA on total polyphenols content of the extracted oils and their diffusion percentages

The diffusion of these molecules in the oil from the olive pulp is very low and only a small quantity of the total phenolic compounds present in olives (Table 7). Nevertheless, we note that this percentage decreases during the ripening from 1.35% at veraison to 0.3% at maturity for control.

Table 7: Influence of exogenous treatment with IAA on the total polyphenol contents of the extracted oils and their diffusion rate during ripening. IAA (N) : Treatment at fruit set; IAA (V) : Treatment at veraison. Values followed by different letters are significantly different (P = 0.05).

Samples	Total polyphenol content of the olives (mg/g olive pulp)			Diffusible total polyphenol content in extracted oils (mg/kg olive pulp)			% diffusion of TP in oils		
	Control	IAA (N)	IAA (V)	Control	IAA (N)	IAA (V)	Control	IAA (N)	IAA (V)
21-Sept.	58.8±1.95^a	57.2±1.5^a	57.4±2^a	804.6±47.9^a	882.5±48.5^a	833.7±34.5^a	1.35±0.11^a	1.54±0.12^a	1.45±0.1^a
03-Oct.	69.1±2.1^a	66.7±2.8^a	70.3±2.5^a	685.5±64^a	726±24.9^a	991.9±43.3^c	1±0.1^a	1.1±0.03^a	1.41±0.02^b
14-Oct.	50.7±2.42^a	49.3±1.8^a	56.6±6.6^b	573.7±54.1^a	624.2±56.8^a	790.6±61.4^c	1.14±0.16^a	1.3±0.13^a	1.4±0.1^b
25-Oct.	52.4±1.51^a	51.6±1.3^a	46.4±8.5^b	446.5±91.1^a	516.4±43.9^a	617.2±85.5^c	0.85±0.15^a	1±0.06^a	1.35±0.24^a
07-Nov.	54.2±1.25^a	54.8±0.6^a	55.9±1.3^a	366.3±73.3^a	315.2±78.8^a	457.3±79.2^b	0.7±0.13^a	0.6±0.15^a	0.82±0.12^b
19-Nov.	66.9±1.7^a	66.6±1.3^a	-	306.2±12.9^a	287.3±13.6^a	-	0.46±0.02^a	0.43±0.03^a	-
05-Déc.	69.9±1.4^a	70.4±0.9^a	-	216.1±74.9	223.7±77.5^a	-	0.31±0.11^a	0.32±0.11^a	-

The treatment of olives by IAA at fruit set did not show significant differences in the levels of total polyphenols in olives. This treatment to fruit set results in a slight increase of the levels of these compounds in the oils but still not significant. This slight increase is the result of increased distribution of compounds in the oils at veraison (1.54%) and tends to the same value of control at maturity (0.3%).

However, the exogenous treatment of olives by the IAA at veraison causes a significant increase in diffusible polyphenols extracted oils throughout the ripening of fruit maturity so that the oils are much richer in polyphenols that control oil (respectively 457 and 216 mg / kg) or a gain of 110%. This richness in polyphenols is mostly due to improved diffusion of phenolic compounds from olive pulp because at maturity, it is found that polyphenols represent 0.8% of the quantity present in olives against 0.3% for the control.

Effect of IAA on cell embrittlement during ripening of the olives

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

At veraison, the pectins component of the cell walls of olives is constituted mainly by insoluble protopectins. The soluble pectins extracted by water and by the Na oxalate represent a small portion of total pectins (Table 8). During the ripening of the drupe, the protopectins contents shows a gradual decline until the value of 25% compared to their initial values. At the same time, there has been a steady increase of soluble pectin contents so that at maturity, the difference between these two pectin fractions becomes very low.

Furthermore, Figure 2 shows the influence of IAA on the evolution of enzyme activities PE and PG olives during ripening. It is noted that both activities usually increase during the ripening: The PE activity increases gradually to a maximum recorded in mid-ripening then down to reach the level observed at veraison. The PG activities decreased during ripening and slowly increase during ripening to reach its maximum at full maturity. The increase in these activities is accompanied by lower levels protopectins and by increased levels of soluble pectins (Table 8).

The treatment of olives by IAA at fruit set causes no significant difference in these enzyme activities during ripening olives. However, the treatment of olives at veraison leads to a fall of contents PE from veraison, then these activities increase and stabilize more or less but at a lesser level than veraison. This decrease is observed in PE control olives towards the end of ripening. Similarly, PG activities are increasing rapidly at the beginning of ripening, thing observed in control olives at the end of ripening.

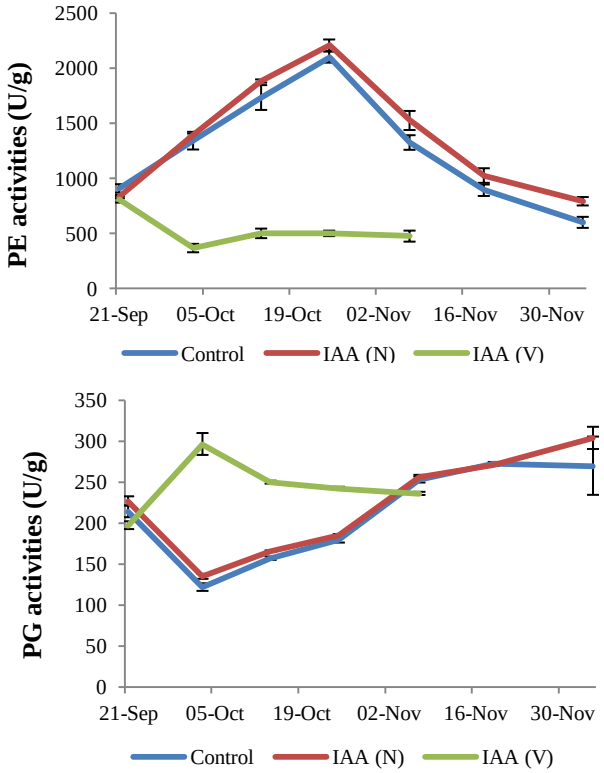

Fig. 2: Influence of IAA on the evolution of the contents of endogenous enzyme activities, polygalacturonase (PG) and pectinestérases activities (PE) during the ripening of olives. IAA (N) : Treatment at fruit set; IAA (V) : Treatment at veraison.

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

At veraison, the cell walls of fruits mainly constituted by protopectins which represent about 90% of all pectins (Table 8). During ripening, the contents protopectins decrease while those of soluble pectin increased so that at maturity, the difference between these two types of pectin decreases and this regardless of the treatment used.

Table 8: Influence of IAA on the contents of various fractions of pectin (µg galacturonic acids/g dry matter) of olives during ripening. IAA (N) : Treatment at fruit set; IAA (V) : Treatment at veraison.

Treatment / Harvest date	Soluble pectins (µg galacturonic acids/g dry matter)			Protopectins (µg galacturonic acids/g dry matter)		
	Control	**IAA (N)**	**(IAA) (V)**	**Control**	**IAA (N)**	**IAA (V)**
S1 (21/09/14)	1846	1825	1339	18604	20255	17788
S2 (03/10/14)	1928	1757	1982	12888	14797	11408
S3 (14/10/14)	1771	2122	2280	11613	11986	8309
S4 (25/11/14)	2525	2316	2411	9288	10174	5571
S5 (07/11/14)	2934	2620	2722	7150	9011	3733
S6 (19/11/14)	3011	2780	-	6239	7931	-
S7 (05/12/14)	3198	3058	-	4572	4949	-

The application of IAA at fruit set will drastically increase the levels of protopectins during ripening olives. From veraison, these insoluble pectin decreases during the maturation of the olives so that at maturity the total pectic substances reach their minimum. In addition, throughout the period of ripening, these values remain higher than those in the control olives. Contrariwise, the treatment at veraison results in very rapid and intense degradation protopectins (over 80%) from ripening so that after a half months, the gap between the protopectins and pectins soluble disappears. Under these conditions, the protopectins reach low levels early compared to control and even a grade lower than that recorded at total maturity of the control.

DISCUSSION

The application of the IAA on olive trees Moroccan Picholine variety shows significant differences on growth and maturation of olives depending on whether the treatment is effected at fruit set or at veraison. There is firstly a significant influence on fruit yield of the olive trees treated versus control. This parameter is very important since the rate of the fructification determines the quality and regularity of fruits at the time of harvesting (Baab & Lafer, 2005). However, the treatment of olive trees by the IAA allows a reduction in fruit drop and therefore a fruit yield gain versus control estimated to about 30% when applied at fruit set and 11% in the case of its application at veraison. These results confirm the work of Saeed *et al.* (2013) on the date palm which state that the application of a treatment by the IAA at a concentration of 150 ppm results in the reduction of the drop in fruits estimated at 17% and 5% respectively for varieties Dhakk and Gulistan which allowed researchers to conclude as to the effect of the IAA in the significant reduction in fruit drop (Saeed *et al.*, 2013). The same finding was made by Azhar *et al.* (2008) and El-Shewy (1999) in their work respectively on citrus and guava. Very significant changes in both morphological, physiological and biochemical accompany this increase in yield: It is observed in the case of treatment at fruit set, increasing the diameter and weight of fruits observed from ripening so that at maturity, these two parameters reach significantly higher values than the control. We also notice an increase in these parameters but less than treatment at veraison. These results are in agreement with other previous studies on the effect of the IAA on the weight of several fruits (Saeed *et al.*, 2013; Saraswathi *et al.*, 2003; Prasad and Pathak, 1994; Singh, 1980) and also confirming the classification of the IAA among phytohormones type growth stimulator. This increase in weight accompanied by a reduction of water content during maturation has the effect of increasing the rate of fat in the treated olives, both at fruit set and at veraison, compared with control olives (24% and 20% respectively).

However, the application of the IAA to fruit set did not bring about changes in periods of growth and development drupes or in the evolution of the anthocyanin synthesis. On the contrary, the application of the IAA at veraison causes a very early maturation estimated at one month before the control olives. This precocity was accompanied by a significant and rapid increase of levels of anthocyanins which reach full maturity significantly higher values compared to the control.

Various studies have been conducted on the effect of the IAA on fruit ripening. These studies have shown that this effect depends on the penetration and diffusion of this phytohormone in the fruit: some studies on tomato, pear and banana indicate that treatments auxin may yield some delay in fruit ripening and softening (Abdel-Kader *et al.*, 1966; Babbitt *et al.*, 1972; Frenkel and Dyck, 1973; McGlasson *et al.*, 1978; Tingwa and Young, 1975; Vendrell, 1969) and delays the onset of anthocyanins in cherries (Kondo *et al.*, 2000). Other studies have shown that anti-auxins have the opposite effect (Frenkel and Haard, 1973).

To obtain the known results for the treatment of auxin, however, vacuum infiltration of the IAA is needed in the fruit (Frenkel and Dyck, 1973; Vendrell, 1969; Tingwa and Young, 1975) or prolonged immersion whole fruit (Abdel-Kader *et al.*, 1966), with a resulting uncertainty with respect to the absorption and distribution (Vendrell, 1970). These techniques also generally require that the fruit is harvested before treatment. While the infusion of a concentration of 10^{-2} M of the IAA in avocado fruit accelerates the ripening and inhibits abscission fruits (Adato and Gazit, 1976). This finding refutes the possibility that the acceleration of fruit ripening caused by auxin is the result of an irregular penetration and diffusion in the fruit (McGlasson, 1970). This latest finding supports the precocity observed in our case when treating olive trees by the IAA at veraison, while the previous conclusion covers the results obtained in the case of the treatment of olive trees at fruit set.

The location of fat in cells of the olives has been the subject of numerous works: According to B. Rangel *et al.*, (1997) the fat accumulates in the cytoplasm of cells of oil-bearing mesocarp, endocarp, and epicarp. According to Ranalli *et al.*, (2001) this fat is localized in the cytoplasm in bound form but also in the vacuoles in free form. The cell maturity is itself related to the degree of fragility of cell membranes and walls which constitute a physical barrier preventing its flow. The fragility of plant cells is estimated by the contents of protopectins which give rigidity to the cell walls. In our case, regardless of the processing effected, these protopectins decrease during ripening olives but in a very intense and rapidly among olives treated at veraison and less intense and slow manner in olives treated at fruit set. This decrease of the contents of protopectin promotes softening of the parietal membrane structures and the fruit thus facilitating the flow of fat. These results are in disagreement with the work of Mínguez-Mosquera *et al.*, (2002) who observed that the rate of protopectins olives cv. Hojiblanca remains stable during ripening. Furthermore, pectin degradation is related to the endogenous pectolytic activities olives. These enzymes convert protopectins on the soluble substances first by demethylation using pectinesterases activities, then degradation to smaller chains of galacturonic acids with polygalacturonase. These physiological events that are influenced by the period of application of the IAA, result in more or less intense embrittlement of the parietal structures and release of cell contents causing a flow of fat estimated 84% at full maturity olives when they are treated by the IAA at veraison. On this date, control olives have not yet reached maturity and they have only a percentage of flow estimated at 77%. When olives are treated at fruit set, the IAA does not result in changes to these pectolytic activities delaying the precocity of maturation and preventing the flow 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 (Amiot *et al.*, 1986). During ripening, the appearance of anthocyanins leads to a very significant reduction in oleuropein (Gómez-Rico *et al.*, 2009; Servili *et al.*, 2004; Bianco *et al.*, 1999) 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 (Bianco *et al.*, 2001; Gómez-Rico *et al.*, 2009). However, the IAA is stimulatory growth phytohormone leads to an increase of the plasticity of the cell wall according to the principle of "embrittlement-deposit". In addition, it is known that auxin leads elongation cell due to the stretching of the cell wall. Knowing that from the fruit set, olives come into intense growth phase, and if at that time the cells receive an activator of auxin-type growth, we witness the deposit of other polysaccharide molecules interspersed between those formants already parietal structures resulting increased contents of pectins parietal. At maturity, this increase makes difficult the release of phenolic compounds. Of this fact, we see that the treatment of olive trees by the IAA at fruit set will affect more on the release of diffusible polyphenols in olive oil extracted following an increase of cell wall polysaccharides awkward releasing these phenolic compounds. Furthermore, is found in the case of olive trees treatment at veraison that the IAA is not playing its role of phytohormone stimulator of growth since at this stage, fruit growth is substantially complete and that the diameter of the olives depends of water content and fat content. In addition, it is found that the IAA sprayed at veraison causes stimulation pectolytic activities PG and PE which result in the degradation of the parietal structures thus facilitating the release of the phenolic compounds. All this results in a precocity harvest olives observed in treated the IAA at veraison and enrichment of polyphenols in oils. These results are in agreement with the work of Amrani Joutei who observed increased contents of pectins parietal grapes treated with IAA at fruit set limiting the release of phenolic compounds, and the absence of the stimulatory effect of growth when IAA is applied at veraison causing degradation of parietal pectins and an increase in the release of phenolic compounds (Amrani Joutei *et al.*, 2004).

CONCLUSION

The influence of the exogenous application of the IAA was investigated on cellular maturity and extractability of the fat and phenolic compounds during the ripening of olives from the Moroccan Picholine variety. In this study, we found that the application of exogenous IAA at fruit set induces stimulation of growth drupes and increased contents of protopectins preventing early softening of olives, this would decrease the extractability of the matter fat and diffusion of phenolic compounds in virgin olive oil. Contrariwise, the treatment of olive trees at veraison increases pectolytic activities promoting softening of the fruit which has an consequences precocity of ripening and facilitate the extractability of olive fats with a good diffusion of PC in virgin olive oil.

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REFERENCES

Abdel-Kader AS, Morris LL, and Maxie EC. Effect of growth regulating substances on ripening and shelf-life of tomatoes. Hortscience, 1966; 1:90–91.

Adato Itzhak and Gazit Samuel. Response of Harvested Avocado Fruits to Supply of Indole-3-acetic Acid, Gibberellic Acid, and Abscisic Acid. J. Agric. Food Chem. 1976; Vol. 24, No. 6.

Aenor, Asociación Española de Normalización y Certificación. Cuerpos Grasos. Determinación del contenido en material grasa total de la aceituna (Norma UNE 55030, Madrid, España, 1961).

Aenor, Asociación Española de Normalización y Certificación. Materias Grasas. Humedad y materias volatiles (Mètodo de la estufa de aire) (Norma UNE 55-020-73, Madrid, España, 1973).

Amiot MJ, Fleuriet A and Macheix, JJ. Importance and evolution of phenolic compounds in olive during growth and maturation. J. Agric. Food Chem., 1986; 34, 823–826.

Amrani Joutei K, Ouazzani Chahdi F, Saucier C, El Hassimi Sow M, Douya D et Glories Y. Influence de l'AIA et de l'ABA sur la maturité des raisins et sur la libération des tanins et des anthocyanes au cours de la macération. ALAWAMIA, 2004; 112 Vol. 1 N° 4.

Azher MN, Ahmad W, Ahmad S and Khan MM. Role of growth regulators on pre harvest fruit drop, yield and quality in kinnow mandarin. Pak. J. Bot. 2008; 40(5): 1971-1981.

Baab G & Lafer G. 2005. Kernobst. Harmonisches Wachstum-optimaler Ertrag. Österreichischer Agrarverlag, Leopoldsdorf, 163 p.

Babbitt JK, Powers MJ and Patterson ME. Effects of growthregulators on cellulase, polygalacturonase, respiration, color, and texture of ripening tomatoes. J. Amer. Soc. Hort. Sci. 1972; 98:77–81.

Bangerth, F. 1983. Hormonal and chemical preharvest treatments that influence postharvest quality, maturity, and storeability of fruit, p. 331–354. In: M. Lieberman (ed.). Post-harvest physiology and crop preservation. Plenum, New York.

Baron A. 1984. Norme française homologuée de détermination des substances pectiques T2. I/MGS/CSM, 0314 et 0607700.

Bianco A, Coccioli F, Guiso M and Marra C. The occurrence in olive oil of a new class of phenolic compounds: Hydroxy-isochromans. Food Chem. 2001; 77, 405–411.

Bianco A D, Muzzalupo I, Piperno A, Romeo G and Uccella N. Bioactive derivatives of oleuropein from olive fruits. J. Agric. Food Chem. 1999; 47, 3531–3534.

Brenes M, Rejano L, Garcia P, Sanchez HA and Garrido A. Biochemical changes in phenolic compounds during Spanish-style green olive processing. Journal of Agricultural and Food Chemistry, 1995; 43, 2702-2706.

Buta JG and Spaulding DW. Changes in indole-3-acetic acid and abscisic acid levels during tomato (Lycopersicon esculentum Mill.) fruit development and ripening. J. Plant Growth Regulat.1994;13:163-166.

Catalano L, Franco I, De Nobili M and Leita L. 1999. Polyphenols in olive mill waste waters and their depuration plant effluents: a comparaison of the Folin-Ciocalteau and HPLC methods. *Agrochimica*, 43, pp. 193-205.

El-Shewy AA. Response of Guava trees to some chemical substances spray. Ann of Agric Sci. Moshtohor. 1999; 37(3): 1649-1661.

Frenkel C and Haard NF. Initiation of ripening in Bartlett pears with an antiauxin (p-chlorophenoxy)isobutyric acid. Plant Physiol. 1973; 52:380–384.

Frenkel, C. and R. Dyck. Auxin inhibition of ripening in Bartlett pears. Plant Physiol. 1973; 51:6–9.

Frenkel C. Involvement of peroxidase and indole-3-acetic acid oxidase isozymes from pear, tomato, and blueberry fruit in ripening. Plant Physiol. 1972; 49: 757-763.

Gómez-Rico A, Inarejos-Garcıá AM, Salvador MD and Fregapane G. Effect of malaxation conditions on phenol and volatile profiles in olive paste and the corresponding virgin olive oils (*Olea europaea* L. Cv. Cornicabra). J. Agric. Food Chem. 2009; 57, 3587–3595.

Hanson E. Post-harvest physiology of fruits. Annu. Rev. Plant Physiol. 1966; 17: 459-480.

Jesús Tovar M, Paz Romero M, Joan Girona and José Motilva M. L-Phenylalamine ammonia-lyase activity and concentration of phenolics in developing olive (*Olea europaea* L. cv Arbequina) fruit grown under different irrigation regimes. Journal of the Science of Food and Agriculture. 2002; 82: 892-898.

Klee HJ, Giovannoni JJ. Genetics and control of tomato fruit ripening and quality attributes. Annual Review of Genetics.2011;45,41-59.

Kondo S, Hayata Y and Iwasaki N. Effects of indole-3-acetic acid and gibberellins on fruit development and maturation of sweet cherries. Acta Hortic. 2000; 514, 75-82.

Mapson LW. Biosynthesis of ethylene and the ripening of fruit. Endeavor. 1970; 29: 29-33.

McGlasson WB, Wade NL and Adato J. 1978. Phytohormones and fruit ripening, p. 447–493. In: D.S. Letham, P.B. Goodwin and T.J. Higgins (eds.). Phytohormones and related compounds. A comprehensive treatise, vol. 2. Elsevier, Amsterdam.

McGlasson WB. 1970. The Biochemistry of Fruits and Their Products. Vol. 1, Hulme, A. C., Ed., Academic Press, London and New York, pp 475-519.

Miller AN. Changes in auxin concentration in developing fruits and seeds of non-endospermic seed species. Plant Growth Regulat. Soc. Amer. Quarterly, 1990; 18:166–176.

Miller AN, Walsh CS and Cohen JD. Measurement of indole- 3-acetic acid in peach fruits (Prunus persica L. Batsch cv. Redhaven) during ripening. Plant Physiol. 1987; 84:491–494.

Mínguez-Mosquera M Isabel, Gallardo-Guerrero Lourdes, and Roca María. Pectinesterase and Polygalacturonase in Changes of Pectic Matter in Olives (cv. Hojiblanca) Intended for Milling. JAOCS, 2002; Vol. 79, n°1.

Prasad A and Pathak RA. Effect of NAA on fruit retention,size,repening and quality of mango cv. Dashehari. Allahabad Agric. Inst. India. Allahabad Farmer. 1974; 46(2): 125-128.

Ranalli A, Malfatti A, and Cabras P. Composition and Quality of Pressed Virgin Olive Oils Extracted with a New Enzyme Processing Aid. Sensory and Nutritive Qualities of Food. Journal of Food Science —2001; Vol. 66, No. 2.

Rangel B, Platt KA and Thomson WW. Ultrastructural aspects of the cytoplasmic origin and accumulation of oil in olive fruit (*Olea europaea*). - Physiol. Plant. 1997; 101: 109-114.

Ribereau-Gayon P and Stonestreet E. Le dosage des anthocyanes dans le vin rouge. Bull. Soc. Chim., 1965; 9, 2649-2652.

Robertson GL. The fractional extraction and quantitative determination of pectic substances in grapes and musts. Am. J. Enol. Vitic., 1979; Vol 30, 3, 182-186.

Saeed A, Sajid M, Asif L, Nazeer A, Junaid M, Nasir M and Umair M. Effect of indole acetic acid (AIA) on fruit drop and fruit quality of date palm cultivars. Pure Appl. Bio., 2013; 2(1): 1-6.

Saraswathi T, Rangasamy P and Azhakiamanavalan RS. Effect of pre harvest spray of growth regulators on fruit retention and quality of mandarins (Citrus reticulata Balanco). South Ind. Hort. 2003; 51(1/6): 110-112.

Saulnier L and Thibault JF. Extraction and characterization of pectic substances from pulp of grape berries. Carbohydrate Polymers. 1987; 7, 329-343.

Servili M, Selvaggini R, Esposto S, Taticchi A, GianFrancesco Montedoro and Guido Morozzi. Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspect of production that affect their occurence in the oil. J. Chromatogr. 2004; 1054, 113–127.

Seymour GB, Ostergaard L, Chapman NH, Knapp S, Martin C.. Fruit development and ripening. Annual Review of Plant Biology, 2013; 64, 219–241.

Singh AR. Effect of foliar sprays of nitrogen and growth regulators on the physicchemical composition of mango. Plant sci. 1980; 8: 7581.

Somogyi M. Notes on sugar determination. J. Biol. Chem. 1952; 195, 19.

Tingwa PO and Young RE. The effect of indole-3-acetic acid and other growth regulators on the ripening of avocado fruits. Plant Physiol. 1975; 55:937–940.

Vázquez-Roncero A, Janer del Valle C and Janer del Valle ML. Determinación de polifenoles totales del aceite de oliva. Grases Aceites, 1973; 22, 350-355.

Vendrell M. Relationship between internal distribution of exogenous auxins and accelerated ripening of banana fruit. Austral. J. Biol. Sci. 1970; 23:1133–1142.

Vendrell M. Reversion of senescence: Effects of 2,4-dichlorophenoxyacetic acid and indoleacetic acid on respiration, ethylene production, and ripening of banana fruit slices. Austral. J. Biol. Sci. 1969; 22:601–610.

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