Middle East Journal of Agriculture Research
ISSN 2077-4605

Volume : 06 | Issue : 03| July-June | 2017
Pages:605-616

Physiological and Anatomical Studies on Olive Propagation by Grafting

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Received: 20 April 2017 / Accepted: 20 June 2017 / Publication date: 10 July 2017

ABSTRACT

This investigation was carried out during both 2013-2014 and 2014-2015 seasons to study the effect of pre-grafting insertion of the, Kalamata and Dolce, olive scion basis in growth regulator solution treatments, just before their cleft grafting onto the Chemlali seedling rootstocks. Different growth regulators and their combinations were used. The influence was evaluated through the response of grafting success percentage, determination of leaf some chemical constituents, as well as, the anatomical examination of scion-stock union zone. The obtained results exhibited that the high grafting success of grafted olive cvs. Kalamata and Dolce transplants as a result of the different tested growth regulator treatments were accompanied with obvious changes in some anatomical features of scion-stocks region i.e., thickness of periderm, Cortex, Vascular tissues (Xylem and Phloem) besides an increase in union zone thickness and cambium tissue accompanied by an increase in the formed vascular tissues. However, the best results in this concern was the treatment of IBA at 50 mg/L+kinetin at 5 mg/L followed by kinetin at 10 mg/L and IBA at 100 and 50 mg/L as compared with the other treatments and control.

Key words: pre-grafting, olive scion, growth regulators

Introduction

Olive, *Olea europea* L., is a member of *Oleaceae* family. It is an evergreen tree indigenous to the Mediterranean region, where weather conditions are most suited for olive growth and fruiting.

Spain, Italy, Greece, Syria, Turkey, Tunisia, Morocco, Portugal, Algeria, Jordan, Palestine, and France are among the major olive production countries. In Egypt, olive cultivation increased considerably during the last few decades and there are many newly introduced cultivars that resulted in the extension of olive plantations in newly reclaimed areas.

Olive tree can thrive successfully on many arid and semi-arid lands, where dry weather, limited irrigation water and water salinity prevail (Bailey, 1961). Therefore, olive cultivation can play an important role in the economy of such areas, in Egypt: e.g. in Sinai, in north western desert and El-Wady El-Gedid ..etc where such land is unsuitable for many other crops. Also, olive plantations in such areas can contribute to soil conservation and combat problems of environment and soil protection that are currently of concern to nations authorities and organizations (Denis, 1977).

According to the statistics of Food and Agriculture Organization (FAO, 2015) in 2014 the total area of olive trees plantations in Egypt is about (152432 Fadden), producing (558610 Tons) of fruit.

Olive industry, in Egypt, is based on the production of oil and canned ripe olives (green ripe and black ripe). However, the total Egyptian production does not satisfy the needs of the local market. The output of these productions should increase to limit importation, with the hope to satisfy the local market, later on.

Large areas in Sinai, the western coastal areas and different oasis of the western desert are most suited for profitable olive production. Massive propagation of suitable cultivars is needed to satisfy the needs of planting such large areas.

Stem cuttings are considered the most simple and economical method of olive propagation. But unfortunately, some olive cultivars, such as the Kalamata cv. cannot be propagated easily by stem cuttings (El-sayed *et al.,* 1995). In the meantime, large number of existing olive trees, in many producing areas, need to be top-grafted by many newly introduced and more productive or commercially needed cultivars.

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Accordingly, the main target of the present investigation was to study the use of top-grafting coupled with dipping of scion base, just before grafting, in some growth regulators solutions to study the effect of such treatment on enhansing the success of grafting through scion/stock integration and union, as well as, the subsequent growth and development of the grafted olive cultivars under study.

Materials and Methods

The present study was carried out in the green house of the Experimental Farm of Horticulture Department, Faculty of Agriculture, Benha University, Qalyubia Governorate, Egypt, during two successive seasons of (2013-2014 and 2014-2015).

The aim of this investigation was to study the influence of the two investigated factors i.e., olive cultivars (Kalamata and Dolce) and pregrafting treatments, of olive scions bases, by some growth regulator treatments, just before their insertion onto the seedling rootstocks by cleft grafting.

Grafting procedures

1- Preparation of chemlali seedling rootstock:

The preparation of Chemlali seedling rootstock was started during early-to mid-Desember of years 2013-2014 and 2014-2015 for the 1st and 2nd experimental seasons, respectively. One year old Chemlali olive, healthy and disease free seedlings were chosen, approximately similar in their growth vigour, with suitable stem thickness (more than 0.6 cm diameter) measured at 20 cm height from soil surface.

The selected Chemlali olive seedlings were transplanted in black perforated plastic bags, each containing 5 kg planting media mixture (clay and sand of equal proportions by volume).

2- Preparation of scion sticks:

From a private olive orchard at El-khatatba region, Minofia Governorate, Egypt, one Kalamata and one Dolce olive trees were selected as a source of scion sticks from each cultivar for cleft grafting on the previously prepared Chemlali rootstock seedlings. The selected Dolce and Kalamata trees were healthy, productive and free of known diseases.

After preparation of scion sticks from each scion cultivar, careful precausions were taken for maintaining the moisture content of the prepared scion sticks during their transportation from the olive orchard at El-khatatba to the Faculty of Agriculture nursery at Moshtohor.

3- Growth regulator treatments:

Prior to the grafting process, growth regulator soluations, at the needed concentrations were prepared, and the following eight growth regulator treatments were ready:
1-IBA at 50 mg/L
2-IBA at 100 mg/L
3-Kinetin at 5 mg/L
4-Kinetin at 10 mg/L
5-NAA at 50 mg/L
6-NAA at 100 mg/L
7-IBA at 50 mg/L+ Kinetin at 5 mg/L
8-Distilled water (as control)

4- The grafting process:

The grafting processes were carried out in mid-February of the years of 2014 and 2015.
a – The Chemlali rootstock seedlings were trimmed and the top was cut-off at 20-25cm from soil surface, then a vertical cut about 5 cm long is made in the rootstock and pried open.

b- A scion stick 10 to 15 cm. long was selected, preferably of the same diameter as the rootstock, then a 2-sided wedge of the same length as the cut in the rootstock was made at the base of the scion.
c- The scion base was dipped in one of the growth regulator treatments for fifteen seconds –just before being inserted onto the rootstock, and checked to be sure that cut surfaces are in good contact and the cambial layers are aligned on at least one side.
d- The wrap was made in an upward spiral with the end tucked under the final turn and pulled taut.

All grafting transplanted for each treatment and cultivar received the same amount of the N: P: K fertilizer mixture and were regularly irrigated.

The experimental design:

The sixteen investigated treatments (2 cultivars; Kalamata and Dolce X eight growth regulator treatments) were arranged in a complete randomized block design with three replications was employed. Each replicate was represented by 10 cleft grafted olive scion/stock combinations, besides two additional ones, acting as an available reserve. Consequently, 240 grafted seedlings from each cultivar (Kalamata and Dolce "2×240=480") were used.

The performance of the two olive scion cultivars (Kalamata and Dolce) in response to the various eight regulator treatments were evaluated through the determination of grafting success percentage, leaf some chemical constituents of the growth scion and the scion/stock union zone for each treatment and cultivar was anatomically examined to detect any resulted differences to be explained.

1. Grafting success percentage

2- Some chemical constituents:

2.1- Total indoles:

Total indoles were determined by using the test of P–dimethyl-aminobenzaldehyd (Larson *et al.,* 1962) and estimated colorimetrically. The concentrations were calculated from a standard curve of indole acetic acid.

2.2- Total phenols:

Folin and Ciocalten colorimetric method (A.O.A.C. 1985) was used at 700 um wave length to be determined colorimetrically. The concentration of total soluble phenols was calculated from a standard curve of pyrogallol.

4. Anatomical studies:

The anatomical examination of the union zone for grafted olive transplants of the two cvs. under study were carried out to study the structure of the newly developed tissues in the previously pepared union zone and to examin the result of scion soaking in different solutions of growth regulators by microscopical investigation of the structure of newly developed tissues in such area (zone). The samples were killed and fixed for at least 48 hrs. in FAA (5 ml formalin, 5 ml glacial acetic acid and 90 ml ethyl alcohol 70%). The callus tissues were washed in 50% ethyl alcohol, dehydrated in a series of ethyl alcohol (70, 90, 95 and absolute), infiltrated in xylene, embedded in paraffin wax of melting point 60-63°C. Cross sections were sectioned to 10-15microns thickness and also prepared and handled with the same recommened procedures of paraffin method and double stained with fast green and safranin, cleared in Xylene and mounted in Canada-balsam (Johanson, 1940). Sections were read to detect histological manifestation of noticeable responses resulted from studied treatments.

The prepared sections were microscopically examined, counts and measurements (μ) were taken using a micrometer eye piece. Averages of readings from 3 slides/ treatment were calculated.

Concerning the anatomical studies of union zone for grafted olive transplants to study the structure of the newly developed tissues in such zone were previously prepared and examined after three months from grafting process.

6. Statistical analysis:

All data obtained during both seasons of study were subjected to analysis of varaiance and significant differences among means were determined according to Snedecor and Corchran (1972). Differences among means for the effect of scion olive cultivars (Kalamata and Dolce) and pregrafting treatments were compared using Duncan multiple range test (Duncan, 1955) at 5% level. The interactions effect between scion olive cultivars and pregrafting treatments were differentiated using L.S.D. method at 5% level.

Results and Discussion

The specific effect of the two investigated factors namely, i.e., olive scion cultivars (Kalamata and Dolce), dipped before grafting in different concentrations of some growth regulators (IBA at 50 &100 mg/L, Kinetin at 5 &10 mg/L, NAA at 50 & 100 mg/L and IBA at 50 mg/L+Kinetin at 5 mg/L) and their possible combinations were studied, at the end of each growth season, pertaining the response of the following parameters:

1- Grafting success percentage

Regarding to specific effect of the two olive scion cultivars (Kalamata and Dolce) on grafting success percentage, data presented in Table (1), reveals that Kalamata olive scions gave statistically the higher success percentage (88.33 & 87.09%) as compared with Dolce scion cultivar as it scored (70.50 & 85.30%) during the first and second seasons, respectively.

Regarding the specific effect of dipping the base of the two olive scion cultivars (Kalamata and Dolce) before grafting in different concentrations of some growth regulators (IBA at 50 &100 mg/L, Kinetin at 5 &10mg/L, NAA at 50 & 100 mg/L and IBA at 50 mg/L+Kinetin at 5 mg/L) on grafting success percentage, Table (1) shows that all dipping treatments statistically increased grafting success percentage when compared with the control (tap water) during the two seasons of study, particularly 50mg/L IBA+5mg/L Kinetin treatment (93.32 & 93.67%), followed descendingly by 10mg/L Kinetin treatment (90.50 & 91.82%) during the first and second seasons, respectively. In addition, 100 mg/L IBA treatment falls in the third order in this respect as it gave (85.70 & 90.15%) followed descendingly by 5mg/L Kinetin treatment (85.50 & 88.85%) during the first and second seasons, respectively.

Concerning the interaction effect of various combinations between two olive scion cultivars and dipping of scion bases before grafting in different concentrations of some growth regulators on grafting success percentage, data in Table (1) demonstrates that the highest success percentage (93.33 & 97.00%) were closely related to Kalamata scions dipped before grafting in 50mg/L IBA+5mg/L Kinetin solution in the first and second seasons, respectively. However, the lowest success percentage (67.70 & 70.70%) were detected by Dolce scions dipped before grafting in tap water (control) in the first season and second season, respectively. All other combinations took an intermediate position between the aforementioned two categories during the two seasons of this study.

The present results regarding the simulative effect of differences among scion cultivars on successful budding or grafting percentage are in general agreement with the findings of Al-Safi and Al-Djaili (2000) on apple trees, Ullah *et al*. (2000) on peach, Al-Kayssi (2011) on apricot. Moreover, Kako *et al.,* (2012) studied the effect of different peach cultivars on the percentage of budding success of peach transplants budded on seedling stocks of peach. They indicated that the cultivar had a significant effect on budding success percentage when Silver King cultivar was significantly superior upon Coronet cultivar but did not significantly differ from May Grand cultivar .The highest budding success percentage (69.52%) was achieved from Silver king cultivar. In addition, Hussain *et al.,* (2016) concluded that used Frantoio olive cultivar as a scion for graft in the month of July showed

maximum sprouting followed by same cultivar when grafted in the month of June, while the minimum graft sprouting percentages were showed by Leccino cv. followed by Moresca both grafted in the month of June. The Frantoio cv. may develop vascular tissues in a graft union region swiftly compared to other cultivars, which ensures the transport of water, nutrients and plant hormones thus plant development.

Furthermore, obtained results regarding the positive effect of investigated growth regulator treatments on budding or grafting success percentage as influenced by immersing scions in some growth regulator solutions i.e., Edriss and Burger (1984) on 'Mexican' lime, 'Valencia' orange and 'Star Ruby' grapefruit rootstocks, Starrantino *et al.*, (1986) on three scions of citrus, Yates (1992) on 'Desirable' pecan, Fang and Yingzi (1997) on grapevine. Nunes *et al.,* (2005) on micro-grafting technique in apple, Abd-Alwahaab *et al.,* (2011) on citrus and Kako *et al.,* (2012 and 2015) on different peach cultivars.

Moreover, AL-Safi (2002) who studied the effect of auxin on the growth of transplants of three local apple cultivars (Ajamy, Sharaby and Kuffi) and declared that the treatment with IAA at 25 and 50 mg/L significantly increased budding success percentage as compared with the control. While, Zenginbal and Esitken (2016) concluded that application of 4000mg/L IBA increased the graft sprouting rate of mulberries.

Table 1: Effect of scion cultivar, growth regulator treatments and their interaction on grafting success percentage.

Grafting success %						
Treatment	Frist season (2013-2014)			Second season (2014-2015)		
	Kalamata	Dolce	Mean	Kalamata	Dolce	Mean
IBA at 50 mg/L	90.70	70.33	80.52 CD	87.33	87.33	87.33 C
IBA at 100 mg/L	90.70	80.70	85.70 BC	90.00	90.30	90.15 ABC
Kinetin at 5 mg/L	90.30	80.70	85.50 BC	90.00	87.70	88.85 BC
Kinetin at 10 mg/L	93.30	87.70	90.50 AB	93.30	90.33	91.82 AB
NAA at 50 mg/L	80.70	70.30	75.50 DE	80.70	80.67	80.67 D
NAA at 100 mg/L	87.70	77.30	82.50 C	80.70	85.00	82.85 D
IBA at 50mg/L+ kinetin at 5mg/L	93.33	93.30	93.32 A	97.00	90.33	93.67 A
Control	80.30	67.70	74.00 E	77.70	70.70	74.20 E
Mean	88.33 A	70.50 B		87.09 A	85.30 B	
L.S.D for interaction at 5%	7.664			5.063		

Means with in the same column or row followed by the same letter (s) were insignificantly affected using Duncan Multiple Range test at the probability of 5%.

2- Leaf some chemical constituents:

2.1- Total indoles (mg/100g leaves F.W)

Regarding the specific effect of the two investigated olive scion cultivars (Kalamata and Dolce) on total indoles content, data presented in Table (2), reflects that Kalamata olive scions had a higher value of total indoles than the other investigated olive scion (Dolce) during 2013 - 2014 and 2014 - 2015 seasons.

Concerning the specific effect of dipping two olive scion cultivars before grafting in different concentrations of some growth regulators on total indoles content, Table (2) shows that all dipped treatments succeeded in increasing total indoles content when compared with the control (tap water) during the two seasons of study, particularly 50mg/L IBA+5mg/L Kinetin treatment, followed descendingly by 100mg/L IBA treatment. Anyhow, the differences between these treatments were so small to reach the significance level. In addition, control treatment scored the lowest total indoles content followed ascendingly by 5mg/L Kinetin treatment without significant differences.

Considering the interaction effect of various combinations between the two olive scion cultivars and dipping scions before grafting in different concentrations of some growth regulators on total indoles content, data in Table (2) indicates that the highest values of total indoles content were reported by Kalamata scions which dipped before grafting in 100mg/L IBA and 50mg/L IBA+5mg/L Kinetin solutions in both seasons. On reverse, the lowest values of total indoles content during the two seasons of this study were closely related to Dolce scions dipped before grafting in tap water

(control). Other combinations took an intermediate position between the aforementioned two categories.

Table 2: Effect of scion cultivar, growth regulator treatments and their interaction on total indoles (mg/100g leaves F.W).

Total indoles (mg/100g leaves F.W)						
Treatment	Frist season (2013-2014)			Second season (2014-2015)		
	Kalamata	Dolce	Mean	Kalamata	Dolce	Mean
IBA at 50 mg/L	220.3	208.1	214.2 ABC	240.3	217.4	228.9 A
IBA at 100 mg/L	230.9	209.7	220.3 A	242.7	218.3	230.5 A
Kinetin at 5 mg/L	196.4	190.2	193.6 D	220.4	190.3	205.4 C
Kinetin at 10 mg/L	210.1	195.7	202.9 BCD	226.1	195.4	210.8 BC
NAA at 50 mg/L	198.7	202.3	200.5 CD	228.9	210.3	219.6 AB
NAA at 100 mg/L	217.6	213.7	215.6 AB	233.6	220.4	227.0 A
IBA at 50mg/L+ kinetin at 5mg/L	223.7	215.5	219.6 A	243.5	220.0	231.8 A
Control	193.4	186.3	189.9 D	212.3	189.2	200.8 C
Mean	211.4 A	202.7 B		231.0 A	207.7 B	
L.S.D for interaction at 5%	19.90			19.89		

Means with in the same column or row followed by the same letter (s) were insignificantly affected using Duncan Multiple Range test at the probability of 5%.

2.2- Total phenols (mg/100g leaves F.W)

As for the specific effect of the two investigated olive scion cultivars (Kalamata and Dolce) on total phenols content, Table (3), reveals that no significant differences between the two olive scion cultivars (Kalamata and Dolce) but, Dolce olive scion had a higher value of total phenols than Kalamata olive scions during the two seasons of this study.

Looking at the specific effect of dipping olive scions before grafting in different concentrations of some growth regulators on total phenols content, it is easy to realize that total phenols content was decreased due to all tested treatments during the two seasons (Table, 3). However, the lowest content of total phenols was gained by 50mg/L IBA+5mg/L Kinetin treatment in the first season and 50mg/L NAA in the second season as compared with the control and other treatments. Irrespective the control, the highest values of this parameter was recorded by the low concentration of kinetin (5mg/L) in both seasons.

Table 3: Effect of scion cultivar, growth regulator treatments and their interaction on total phenols (mg/100g leaves F.W).

Total phenols (mg/100g leaves F.W)						
Treatment	Frist season (2013-2014)			Second season (2014-2015)		
	Kalamata	Dolce	Mean	Kalamata	Dolce	Mean
IBA at 50 mg/L	142.7	146.2	144.4 AB	135.4	147.6	141.5 AB
IBA at 100 mg/L	136.6	142.6	139.6ABCD	132.0	140.5	136.3 AB
Kinetin at 5 mg/L	145.5	152.4	148.9 AB	140.0	145.9	142.9 AB
Kinetin at 10 mg/L	139.2	145.3	142.3 ABC	137.1	142.8	139.9AB
NAA at 50 mg/L	132.7	136.8	134.8 BCD	130.7	131.2	130.9 AB
NAA at 100 mg/L	121.8	130.6	126.2 CD	123.2	130.4	126.8 B
IBA at 50mg/L+ kinetin at 5mg/L	113.9	130.2	122.1 D	120.7	136.4	128.6 B
Control	148.4	158.7	153.6 A	146.5	152.3	149.4 A
Mean	135.1 A	142.8 A		133.2 A	140.9 A	
L.S.D for interaction at 5%	25.27			27.25		

Means with in the same column or row followed by the same letter (s) were insignificantly affected using Duncan Multiple Range test at the probability of 5%.

Focusing on the interaction effect of various combinations between the two olive scion cultivars and dipping scions before grafting in different concentrations of some growth regulators on total phenols content, enclosed data in Table (3) illustrates that during the two seasons the lowest values of total phenols content were detected by Kalamata scions when dipped before grafting in 50mg/L

IBA+5mg/L Kinetin solution. On the other hand, the highest values of total phenols content were registered by Dolce scions when dipped before grafting in tap water (control). Other combinations took an intermediate position between the aforementioned two categories during the two seasons of this study.

4. Anatomical studies: .

4.1-Effect of the applied growth regulators treatments on some anatomical traits in the union zone for olive transplants of Kalamata cv.

Data in Table (4) and Figs (1-a and b) show that 100 mg/L IBA and50mg/L IBA+5mg/L kinetin treatments were the superior treatments for increasing the diameter of whole union section from 5152.30 µ in the control treatment to reach 6636.60 µ in 100 mg/L IBA treatment and 5282.5µ in 50 mg/L IBA+5mg/L kinetin treatment , all other tested treatment were lower than the control, While IBA at 100 mg/L was the only treatment which increased the rootstock thickness from 3408.30 µ in the control to reach to 4778.10 µ, while all other treatments declined it.

Regarding the scion thickness as shown in Table (4) and Figs (1-a and b), IBA at 50 mg/L showed to be the most effective treatment for inducing the highest value as it scored 1557.70 µ, followed by IBA at 50 mg/L+Kinetin at 5mg/L treatment which recorded 1458.00 µ, whereas all other treatments decreased it when compared with the control treatment. As for periderm thickness of rootstock, its increase was shown only in case of kinetin at 10 mg/L as it registered 135.00 µ, yet the remained treatments were lower than the control. Here, also it could be observed that the cortex thickness of rootstock was increased only with control treatment as it reached. 208.80 µ, but it was lower in all other treatments.

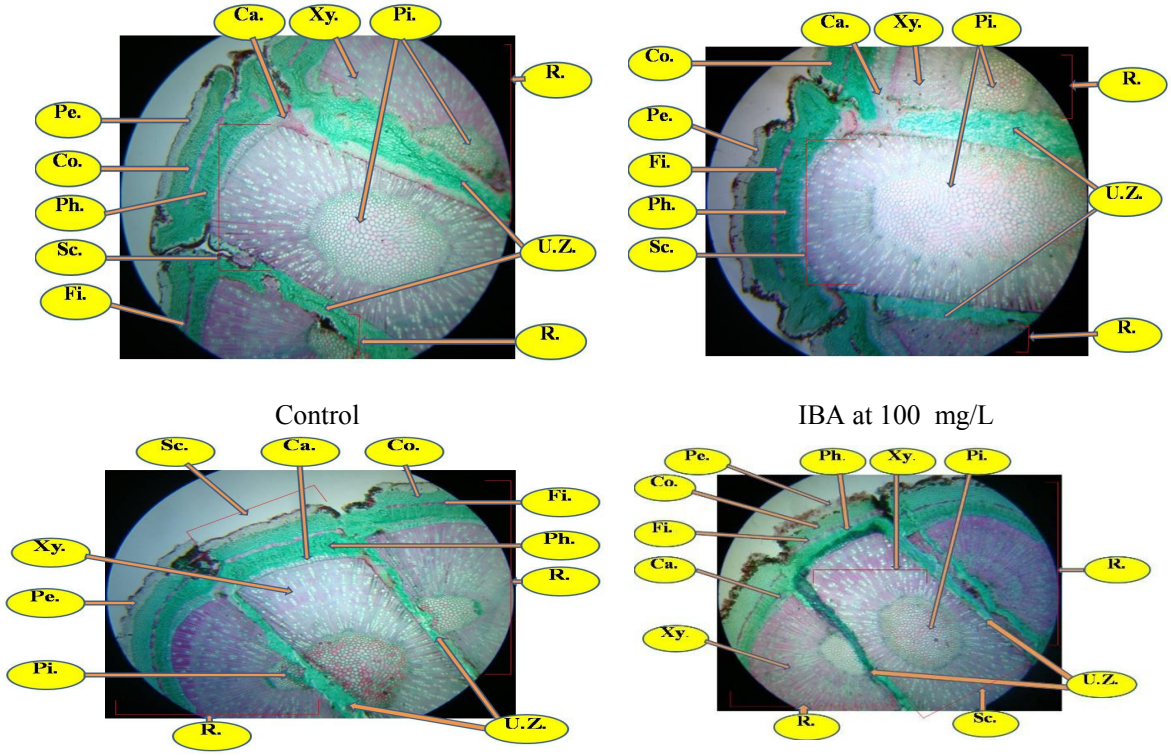
Concerning the number of cortex layers of rootstock, as shown in Table (4) and Figs (1-a and b), it could be clearly observed that the increase in number existed in the control and the IBA at 50 mg/L as they gave the same exact value (9.00 µ), while all other treatments ranged in between 6 and7. As for the fibers thickness, it was increased by control treatment followed by the treatment of IBA at 50 mg/L+ kinetin at 5 mg/L, as they scored 45.90 µ and 44.10 µ, respectively while, the remaining treatments ranged between 27.00 and 40.50 µ.

Moreover, the treatments of NAA at 100 mg/L and IBA at 50 mg/L+ kinetin at 5 mg/L induced the highest phloem tissue thickness of the rootstock as they gave the same value (90.00 µ), while the other treatments were much lower than the control. All tested treatments except NAA at 50 mg/l increased the first union zone thickness with the superiority of IBA+kinetin treatment which induced the highest values (337.5µ) when compared with control treatment (164.50 µ). Referring to the cambium tissue thickness and the xylem tissue thickness of rootstock, all treatments succeeded to increase its thickness as compared with control which scored the lowest values (47.30µ and 526.05, respectively).

With respect of the first union zone thickness, as shown in Table (4) and Figs (1-a and b) it could be obviously observed that it was highest under both IBA at 100 mg/L and NAA at 50 mg/L, followed by kinetin at 5 mg/L and IBA 50mg/L+kinetin 5mg/L. Regarding the vacuole presence and thickness of first union zone, results in Table (4) and Figs (1-a and b) the treatments of IBA 50 mg/L only showed presence of vacuole as well as the control. Furthermore, all studied treatments failed to increase the xylem thickness of scion, except the treatment of IBA 50 mg/L when compared with control. On the opposite, all treatments succeeded in increasing the pith thickness of scion, except for NAA at 100 mg/L when compared with control treatment. Also, all applied treatments induced an increase in the thickness of second union zone, with the superiorty of 50 mg/l IBA+5mg/l kinetin treatment. As for the vacuole thickness of second union zone only the treatments of IBA at 50 mg/L and NAA at 50 mg/L increased it. Regarding the thickness of rootstock pith, it was found that all studied treatments failed to increase it, except for the treatment of IBA at 100 mg/L which increased it as compared with the control treatment.

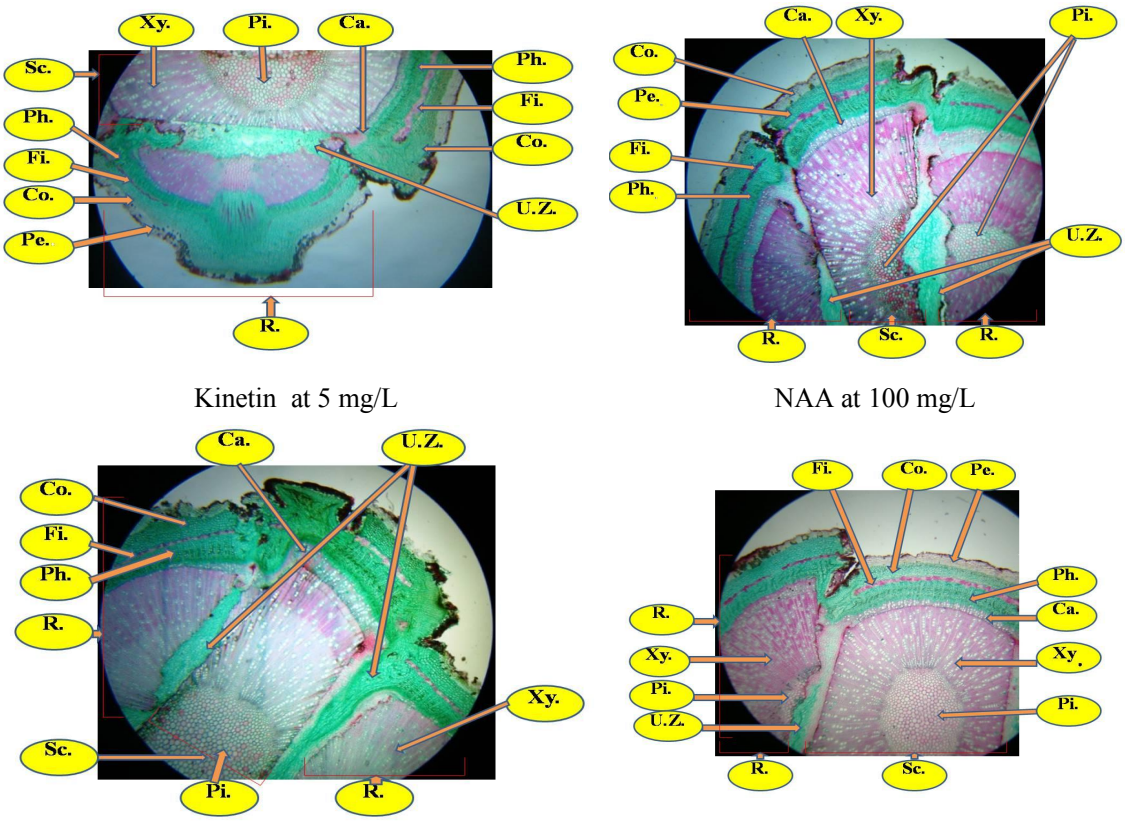
Table 4: Effect of the applied growth regulators treatments on some a natomical traits in the union zone for olive transplants cv. Kalamata.

Treatment / Histological Characters (micron)	Control	IBA at 100mg/L	IBA at 50mg/L	Kinetin at 10mg/L	Kinetin at 5mg/L	NAA at 100mg/L	NAA at 50mg/L	IBA at 50mg/L +kinetin at 5mg/L
Diameter of whole union section	5152.30	6636.60	4886.02	4667.70	4267.30	4130.55	4570.42	5282.50
Scion thickness	1363.50	1399.50	1557.00	1008.00	1089.00	756.00	1242.00	1458.00
Rootstock thickness	3408.30	4778.10	3044.00	3031.20	2628.00	2928.60	3048.32	3115.00
Periderm thickness of rootstock	130.50	117.00	128.70	135.00	99.00	111.60	108.00	64.80
cortex thickness of rootstock	208.80	135.00	190.80	163.80	119.70	126.00	130.50	162.00
No. of cortex layers of rootstock	9.00	6.00	9.00	7.00	7.00	7.00	6.00	7.00
Fibers thickness of rootstock	45.90	34.20	34.20	27.00	27.00	40.50	37.36	44.10
Phloem tissue thickness of rootstock	89.10	42.30	80.10	51.30	45.00	90.00	69.30	90.00
Cambiume tissue thickness	47.30	87.30	62.10	89.50	54.30	43.20	58.50	90.90
Xylem tissue thickness of rootstock	526.05	828.00	810.10	855.00	567.00	754.65	720.00	1358.55
First union zone thickness	164.50	207.00	195.02	291.00	220.00	184.50	145.10	337.5
Vacuole thickness of first union zone	36.00	-	30.40	-	-	-	-	-
No. of vacuole of first union zone	3.00	-	2.00	-	-	-	-	-
Xylem thickness of scion	733.5	526.50	810.00	270.00	243.00	306.00	139.20	450.00
Pith thickness of scion	630.00	873.00	747.00	1008.00	846.00	450.00	738.00	1242.00
Second union zone thickness	216.00	252.00	90.00	337.5	330.30	261.45	135.00	372.00
Vacuole thickness of second union zone	45.00	-	54.00	-	-	39.60	49.50	-
No. of vacuole of second union zone	2.00	-	2.00	-	-	1.00	2.00	-
Pith thickness of root stock	1233.00	1279.80	486.00	738.00	846.00	450.00	621.00	504.00
Thickness of necrotic layer	-	-	-	-	-	-	-	-



Where: Sc.= Scion thickness, R.= Rootstock, Pe.= Periderm, Co.= Cortex, Fi.= Fibers, Ph.= Phloem, Ca.= Cambiume, Xy.= Xylem, U.Z.= Union Zone, Pi.= Pith.

Fig (1.a): Effect of the applied growth regulators treatments on some anatomical traits in the union zone for olive transplants cv. Kalamata.



Where: Sc.= Scion thickness, R.= Rootstock, Pe.= Periderm, Co.= Cortex, Fi.= Fibers, Ph.= Phloem, Ca.= Cambiume, Xy.= Xylem, U.Z.= Union Zone, Pi.= Pith.

Fig (1.b): Effect of the applied growth regulators treatments on some anatomical traits in the union zone for olive transplants cv. Kalamata.

4.2-Effect of the applied growth regulators treatments on some anatomical traits in the union zone for olive transplants Dolce cv.

Data presented in Table (5) and Figs (2-a and b) indicate that all tested applications of growth regulators succeeded in increasing the diameter of whole section as compared with the control treatment. However, the highest values were scored by 50mg/L NAA treatment as it gave 6598.04 µ followed by 10 mg/L kinetin treatment which scored 5656.00 µ.

Moreover, scion thickness was trearted by 50 mg/L NAA reached (2439.00 µ) followed by 10mg/L kinetin treatment (1980.00 µ), while the lowest values of scion thickness were registered by IBA at 50 and 100 mg/L as they gave the same exact value (990.00 µ). Also, it could be observed that all applied growth regulators succeeded in increasing the thickness of rootstock and cortex of rootstock, except for the treatment of IBA at 50 mg/L + kinetin at 5 mg/L which decreased it. However, the highest value of rootstock thickness was recorded by 50 mg/L NAA treatment (3980.00 µ). Data, outlined in Table (5) and Figs (1-a and b) showed that all applied treatments of growth regulators increased the number of cortex layer of rootstock especially 100 mg/L NAA treatment, followed by 10 mg/L kinetin treatment as they scored 12.00 and 11.00, respectively. Furthermore, IBA at 100 mg/L proved to be the most effective treatment for inducing the thickness increase of rootstock fibers as it gave 36.00 µ, followed by NAA at 50 mg/L which gave 31.00 µ. On the reverse the lowest thickness of rootstock fibers was gained by treatment of IBA at 50 mg/L+kinetin at 5 mg/L as it recorded 25.20 µ. All other treatments occupied an intermediate position between the aforementioned treatments. In addition, all applied growth regulators treatments increased phloem tissue thickness of rootstock, except for 10 mg/L kinetin treated scions, which decreased it. However,

the highest values of this parameter were scored by IBA at 50 mg/L + kinetin at 5 mg/L. Additionally, all applied growth regulator treatments increased cambium tissue thickness of rootstock as compared with control treatment. However, the highest values were scorded by 50 mg/l IBA+5 mg/l kinetin treatment as it gave (64.80µ) followed by 10mg/l kinetin treatment. All growth regulator treatments increased xylem tissue thickness of rootstock as compared with control treatment, particularly 50 mg/l IBA+5 mg/l kinetin treatment transplants as it gave 1053.00 µ. Also, the highest value of first union zone thickness was scored by 50 mg/l IBA+5 mg/l kinetin as it gave 189.00 µ. Moreover, the highest values of vacuole of first union zone were recorded by control treatment. In addition, the highest thickness of scion xylem and pith were scored by 5 mg/L kinetin treatment followed by 50 mg/l IBA +5mg/l kinetin treatment. The highest thickness of second union zone was registered by 5 mg/L kinetin treatment as it gave 360.00 µ. Furthermore, the highest values of vacuole of second union zone were recorded by 50 mg/L IBA treatment as it gave 77.5µ while, the highest pith thickness of rootstock was gained by 50 mg/L NAA treatment. Besides, all applied growth regulator treatments decreased thickness of necrotic layer as compared with control treatment, except for 100 mg/L IBA treatment which increased it.

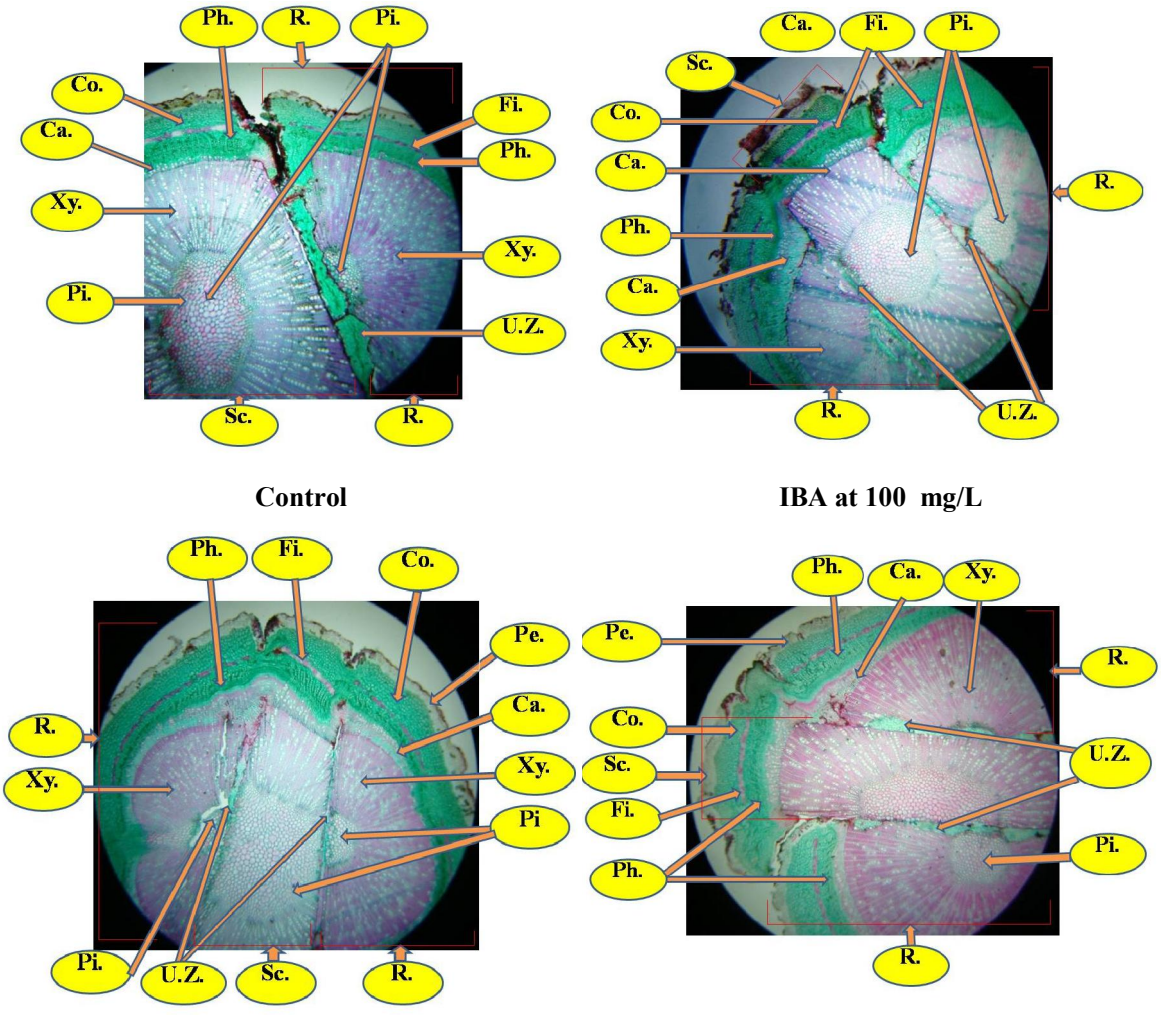
Increasing of xylem and phloem tissues means that the translocation of crude nutrients and water from soil to leaves, as well as, the translocation of sugar and other bio-constituents from leaves to other plant parts are being improved (Marschner, 1995). That is directly could be reflected upon the vigorous growth of such treated plants.

Also, it could be noticed that the increase in diameter of whole section of stem was reflected upon different tissues comprising the whole section. Since, thickness of each cuticle layer, epidermis, cortex (collenchyma and parenchyma tissues) and pith parenchyma layers, as well as, the dimensions of vascular bundles. Moreover, thickness of phloem tissues, of cambial region and of xylem tissue, number of xylem vessels/vascular bundle and diameter of the widest xylem vessel were greatly increased compared with the control. Also, IBA at 50mg/L+kinetin at 5mg/L, kinetin at 10 and 5mg/L and IBA at 100 mg/L treatments were more pronounced in this respect.

In general, the stimulatory effects of applied treatments upon the anatomy features of treated plants could be attributed to the effect upon cambium activity. Increment of cambium activity could mainly be attributed to the increase of endogenous hormones level especially cytokinins and auxins, (Sotiropoulos *et al.,* 2002) as well as the findings of the present study.

Table 5: Effect of the applied growth regulators treatments on some anatomical traits in the union zone for olive transplants cv. Dolce.

Treatment / Histological Characters (micron)	Control	IBA at 100mg/L	IBA at 50mg/L	Kinetin at 10mg/L	Kinetin at 5mg/L	NAA at 100mg/L	NAA at 50mg/L	IBA at 50mg/L +kinetin at 5mg/L
Diameter of whole union section	4094.1	4394.70	4373.10	5656.00	4879.55	4266.00	6528.4	4732.6
Scion thickness	1170.00	990.00	990.00	1980.00	1080.00	1035.00	2439.00	1836.00
Rootstock thickness	2658.60	3053.70	3202.20	3323.6	3503.00	2988.00	3980.00	2347.60
Periderm thickness of rootstock	148.50	90.00	112.50	108.00	144.00	117.00	152.00	99.00
Cortex thickness of rootstock	180.00	216.00	180.00	202.50	207.00	333.71	198.00	170.00
No.of cortex layer of rootstock	7.00	9.00	8.00	11.00	9.00	12.00	8.00	9.00
Fibers thickness of rootstock	28.80	36.00	27.00	28.80	27.00	18.00	31.00	25.20
Phloem tissue thickness of rootstock	88.20	90.00	90.00	63.00	90.00	90.00	90.00	108.00
Cambiume tissue thickness	28.80	54.00	39.60	57.60	54.00	36.00	38.70	64.80
Xylem tissue thickness of rootstock	504.00	990.00	913.50	1017.00	540.00	810.00	882.00	1053.00
First union zone thickness	112.5	171.00	135.00	117.00	94.00	108.00	90.00	189.00
Vacuole thickness of first union zone	53.15	-	50.00	25.00	25.00	17.00	-	-
No.of vacuole of first union zone	2.00	-	1.00	2.00	2.00	1.00	-	-
Xylem thickness of scion	540.00	1188.00	210.00	1233.00	1312.20	180.00	180.00	1286.00
Pith thickness of scion	630.00	810.00	990.00	747.00	1080	855.00	648.00	1071.00
Second union zone thickness	153.00	180.00	45.90	235.40	202.50	135.00	89.40	360.00
Vacuole thickness of second union zone	50.00	73.00	77.5	-	-	-	-	-
No.of vacuole of second union zone	1.00	1.00	2.00	-	-	-	-	-
Pith thickness of root stock	630.00	247.50	270.00	306.00	531.00	180.00	765.00	387.00
Thickness of necrotic layer	112.50	144.00	-	18.00	-	90.00	58.50	58.50



Where: Sc.= Scion thickness, R.= Rootstock, Pe.= Periderm, Co.= Cortex, Fi.= Fibers, Ph.= Phloem, Ca.= Cambiume, Xy.= Xylem, U.Z.= Union Zone, Pi.= Pith.

Fig 2.a: Effect of the applied growth regulators treatments on some anatomical traits in the union zone for olive transplants cv. Dolce.

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