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## **PHYSIOLOGICAL STUDIES ON ROOTING OF EASY AND DIFFICULT TO ROOT OLIVE CUTTINGS**

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### **ABSTRACT**

During 2009 and 2010 seasons, total carbohydrates, N, Zn, C/N and endogenous hormones (IAA, GA<sub>3</sub> AND ABA) were determined in soft cuttings of easy (Picual olive) and difficult (Kalamata) to root cuttings before planting. These cutting were dipped in solutions of Zn sulphate at 0.0 ppm and IBA at 1000, 2000 or 4000 ppm for one minute.

Results showed that easy to root cuttings (Picual olive) characterized by a higher values of carbohydrates, C/N, Zn IAA, and had a lower level for N, GA<sub>3</sub> and ABA. A remarkable increase was observed for N, GA<sub>3</sub> and ABA in difficult to root Kalamata olive cuttings. The ability of different olive cv. cuttings to highly root was associated with the balance occurred between three endogenous hormone like substances namely IAA, GA<sub>3</sub> and ABA as well as C/N and higher own content from Zn.

Dipping easy (Picual) and difficult (Kalamata) to root olive cuttings in solutions containing IBA at 1000 to 4000 ppm plus Zn at 0.0 ppm was essential for enhancing rooting %, distribution and length of root and survival % comparing with using each compound alone. The beneficial effects of IBA and Zn on rootability were greatly appeared in difficult to root Kalamata olive cutting.

For enhancing rootability of hard to root Kalamata olive cutting, it is recommended to chip soft cuttings taken from middle

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portions of current shoots in any indolic and Zn compounds at 2000 and 500 ppm, respectively.

### INTRODUCTION

The balance naturally occurring between various endogenous hormone- like substances as well as N, carbohydrates and zinc in fruit tree cuttings plays an important role in success of rooting process. Why certain cuttings are hard to root?. The answer emphasized the importance of exiting highly specific root forming substances (Singh, 1983). During the formation of roots in cuttings, the first step is the differentiation depending upon the proportion of indole acetic acid (IAA) to the other natural hormones i.e. gibberellic acid (GA<sub>3</sub>) and abscisic acid (ABA) present in the cuttings (Hess, 1963). The same author reported that when IAA was applied high root primordial developed. Tissues of easy- to- root fruit tree cuttings repeatedly showed to have a higher IAA than those difficult to- root cuttings (Hess, 1962 and 1965). The natural auxin IAA had been thought to enhance enzyme activity, thus, increase starch hydrolysis and facilitate mobilization (Balo *et al.*, 1970 and Kakbadze, 1970). The time of year in which cuttings are taken from their parent trees, the age of cuttings and the shoot position have a strong influence on the rooting ability (Krause, 1962; Robitaille and Janick, 1979; Good Lane, 198 and El- Morsy, 1988).

Chemical composition of the cuttings in related to their physiological behaviour and rooting response. The natural balance occurred among carbohydrates and nitrogen as well as zinc content in stem cuttings after been taking from mother plants effectively controlled and governed the success of rooting process. In addition, the value of C/N in these cutting is considered an essential factor. Zinc is a component of a number of enzyme and acts as an enzyme cofactor this element is required for the formation of tryptophan, which is a precursor for the auxin IAA.

Salisbury and Ross 1969; Steward, 1969; Wilkins, 1969; Leppold and Kriedemann, 1977; Bonner and Varmer, 1976; Street and Helgiopik, 1977; Thimann, 1977; Moore, 1979; Hartman and

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Kester, 1983; El- Morsy *et al.*, 1993; Seagle *et al.*, 1990; Reiley and Shry, 1997; Swietlik, 1999 Nelson (2001) and Skirvin (2004).

Many authors tried propagation of some olive cvs by using stem cuttings some of them reported that rooting of stem cuttings of the prime and important olive cvs did not gave satisfactory results especially when untreated with hormones. (Webber, 1942; Ruehle, 1946; Rathore, *et al.*, 1970; Porlingis and Therios, 1976; Blommaert, *et al.*, 1979; Eid, 1980; Kilany and Gaber, 1986).

Stem cuttings is considered to be the most simple and economical method of propagation. It is important, particularly in horticulture for mass production of improved plant material in a short time and to perpetuate the characteristics of the parent plants. The rooting potential of cuttings of different plant species, however varies considerably. Some of them root easily while others can not root even with the application of growth substances (Hartmann and Kester, 1983; Reddy and Singh, 1988; El- Konaissi, 1989; Debnath and Maiti, 1990; Abo- Rawash, *et al.*, 1993; Singh, *et al.*, 1990 and Nelson, 1999).

The target of this study was finding out the answer about the definite question, why some olive cvs especially Kalamata are hard to root? Chemical analysis of easy (El- Shamy El- Agazy) and hard (Kalamata) to root cuttings before planting was carried out to suggest the appropriate treatment for inducing good rooting and survival %. The rooting potential of these cuttings with the application of IAA and zinc in sulphate form was also examined.

## **MATERIALS AND METHODS**

This study was carried out during the two successive seasons of 2009 and 2010 in a private orchard at Sakaka region, El- Goof, Saudia Arabia. Shoots moderate in vigour of the current season were collected from two olive cvs (*Olea europea*), Picual as a readily to root cv. and Kalamata as a hard to root cv.

The trees were of about ten years old, soft cuttings of both olive cvs were taken at mid June and prepared of about 10 cm. long and 0.9 cm in diameter which include five nodes and two leaves. They were

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taken from middle sections shoots. Two parts sections were included, The first part was to determine carbohydrates, nitrogen, zinc, IAA, GA<sub>r</sub> and ABA of soft stem cuttings for both olive cvs before carrying out rooting experiment (second part). At the end of the first part of this study and according to the obtained resulted, the suggestions for using IAA and Zinc for promoting rootability of hard to root cuttings of Kalamata olive cv. were arised (on the basis lower content of IAA and Zn in such cuttings comparing with easy to root cuttings content). In the second part, cuttings of both olive cvs were dipped in solution of Zn- sulphate at 000 ppm and IAA at 1000, 2000 or 3000 ppm for one minute before being subjected to an intermittent mist system.

### The following treatments were involved:-

- 1- Control (undipped in IAA and Zn)
- 2- Dipping cuttings in Zn solution of 000 ppm (0.0 g/l)
- 3- Dipping cuttings in IBA solution at 1000 ppm (0.0 g/l)
- 4- Dipping cuttings in IBA solution at 2000 ppm (0.0 g/l)
- 5- Dipping cuttings in IBA solution at 3000 ppm (0.0 g/l)
- 6- Dipping cuttings in IBA solution at 1000 ppm + Zn at 000 ppm
- 7- Dipping cuttings in IBA solution at 2000 ppm + Zn at 000 ppm
- 8- Dipping cuttings in IBA solution at 3000 ppm + Zn at 000 ppm

Solution of IAA was prepared by dissolving the assigned amounts in 00 % ethyl alcohol. Control treatment involved the application of water with ethyl alcohol at 00 %. N in both seasons. All cuttings for both olive cvs after IBA treatments were planted at a depth of five cm. in plastic flats filled with a mixture of vermiculite peat moss and sand (1: 1: 1 by volume).

The flats were kept for 30 days in the shade house under intermittent mist, operating during day house was according to seasonal and daily weather conditions, within a range of 2.0 to 0.0 min. between sprays and 0- 4.0 sec. of mist duration. Cuttings were held at greenhouse for another one month. Each treatment was replicated five times, ten cuttings per replicate.

The experiment was set up according to the factorial complete randomized design. The first factor (A) was the two olive cvs and the

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second factor (B) was the eight IBA and Zn treatment. Total carbohydrates, nitrogen and Zn were determined in the basal portion of cuttings according to the procedures outlined in Piper, (1950) and A.O.A.C, (1990) and C/N was calculated. For the determination of endogenous hormones IAA, GA<sub>r</sub> and ABA, samples from the basal portion of cuttings were taken and kept in methanol at -20 °C until fractionation. The procedures of Fadl and Hartmann, (1967) for fractionation were followed to separate these natural hormones.

At the end of experiment (second part) (8 weeks after planting) the following parameters were measured:-

- 1- Percentage of rooting
- 2- Number of roots per cutting
- 3- Root length (cm.)
- 4- Root distribution (cm<sup>3</sup>)
- 5- Percentage of survival

Obtained data of this work were statistically compared using New L.S.D test according to Gomez and Gomez, (1985)

## RESULTS AND DISCUSSION

### 1- Endogenous hormones namely IAA, GA<sub>r</sub> and ABA as well as carbohydrates, nitrogen C/N and Zn in easy and difficult to root olive cuttings:-

It is clear from the data in Table 1 that in easy to root Picual olive cuttings the endogenous IAA, total carbohydrates, C/N and Zn were the highest, whereas, the other hormones i.e GA<sub>r</sub> and ABA as well as N were the lowest comparing with those in Kalamata cuttings (hard to root olive cuttings). The highest level was concomitant with its ability to root as nextly will be discussed and the vice versa for the other difficult to root olive cv namely Kalamata. Therefore, it could be concluded that IAA is possibly among the factors that controlling rooting of the olive stem cuttings. In this respect Hess (1962 and 1963) found that rooting was almost related in most cases to the presence and accumulation of the natural auxin IAA in the basis of cuttings. Swietlik (1999) stated that Zn is a precursor for the auxin IAA. Skirvin (2004) emphasized the beneficial effect of carbohydrates

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on forming of root primordial. This conclusion is in parallel with that found by Hartmann and Kaster (1983); Singh (1983); Seagle, *et al.*, (1990); Reiley and Shry (1997) and Nelson (2000).

**Table 1: Endogenous hormones (mg/g F.W) and nutritional status on easy and difficult to root olive cv cuttings during 2009 and 2010 seasons.**

Parameters	2009		2010	
	(Picual)	(Kalamata)	(Picual)	(Kalamata)
IAA	0.962	0.329	0.999	0.400
GA <sub>3</sub>	0.790	3.770	0.800	4.010
ABA	0.005	0.031	0.008	0.041
Carbohydrates%	20.0	12	19.5	11.9
N %	2.0	2.99	1.97	3.00
C/N	10/1	40.1/1	9.90/1	39.7/1
Zn (ppm)	80.9	22.0	96.3	23.3

However, the lower concentration of both ABA and GA<sub>3</sub> in Picual olive cutting and the greatest values for both hormones in Kalamata olive cuttings may answer the question why Kalamata olive cuttings are hard to root. The inhibition of GA<sub>3</sub> to root formation is a direct local effect that prevents the early cell division involved in transformative of mature stem tissues to a meri-stematic condition (Brian *et al.*, 1960). Gibberellic acid has a function in regulating nucleic acid and protein synthesis and may be a suppressing for root initiation (Moore, 1979). Lowering the natural level of GA<sub>3</sub> in the tissues should stimulate adventitious root formation in cuttings (Hartmann and Kester, 1983). The lower concentration of ABA in the easy vine to- root vine cuttings may indicate that this plant regulator do not play an important role in root initiation. This finding confirmed the work of Bonner and Varmer (1976). In fact, reports on the effect of ABA on adventitious root formation are contradictory (Basu *et al.*, 1970) apparently depending upon the concentration and the nutritional status of the parents which the cuttings are taken from (Hartmann and Kester, 1983).

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The relationship between the chemical composition of the cuttings and the rooting ability are in line with the finding of Fadl and Hartmann (1967); Moore (1979); and Reily and Shry (1997).

### - Root characters and survival %:-

As shown in Tables 2, 3 and 4 great and significant differences in the percentage of rooting, root distribution number of roots/ cutting, root length and survival % were existed among Picual and Kalamata olive cuttings. The maximum values of these parameters were recorded on easy to root Picual olive cutting comparing with those produced from difficult to root Kalamata olive cuttings.

**Table 2: Effect of dipping in IBA and Zn solution on the percentage of rooting in easy and difficult root olive cuttings during 2009 and 2010 seasons.**

IBA and Zn Treatments (B)	2009			2010		
	Olive cvs (A)					
	Percentage of rooting					
	a <sub>1</sub> Picual	a <sub>2</sub> Kalamata	a <sub>3</sub> Mean (B)	a <sub>1</sub> Picual	a <sub>2</sub> Kalamata	a <sub>3</sub> Mean (B)
b <sub>1</sub> 0.0 ppm	60.0	22.2	41.1	22.2	22.1	22.2
b <sub>2</sub> Zn at 0.0 ppm	61.9	29.9	45.9	64.0	30.3	47.4
b <sub>3</sub> IBA at 1000 ppm	63.0	33.3	48.2	66.9	34.0	51.7
B <sub>4</sub> IBA at 2000 ppm	64.9	35.0	50.0	68.9	36.0	52.7
B <sub>5</sub> IBA at 3000 ppm	65.0	36.0	50.5	69.0	37.0	53.0
b <sub>6</sub> IBA at 1000 ppm +Zn	68.0	41.0	54.5	72.2	41.9	57.1
b <sub>7</sub> IBA at 2000 ppm +Zn	70.0	49.0	62.3	79.3	50.2	64.8
b <sub>8</sub> IBA at 3000 ppm +Zn	70.2	50.0	62.6	80.0	50.0	65.3
Mean (A)	66.6	37.1		70.4	37.8	
New L.S.D at 5%	A	B	AB	A	B	AB

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**Table 3: Effect of dipping in IBA and Zn solution on No. of roots/ cutting and root length (cm.) in easy and difficult to root olive cuttings during 2009 and 2010 seasons.**

IBA and Zn Treatments (B)	2009			2010		
	Olive cvs (A)					
	No. of roots/ cutting					
	a <sub>1</sub> Picual	a <sub>2</sub> Kalamata	a <sub>3</sub> Mean (B)	a <sub>1</sub> Picual	a <sub>2</sub> Kalamata	a <sub>3</sub> Mean (B)
b <sub>1</sub> 0.0 ppm	18.0	3.0	10.0	19.2	3.3	11.3
b <sub>2</sub> Zn at 0.00 ppm	19.4	4.1	11.8	21.0	4.0	12.8
b <sub>3</sub> IBA at 1000 ppm	20.7	0.2	13.0	22.0	0.2	14.1
B <sub>1</sub> IBA at 2000 ppm	21.8	6.3	14.1	24.0	6.8	15.4
B <sub>2</sub> IBA at 4000 ppm	22.0	6.4	14.2	24.3	7.0	15.7
b <sub>4</sub> IBA at 1000 ppm +Zn	23.3	7.9	15.6	26.0	8.3	17.2
b <sub>5</sub> IBA at 2000 ppm +Zn	25.3	10.2	17.8	27.7	9.4	18.6
b <sub>6</sub> IBA at 4000 ppm +Zn	25.0	10.0	18.0	28.0	9.0	18.8
Mean (A)	22.0	6.7		24.1	6.8	
New L.S.D at 5%	A	B	AB	A	B	AB
	1.0	1.0	1.4	1.0	1.0	1.4
Character	Root length (cm.)					
b <sub>1</sub> 0.0 ppm	10.0	1.8	0.9	11.1	2.0	6.6
b <sub>2</sub> Zn at 0.00 ppm	10.7	2.0	6.6	11.9	2.6	7.3
b <sub>3</sub> IBA at 1000 ppm	11.0	3.0	7.0	12.0	3.2	7.9
B <sub>1</sub> IBA at 2000 ppm	12.1	4.0	8.3	13.8	4.0	8.9
B <sub>2</sub> IBA at 4000 ppm	12.2	4.6	8.4	14.0	4.1	9.1
b <sub>4</sub> IBA at 1000 ppm +Zn	12.9	0.3	9.1	14.9	4.9	9.9
b <sub>5</sub> IBA at 2000 ppm +Zn	14.0	6.0	10.0	15.9	0.6	10.8
b <sub>6</sub> IBA at 4000 ppm +Zn	14.1	6.1	10.1	16.0	0.8	10.9
Mean (A)	12.2	4.3		13.8	4.0	
New L.S.D at 5%	A	B	AB	A	B	AB
	0.5	0.6	0.8	0.4	0.5	0.7

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Table 4: Effect of dipping in IBA and Zn solution on root distribution (cm<sup>3</sup>) and survival % in easy and difficult to root olive cuttings during 2009 and 2010 seasons.

IBA and Zn Treatments (B)	2009			2010		
	Olive cvs (A)					
	Root distribution (cm <sup>3</sup> )					
	a <sub>1</sub> Picual	a <sub>2</sub> Kalamata	a <sub>3</sub> Mean (B)	a <sub>1</sub> Picual	a <sub>2</sub> Kalamata	a <sub>3</sub> Mean (B)
b <sub>1</sub> 0.0 ppm	66.2	8.2	37.2	69.3	10.0	39.7
b <sub>2</sub> Zn at 0.0 ppm	68.9	9.6	39.3	71.3	11.0	41.4
b <sub>3</sub> IBA at 1000 ppm	71.0	11.6	41.3	73.4	13.3	43.4
B <sub>4</sub> IBA at 2000 ppm	73.3	13.6	43.0	75.0	15.0	45.0
B <sub>5</sub> IBA at 3000 ppm	74.0	14.0	44.0	75.0	15.1	45.3
b <sub>1</sub> IBA at 1000 ppm +Zn	80.0	16.0	48.0	79.9	17.0	48.0
b <sub>2</sub> IBA at 2000 ppm +Zn	82.0	19.0	51.0	83.0	20.0	51.0
b <sub>3</sub> IBA at 3000 ppm +Zn	82.7	20.0	51.4	83.3	20.2	51.8
Mean (A)	74.8	14.1		76.3	15.3	
New L.S.D at 5%	A	B	AB	A	B	AB
	1.0	1.4	2.0	1.6	1.7	2.0
Character	Survival %					
b <sub>1</sub> 0.0 ppm	70.0	16.6	43.3	71.0	19.0	43.3
b <sub>2</sub> Zn at 0.0 ppm	72.0	18.6	45.3	73.0	20.0	46.0
b <sub>3</sub> IBA at 1000 ppm	73.0	26.0	49.8	75.2	27.0	51.1
B <sub>4</sub> IBA at 2000 ppm	75.0	29.6	52.3	77.3	30.0	53.7
B <sub>5</sub> IBA at 3000 ppm	75.0	32.0	53.8	78.0	33.0	55.0
b <sub>1</sub> IBA at 1000 ppm +Zn	77.2	33.6	55.4	79.9	35.0	57.0
b <sub>2</sub> IBA at 2000 ppm +Zn	81.0	38.0	59.0	84.0	40.0	62.0
b <sub>3</sub> IBA at 3000 ppm +Zn	81.3	38.3	59.8	84.0	40.0	62.0
Mean (A)	75.7	29.1		77.9	30.1	
New L.S.D at 5%	A	B	AB	A	B	AB
	1.0	1.0	2.1	1.6	1.7	2.4

Dipping olive cuttings in a solution containing IBA at 1000 to 3000 ppm beside Zn at 0.0 ppm in sulphate form significantly promoted all characters of root as well as survival % compared with using each compound alone. All IBA and Zn treatments caused

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significant promotion on these parameters compared with the control treatment. The promotion on the rooting ability was associated with the increase in IBA concentrations from 1000 to 4000 ppm. No significant stimulation was observed when IBA concentrations were increased from 2000 to 4000 ppm.

The interaction between olive cvs and Zn and IBA treatments had significant effects on roots and survival %. The promotive effects of IBA and Zn on rooting were clearly shown in those hard to root cuttings of olive cv. Kalamata. The maximum rooting %, root distribution, root length, number of roots and survival % were attained when Picual olive cuttings were dipped in solution containing 2000 ppm IBA plus zinc at 200 ppm. Treating Kalamata olive cuttings with IBA and Zn effectively solved the problem of poor rooting in such olive cv. cuttings. These results were true during the two experimental seasons.

The presence of IAA, carbohydrates, C/N and Zn at a higher level in easy to root cuttings and at a lower levels in hard to root cuttings could ascertain the view about the possible contribution of the compound with rooting ability of the various olive cvs. Basu *et al.*, (1970) stated that easy to root cutting had low level of GA<sub>r</sub> and ABA which are inhibitors of root formation. It may be concluded that there are several important factors related to the root ability of cuttings these factors are generally higher value of IAA carbohydrates and Zn and lower content of GA<sub>r</sub>, ABA and N in olive cuttings. The positive effects of IAA and Zn in enhancing root primordial and activity of different enzymes. The same trend was observed by Abou Rawash *et al.*, (1993) and Nelson, (1999).

As a conclusion, these results confirmed that it is possible for solving the poor of rooting in different olive cvs by dipping cuttings in solution containing IBA at 2000 ppm plus Zn at 200 ppm for one minute. Hormonal and nutritional status of the cutting are considered important factors controlling rooting of fruit species.

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## دراسات فسيولوجية علي تجذير عقل الزيتون السهلة والصعبة التجذير

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تم تقدير محتوى العقل الساقية الغضة السهلة التجذير للزيتون البيكوال وكذلك الصعبة التجذير للزيتون الكلاماتا قبل زراعتها من الكربوهيدرات الكلية والنيتروجين والنسبة ما بين الكربوهيدرات والنيتروجين والزنك والهرمونات الداخلية (اندول حامض الخليك والجبرلين وحامض الابسيسك) وذلك خلال موسمي ٢٠٠٩، ٢٠١٠ ولقد تم غمس العقل في محاليل من سلفات الزنك بتركيز ٥٠٠ جزء في المليون وأندول حامض البيوتريك بتركيز ما بين ١٠٠٠ الي ٤٠٠٠ جزء في المليون لمدة دقيقة واحدة.

أشارت نتائج الدراسة الي أن العقل السهلة التجذير للزيتون البيكوال كان محتواها عالي من الكربوهيدرات والنسبة ما بين الكربوهيدرات والنيتروجين والزنك وأندول حامض الخليك ومحتواها منخفض من النيتروجين والجبرلين وحامض الابسيسك وكان هناك زيادة واضحة في محتوى العقل الصعبة التجذير للزيتون الكلاماتا من النيتروجين ومن الكربوهيدرات + النيتروجين والجبرلين وحامض الابسيسك ونسبة منخفضة من الابسيسك وارتبطت قابلية عقل الزيتون للتجذير بالاتزان الحادث بين الهرمونات الداخلية الثلاثة وهي اندول حامض الخليك والجبرلين وحامض الابسيسك كذلك مع النسبة ما بين الكربوهيدرات والنيتروجين وكذلك محتواها العالي من عنصر الزنك.

انه من الأهمية بمكان من أن غمس العقل السهلة التجذير للزيتون البيكوال والصعبة التجذير للزيتون الكلاماتا في محاليل تحتوي علي اندول حامض البيوتريك بتركيز ما بين ١٠٠٠ الي ٤٠٠٠ جزء في المليون مع الزنك بتركيز ٥٠٠ جزء في المليون وذلك لأجل تحسين النسبة المئوية للتجذير وانتشار وطول الجذر والنسبة المئوية للنجاح وذلك بالمقارنة باستخدام أي من المادتين بمفرده ولقد ظهرت أهمية اندول حامض البيوتريك والزنك خصوصا في تجذير العقل الصعبة التجذير للزيتون كلاماتا.

لأجل تحسين تجذير العقل الصعبة التجذير للزيتون كلاماتا فإنه يوصي بغمس العقل الغضة الوسطية في أندول حامض البيوتريك وسلفات الزنك بتركيز ٢٠٠٠، ٥٠٠ جزء في المليون علي التوالي.