

## Study of Main Factors Influencing Olive Propagation

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**Abstract:** From 2009-2011, several experiments were carried out at the propagation Station in Tirana having as objective the study of the influence of endogenous and exogenous factors on two olive cultivars, “White olive of Tirana”, (BT) and “The black of Tirana” (ZT). The green cuttings are treated by different concentration of IBA at several stages of their meristematic activity. Induced rhizogenesis after callus tissue formation was influenced significantly (LSD.2.11 HSD,  $q=0.05$ ) by the presence of inflorescence (13.3%). The apical green cuttings differed from medial part and basal ends respectively 8.8% and 13.3%. In May, the IBA concentrations 3 g/l<sup>1</sup> has improved significantly rooting at studied cultivars, 20.2% (BT) and 9.4 % (ZT). While in October and February period, the treatment by IBA at concentration of 5 g/l<sup>1</sup> improved the rooting capacity by 11.7% (BT) and 7.7 % (ZT) compared to the results achieved by using the concentration 3 g/l<sup>1</sup> of IBA. The cultivar affects and presents a strong relation with the IBA concentration ( $r = 0,689$ ), and with the period ( $r = 0,888$ ). Defoliation percentage is specially related to the period ( $r = 0,557$ ) and the other techniques applied. The factors grouping highly influenced rooting percentage of olive cultivars, by a regression coefficient of  $R^2 = 0,7084$ .

**Key words:** *Cultivar, stimulation, rooting capacity, Olea europea, autochthonous.*

### Introduction

The “mist propagation” technique, has a high propagation coefficient, produces a powerful and resistant seedling to diseases, but its efficiency is influenced by different factors such as endogenous hormonal stimulants (Fiorino *et al.* 1980) exogenous hormonal stimulants (Leva *et al.* 1992) hormonal acid concentrations (Bartolini *et al.* 1989), the nature of the green cutting and its place in the sprig the time applied for propagation, etc. (Caballero J.M. 1983). A lot of researches for the propagation of the olive varieties have proved different rooting capacities from one variety to the other. Meanwhile a lot of hormonal acids have been experimented with several hydro alcoholic concentrations and the obtained results have varied according to the genotype used. (Cimat, 1989). For these reasons, in the center of olive propagation in Pezë e vogël, research was carried out for several important factors in two olive cultivars in the area of Tirana; “i Bardhë i Tiranës” (BT) and “i Zi i Tiranës (UZ).

### Material and method

#### Experimental scheme

(a) Study on the concentration of IBA regarding the meristematic activity of the vegetal material. The green cuttings of both olive cultivars the “i Bardhë i Tiranës” (BT) and “i Zi i Tiranës” (ZT) were proved through the method of “mist propagation”, for their rooting capacity in correlation with four concentrations of the Indole-3-butyric acid (IBA) and a Treatment (Control), without a hormone; in four moments of the meristematic activity (5th February, 5th May, 5th September, and 5th December). The scheme consisted of 12 treatments, applied over a period of time encompassing 4 terms: (1) BT Control, (2) ZT Control, (3) BT IBA 1g l<sup>1</sup> (4) ZT IBA 1g l<sup>1</sup>, (5) BT IBA 3g l<sup>1</sup>, (6) ZT IBA 3g l<sup>1</sup>; (7) BT IBA 5g l<sup>1</sup>, (8) ZT IBA 5g l<sup>1</sup>, (9) BT IBA 8g l<sup>1</sup>, (10) ZT IBA 8g l<sup>1</sup> (11) BT IBA 3g l<sup>1</sup>Talc, (12) ZT IBA 3g l<sup>1</sup> Talc ;

(b) Study of several endogenous and exogenous factors on their rooting capacity. 13 factors were proved at the same concentration and term [7 endogenous and 6 exogenous]. On the 8-10 March, of

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the years 2008, 2009, 2010; the following scheme was experimented on the “i Bardhi Tiranes” (BT) and “i Ziu Tiranes” (ZT)) olive trees: (1) apical cutting, (2) medial cutting, (3) basal cutting, (4) cutting with flowers, (5) cutting without flowers, (6) Centennial parent tree, (7) New parent tree, (8) IBA C<sub>12</sub>H<sub>13</sub>NO<sub>2</sub>, (9) IBA C<sub>12</sub>H<sub>13</sub>NO<sub>2</sub>K, (10) IBA/ANA, (11) IBA-GA<sup>3</sup>, (12) IBA-TALC, (13) IBA-BAP

**Vegetal material:** the green cuttings were extracted from olive trees in “Rapaçeç”, Tiranë. The green cuttings were 8-10 cm long with two couple leaves in the apical part. [Figure 1]. Each treatment had 100 cuttings, (4 repetitions x 25 cuttings). Treatment in 5 seconds. In each hydro alcoholic concentration: alcohol comprised 24% and H<sub>2</sub>O, 76%.

**Humidity and temperature regimes:** the planting was carried out in a nebulisation bank (type SHB,) with perlite substrate. The temperature in the environment of the bank was kept 20°C, whereas in the substrate 24°C (± 1°C), through a furnace with two pumps Grundfos, three Thermostats, one Flusostat and other equipment of control. The mist was realized for 5-7 seconds, each 15 Wh/m<sup>2</sup>, in function of the solar radiation. The light was preserved 4500 lux, 12-13 hours/ day (Gonda *et al.*, 2006).

**Research indices and statistic analysis:** Rooting percentage, (cutting with roots expressed in %). statistic analysis: performed with software, referred to 100 green cuttings for each treatment. Variance demonstration, Correlation coefficient of independent factors, regress analysis and statistical importance (Jmp. Sas/stat. 2008; Rodríguez *et al.* 2008).

## Results

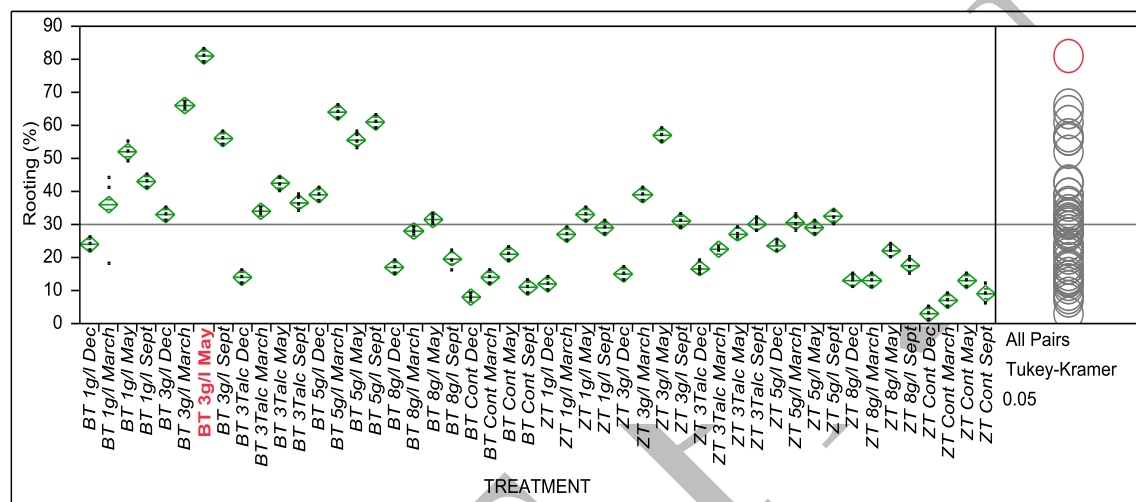
**Rhizogenesis process: Morphological exchanges:** At the beginning after the cutting, there is a physiological disorder and to normalize this state, the cutting reacts energetically with the rapid division of cells and the heal of the wound. The most activated tissue in the formation of the callus is the cambium. Callus formation is done through all the live cells found under the cut area (Figure 1). The first symptoms are an increase of the cells of the cortical parenchyma and the parenchyma of the phloem, whereas the other cells do not change the state. Step by step they are augmented, and put a pressure on the sclerenchyma ring. Later they are unified as a mass of hyperplastic tissues. Whereas the cells of the periderm, suber, and the rays of the secondary phloem are preserved. Within the callus, next to the vascular cambium and the parenchyma tic phloem emerge cellular groups that develop in two directions (external-internal), with a greater meristem tic activity (Bartolini *et al.* 1989). The skin covering the internal cellular cones which form the vascular cylinder is distinguished. The vascular tissues are immediately interconnected to the xylem's cutting, which increases towards the periphery where the anatomical structures are distinguished; cortex with the periderm and xylem to the medulla, time which is considered as the outset of sprigs' emergence. (Caballero J.M. 1983)



**Figure 1.** Morphological exchanges and the main stages of the process.

**The study of the IBA concentration in relation to the meristem tic activity of the vegetal material.** It has resulted that the olive cultivars; i Bardhi Tiranës (BT) and i Ziu Tiranës (ZT) under the influence of treatments have expressed different rooting percentage values (*table-3*). The BT olive, under the variant Control and in any treatment had higher rooting values than the ZT olive. (*Tukey test lsd 2.23 q=0.05*). (*Figura-2 & Table-3*)

Figure -2 and table -3, present the variance analysis for 12 treatments applied in 4 terms (March, May, September, December), and the cultivars have a different frequency of the averages, distance certified through Tukey-kramer (*Lsd 2.23, alpha=0.05*) where the variables with the same letter do not have certified differences. Each variable is found in a position with a dominant or inferior sense towards the average line of the 48 treatments (29.7%) (Figure 2)



**Figure-2,** Anova test, to demonstrate the changes in rooting percentage of the BT and ZT cv-s

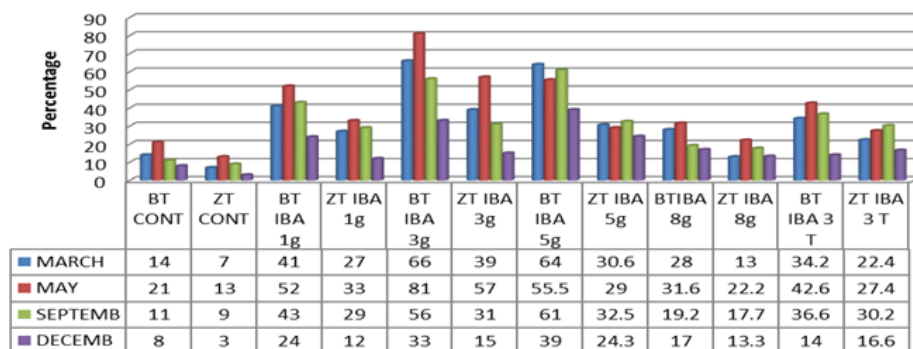
**Tabele-3.** Rooting percentage of the “ i Bardhi Tiranës” olive (BT) and “ i Ziu Tiranës” (ZT)

PERIOD TREATMENT	MARCH (%)	MAY (%)	SEPTEMBER (%)	DECEMBER (%)
BT CONTROL	14.0 ±1.63 stuv	21.0 ±1.63 nopqr	11.0 ±1.63 tuvw	8.0 ±0.81 vwx
ZT CONTROL	7.0 ±1.63 wx	13.0 ±1.63 stuvw	9.0 ±2.44 uvwx	3.0 ±1.63 x
BT IBA 1 g l <sup>-1</sup>	41.0 ±1.08 fgh	52.0 ±2.44 d	43.0 ±1.63 e	24.0 ±1.63 klmno
ZT IBA 1 g l <sup>-1</sup>	27.0 ±1.27 jklmn	33.0 ±1.91 ghij	29.0 ±1.63 ijklm	12.0 lmnop
BT IBA 3 g l <sup>-1</sup>	66.0 ±0.81 b	81.0 ±1.63 a	56.0 ±1.63 cd	33.0 ±1.63 ghij
ZT IBA 3 g l <sup>-1</sup>	39.0 ±1.63 efg	57.0 ±1.63 cd	31.0 ±1.63 hij	15.0 ±1.63 rstu
BT IBA 5 g l <sup>-1</sup>	64.0 ±1.63 b	55.5 ±2.21 cd	61.0 ±1.63 bc	39.0 ±1.63 efg
ZT IBA 5 g l <sup>-1</sup>	30.6 ±2.21 hijk	29.0 ±1.63 ijklm	32.5 ±1.91 ghij	24.3 ±1.25 klmno
BT IBA 8 g l <sup>-1</sup>	28.0 ±0.81 ijklm	31.6 ±1.63 hij	19.2 ±1.29 opqrs	17.0 ±1.63 opqrs
ZT IBA 8 g l <sup>-1</sup>	13.0 ±1.63 stuvw	22.2 ±1.70 mnopq	17.7 ±2.21 opqrst	13.3 ±1.70 qrst
BT IBA Talc 3 g l <sup>-1</sup>	34.2 ±0.81 ghi	42.6 ±1.91 ef	36.6 ±2.21 efgh	14.0 ±1.63 pqrst
ZT IBA Talc 3 g l <sup>-1</sup>	22.4 ±0.57 mnopq	27.4 ±1.25 ijklmn	30.2 ±1.70 hijkl	16.6 ±1.70 pqrst

Levels not connected by same letter are significantly different

Correlations (*cultivar-term=term-concentration*), have highlighted a higher rooting concentration at the “Ulliri Bardhë” (BT), compared to the “Ulliri Zi” (ZT). The best concentrations derive with; IBA3g l<sup>-1</sup> and IBA5g l<sup>-1</sup>. The concentration IBA5g l<sup>-1</sup> has acted better when the cambium was less active, whereas the concentration IBA3g l<sup>-1</sup> in the cases when the activity of the cambium was intensive tested through vegetative dynamic. Treatment 9 and 10, with maximal concentration of IBA8g l<sup>-1</sup>, were extremely toxic for the tissues of the cutting and had lower rooting percentage with obvious

changes compared to the other concentrations of the IBA. In the treatment [12] IBA/Talc, the rooting percentage resulted with differences even below the levels of hydro alcoholic use. The meristem tic activity tested in four moments of the vegetative cycle, (February, May, September, and December) was characterized by changes and was the cause of different natural capacities (Table 4). In May the rooting capacity was higher than in the other terms, because of a more intensive meristem tic activity during this period (Caballero, 1983).

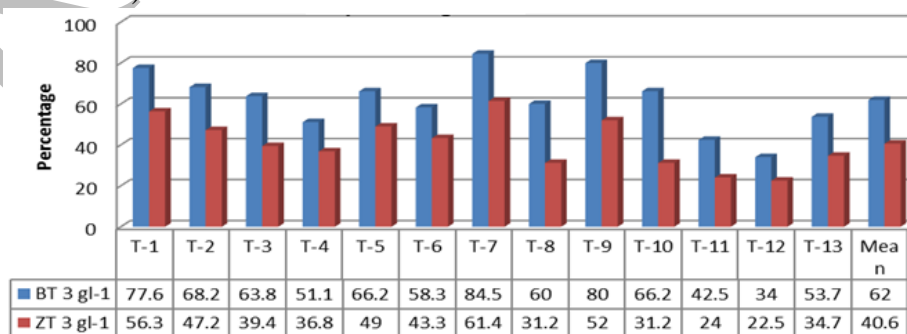


**Figure 4.** IBA influence in different terms on rooting percentage

Both cultivars reacted better under the concentration of IBAs  $3g\ l^{-1}$ , in May and March, while in March and September under the concentration  $5g\ l^{-1}$ . The cultivars had inferior values in December and in some cases even in September. In all the cases the variant Control which represents the natural capacity, expressed a lower percentage compared to the treatments of synthetic stimulant. Whereas the maximal concentration of the Indole butyric acid  $8g\ l^{-1}$  caused toxicity in both cultivars and was accompanied by lower rooting percentage (Ismaili, 2010).

The correlations have displayed the connection between rooting percentage and defoliation percentage (smoothing Spline Fit,  $\lambda=0.1$ ). In any case the application of excessive vapor has caused a high percentage of defoliation expressed in (%). I.e.; a high rooting percentage has had a low defoliation percentage and the opposite; in any case the high percentage of the green cuttings defoliation is accompanied with low rooting.

**Study of some endogenous and exogenous factors on rooting capacity.** Individual evaluation was based on the independent variables and the relation between the varietal tissue potency and the influential factors. Both cultivars “Bardhi Tiranes” and “Ziu Tiranes”, (BT and ZT) were studied during a unique term 8-10 March and at the same concentration of IBA  $3g\ l^{-1}$ , in relation to 13 factors; (i) endogenous treatments; (1,2,3,4,5,6,7) and (ii) exogenous factor treatment; (8,9,10,11,12,13). All he raised hypothesis had positive influences and tendencies for the rooting percentage. However they were not able to modify the genetic character i.e. the varietal rooting capacity. This means that the olive BT, in relation to any factor had much more tendency for rooting (21.4%), compared to the olive ZT (Fiorino *et al.* 1990).



**Figure 5.** Influence of endogenous and exogenous factors on rooting percentage of BT and ZT olive. Treatments: (1) apical cutting, (2) medial cutting, (3) basal cutting, (4) cutting with flowers, (5) cutting without flowers, (6) Centennial tree, (7) New tree, (8)  $C_{12}H_{13}NO_2$ , (9)  $C_{12}H_{13}NO_2K$ , (10) IBA/ANA, (11) IBA- $GA^3$ , (12) IBA TALC, (13) IBA-BAP

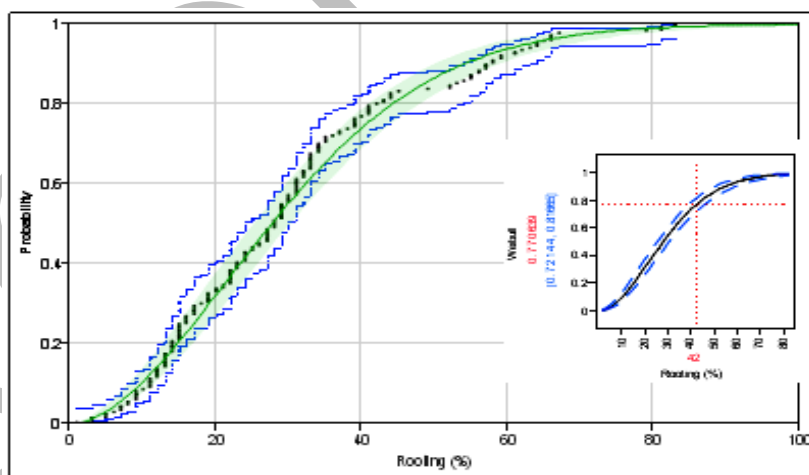
The rooting tendency of the cuttings dealing with new trees was better than when dealing with old ones. The cuttings from new olive trees BT, have a rooting percentage of (84.5%) compared to (61.5%) of the old trees. The mechanisms of transforming a new form tree into old form tree influence their rooting capacity, because the content of the endogenous auxins changes. It is superior the in the new ones accompanied by phenolic, less inhibitor components (Leva *et al* 1992).

Treatments for the position of the cutting upon the sprig *apical, medial, basal* have been characterized by statistically different values *Tukey-kramer. (Lsd 2.19 HSD)*. In March as soon as the meristemic differentiation begins, the endogenous hormones have the tendency to go towards the apical buds. For this reason the apical cuttings have higher rooting concentration BT (77.6%) and ZT (56.3%), compared to the basal segments which are (63.8%) and (39.4%).

When the green cuttings had inflorescence and flowers; rooting capacity was 13.7% lower than in the cases when the cutting was without them. (*average for both cultivars*). The comparison of two products of the IBA and their combinations with ANA and GA<sup>3</sup>, and BAP, offered heterogeneous values. IBA with Potassium (C<sub>12</sub>H<sub>13</sub>NO<sub>2</sub>K) has mainly stimulated the rooting of the cuttings (80%) over (60%) gained with (C<sub>12</sub>H<sub>13</sub>NO<sub>2</sub>), IBA standard. The lowest tendencies for rooting derived from the mix solution IBA/GA<sup>3</sup>, and IBA/Talc. In treatment 10, [IBA/ANA], and treatment 13 [IBA/BAP] the tendencies for rooting were on average better. In each case the hormones caused apical sleep, thus decelerating the bud activity and favouring rhizogenesis, thus eliminating competitiveness in the rooting process. While the wound of the base allows the penetration and formation of the ethylene as a good rooting favourer. The cuttings created a callus and later continued with bad rooting, the cause was that the cells appear without a cytoplasm and the membranes are reabsorbed and destroyed by forming big holes in the hyperplasic mass (Fiorino *et al.* 1990).

Whereas the presence of leaves on top of the green cutting has been an important factor for the success of rooting, a micro photosynthetic process, the stimulation and emergence of root emissions (Caballero, 1983).

**The demonstration of the hypothesis:** the correlative analysis through regression has demonstrated the relation between the two factors and the character of the relation.

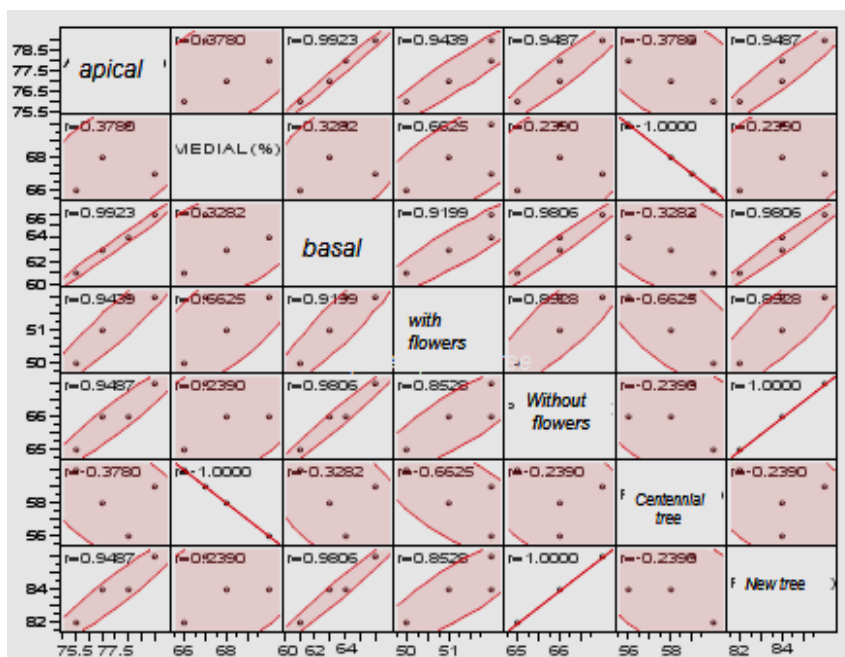


**Figure- 6.** Profile of the distribution of the rooting percentage of the independent factors

The apical cuttings (1) extracted by a new tree (7) have a Correlation Coefficient =0.94, which means that there is a stronger bond between them. Between the treatments; New tree (7) and other factors such as cuttings without flowers (5), basal cuttings (3), cuttings with flowers (4), there are strong positive relations because the value of the correlation coefficient is more than 0.8 in each case (Gonda *et al.* 2006).

For the demonstration of the 13 Hypothesis raised in (Table 8), t,s actual values are given. The hypothesis that the apical cuttings (H0) influence the tendency for rooting is certified because the value (tf>tk 2.2092 > 2), *i.e.* our assumption that the medial and basal cuttings (H1) in March influence more, is not certified. In the same way, the influence of inflorescence (H1) with values tf>tk.

(2.1114 >2) is not acceptable, whereas the application for the propagation of the cuttings without flowers (H0) influences the rooting tendency and is certified.



**Figure 7.** Dendrogram generated by Scatterplot Matrix Multivariate Correlations analysis based on distance computed from pairwise comparisons of independent factors.

The old parent trees (H1) with a value of  $t_f$  (2.4131 > 2), is not acceptable, i.e., the extraction of cuttings from new trees (H0) has influenced the tendency for an increase in the rooting percentage. As far as exogenous factors are concerned; IBA ( $C_{12}H_{13}NO_2K$ ) [H0] has the tendency and favours better rooting than the hypothesis [H1],  $C_{12}H_{13}NO_2$ , IBA/GA3, IBA/ANA, IBA/Talc, and IBA/BAP is certified in this respect (Jmp sas/stat. 2010).

**Table 8.** Equation of regression and the actual values of  $t_s$  for main hypothesis.

	Regression Coefficient	Standard Error	Prob. Level	T-value $H_0 : B = 0$	Decision 95%
Parent tree origin	0.2311	0.05	0.029886	2.2092	Accept H0
Influence of Flowers	0.1517	0.04	0.025331	2.1114	Accept H0
Age of parent tree	0.2341	0.05	0.022793	2.1900	Accept H0
$C_{12}H_{13}NO_2K$	0.3451	0.03	0.013002	2.3445	Accept H0
$C_{12}H_{13}NO_2$	0.0761	0.04	0.073796	1.7625	Accept H1
IBA-Talc	0.0993	0.06	0.081628	1.7311	Accept H1
IBA/ANA	0.1122	0.05	0.019889	2.0562	Accept H0

The accepted hypothesis imply that these acknowledgements are statistically reliable for ( $\alpha=0.05$ ). The lowest value of ( $\alpha$ ), (tabela-8) the lowest possibility to be accepted (H1), when it is real (H0). The concept of  $R^2$  as a proportional coefficient of the variation has fluctuated from (039) to (09) and the line represents 90% of the points between the standard deviations (variables) (Figure 6). I.e., 72-81 % of the treatments has demonstrated a lower tendency than 42%, for rooting which statistically are not acceptable (H0) and do not have efficiency in use (Figure-6). The model regression analysis has demonstrated that the rooting averages are in the ascending slope ( $R^2 = 0.87$ ) ( $y=mx+b$ ), and show - that the treatments have had a constant effect of use, but only about 20% of the treatments have statistical importance (Rodríguez *et al.* 2008).

## Conclusions

The study of the concentrations of the IBA in relation to the meristematic activity of the vegetal material has certified 4 hypothesis; IBA  $3 \text{ g l}^{-1}$  in May, IBA  $5 \text{ g l}^{-1}$  in March, IBA  $5 \text{ g l}^{-1}$  in September and IBA  $1 \text{ g l}^{-1}$  in May. In fact that IBA  $3 \text{ g l}^{-1}$  May and  $5 \text{ g l}^{-1}$  in March & December has the tendency to increase rooting are significantly different.

The study of the exogenous and endogenous factors on rooting capacity has approved above all that the apical cuttings have the tendency to increase rooting percentage. (ii) A parent tree at a new age and (iii) cuttings without the presence of flowers have better tendencies for rooting. (iv) IBA with Potassium has better tendencies that the standard IBA. The combination of IBA/ANA, has given the tendency for important rooting percentage.

The cultivar of the olive BT has better tendencies compared to the ZT, as far as rooting percentage is concerned.

Only 22 % of the hypotheses which are recommended for application were important. Whereas 78% of the raised hypothesis resulted is unimportant.

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