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Foliar Treatment with GA3, BAP, IBA and NAA in the Rooting Process of Green Cuttings of Olive

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A B S T R A C T

This experiment has proved the influence of foliar treatments with four hormones for the process and the percentage of rooting in olive Himara varieties. The method was implemented in September; the cuttings are prepared in the apical part of the sprig. Variants applied are: Control (hydro alcoholic), 4000 ppm IBA basal and four foliar concentrations 50, 100 and 200 ppm for each hormone; BAP, GA_3, IBA and NAA constitute the scheme of this experiment. The results have proved that the treatment basal IBA has favored better rooting process compared with the foliar treatment. IBA foliar were 2.0% higher than NAA treatments and 2.7% compared with GA3 foliar and BAP. Only, IBA basal when it is associated with 100-200 ppm foliar IBA changed versus others. In general, foliar treatments have increased 0.8-2.0%, versus the rooting control percentage, while basal IBA treatments 46.3%. Four hormones have caused different reactions for defoliation whereas for the roots biometrics index are small changes.

Introduction

Albania is the country's oldest in the Mediterranean basin, for the cultivation of olive trees has several important cultivars and it has always created new olive groves, (Forbes *et al*., 1978; Frezzoti, 1930). In these last 15 years in Albania, more research's been conducted for the possibility and multiplication factors involved of green cuttings with mist propagation method. All these researches have indicated many aspects of unclear that require further intensification of research.

Among the research it is very important time of receipt of green piece, (Caballero *et al*., 1986; Bottari *et al*., 1952). The maturity level of sprig, cutting position on the linear length of sprig, type, methods and concentrations of substances rhizogenic, the time necessary for the realization of the rooting process, has great influence on the genetic profile (Fabbri *et al*., 1955; Bottari *et al*., 1952; Fernandes *et al*., 2002; Ismaili, 2010)

Our olive varieties have the various abilities rooting. Being the genetic factor, with difficult rooting varieties continues some research such as: Minimum and maximum

the number of buds and leaves, the effect of treatment with different chemical substance through foliar method, etc. (Hannachi *et al*., 2008; Ismaili, 2016).

To draw strong conclusions clear on the phenomena are taken into consideration research and their formulations on this topic, especially at: the use of treatments auxins, mainly Indole-3 Butyric Acid, allowed standardize the technique and identify alternative methods, with a relative constancy in the results achieved only if conditions remain unchanged pick Caballero (1993), the chemical nature, the dose concentration, retention time in solution, combination with other substances, treatment conditions, etc (Fabbri, (1980); Ismaili *et al*, 2014). The individual genetic ability is another aspect important, especially for varieties resulting difficult.

Therefore, this experiment has done a complete analysis of the relations between olive varieties Himara, with rooting difficult and the auxinic factor, to formulate a correct method for its multiplication. The main goal is the study of stimulant effect by foliar road.

Materials and Methods

The researches of the four hormones IBA, NAA, BAP and GA3 were done in the Olive Experimental Station in the period of 2013-2015. The effect of the method by foliar chemical treatment in correlation with basal treatment AIB 4000 ppm was to study the rhizogenesis process in Olive Himara cultivar.

Plant material

In September 20, the pieces are prepared in the apical part of sprig. All the green pieces are fragile, around 8 cm, prepared from the top of sprig (apical).

An apical meristem supplied with two leaf pairs, was taken from the same tree for three years. The four hormones were applied in three concentrations: 50,100,200ppm, via eight spraying mist form, in any week.

The same scheme was applied during all the terms: 100 cuttings are divided into four repeats for each variant. (i) Control (hydro alcoholic solution). (ii) Basal IBA4000 ppm; (iii) IBA4000+BAP/50,100,200ppm; (iv) IBA4000+GA_3/50, 100, 200ppm; (v) IBA4000+IBA/50, 100, 200ppm; (vi) IBA 4000 + NAA/50,100, 200ppm, constitute the scheme of this experiment.

Butyric acid ($C_{13}H_{12}NO_2$) is the basic treatment 4000 ppm hydro alcoholic solution. Wherein, alcohol comprises 22 % and H_2O, 78%. Hormones for foliar treatments: Naphthalene acetic acid NAA, ($C_{12}H_{10}O_2$) is an organic compound. Gibberellic Acid GA_3 ($C_{19}H_{22}O_{6)}$ is plant growth hormone, in the same time, BAP ($C_{12}H_{11}N_5$).

The dip solution of each treatment was prepared in a Pyrex container, ½ light, temperature 22-23°C. The pieces were sunk into the dip solution (basal part 1 cm), 5 seconds. 100 cuttings were used for each treatment, (4 repetitions x 50 cuttings). A nebulization bank (tip SHB), perlite substrate, are installed within a biological greenhouse. For 70 days ambient temperature 20°C during the day and 18°C at night: in a substrate 24°C (± 1°C) realized through a furnace.

The Humidity is 85%, supplied by launching water mist each 15 wh/m², 3-5 seconds, in function of the active solar radiation. This process was carried out under the functioning of light from an electronic leaf that surveys the percentage of humidity in the bank. The radiance is 6000 lux and 50%

light/14 hours per day. The module of the solar integrator was automatic through a solar integrator SI–20, fog automatically through a solar photovoltaic cellule.

Research Indices

At the end of the rooting process we evaluated, when the roots have ocher color rooting were evaluated percentages, the number and length of roots, the number and length of buds blown and the number of fallen leaves for each piece. Sample data are modeled and is realized descriptive analysis for the variance, Stand Dev, test variability and correlations by jmp software Jmp, SAS (2008).

Results and Discussion

During the rooting phases there have been several physiological processes. After the lesion was healed on both sides of the sclerenchemical ring we notice augmentation of the cortical parenchyma and phloem and numerical increase, which are later transformed into a mass of hyper plastic tissues. Following of this process, differentiation of radices originates from a cell of the primary and secondary ray pith in the area where these are bred with the cambial stratum, (Photo-1, table-1). According to the data in Table 1, basal treatment with IBA 4000 ppm has favored better rhizogenous process compared to Foliar treatments of IBA at the same time to ANA, BAP and GA^3, statistically validated, *prob >F < .0001*.

IBA in the form foliar has favored better the rooting compared to three other stimulants and has been increasing effect simultaneously with the increase of its concentration (0.3% to 2.0%). Treatment basal IBA hydro alcoholic combined at foliar treatments of IBA had higher effects than with ANA, BAP and GA^3 at any concentration.

Basal treatments have stimulated higher percentage rooting compared with foliar treatments. The changes were evaluated by coefficient of variation, table-1. Treatment Control is 18.5% of the different versus the foliar treatments and 16.4% vs. basal treatments. IBA foliar treatments were 2.0% higher than treatments with NAA and 2.7%, compared with GA^3 foliar. Only 4,000 ppm IBA treatment basal when it is associated with 100-200 ppm foliar IBA changed versus others 2.4%. In general, foliar treatments have increased 0.8-2.0%, versus the rooting Control percentage, while basal treatments 46.3%.

IBA 200 ppm foliar treatment has higher effects than those obtained with doses of others stimulants. Higher concentrations road foliar have coincided with a slight increase in the percentage of rooting. In general, foliar treatments have not had the greatest impact on rooting and they have resulted in no statistical difference with control variant results.

Referring to rooting averages, 7.1% foliar treatments and average with ± 0.76 Stand Dev, i.e. are four hormones with their concentrations. IBA basal treatments contributed 46% to 54.6% for rooting, 48.2% average and stand dev. ± 2:22. Results lower has been treatment IBA basal + GA^3 45, 2% whereas; with the best results when accompanied by foliar IBA, is 54.6%. BAP has average 47.6% while; Basal treatment with IBA has been influential 52.6%, while 2.0% foliar treatments.

In general, treatment with NAA at any concentration thereof has resulted in some negative reflexes in the function of leaves and their resistance during the process.

Basal treatments as well have influenced in a positive for the increase of average root for each green cuttings. The average number of large roots has been in treatment IBA / IBA foliar 3.78 roots. Regarding the numbers of roots, Treatments range from IBA / IBA-F to the control treatment, as follows: IBA/IBA>IBA/ANA> IBA/GA^3> IBA/ BAPl>CONTROL.

Statistically, Nr means of roots is 2.48 and mean stand.dev. 0.37. Amplitude 0.05 to 0.87 according to comparisons for all pairs using Tukey-Kramer HSD alpha 0.05 q*3.76983.

Treatment IBA / GA^3 and IBA/BAP stimulated the awakening of buds, a phenomenon which has reduced the percentage of rooting and the number of roots. While the number of buds damaged during the process is caused by foliar treatments based NAA, dominating compared with control Treatment. The number of buds blown and the number of resistant leaves is influenced by these treatments foliar and basal and all these data are described in table 1, Figure 4. The number of major buds blown and the resistant leaves was in treatment GA^3 and BAP, because the averages have statistical differences versus other treatments. Number of defoliated leafy has average 161.9 and dev stand, 6.38 means dev stand, amplitude 114.6 to 205 comparisons for all pairs using Tukey-Kramer HSD alpha 0.05 q*3.76983, have been statistically confirmed.

The roots biometrics has not been strong influences of treatments. The length of the root has fluctuated in amplitude 42 mm to 56 mm, no statistical difference and it is not affected by preparations or their concentrations.

Basal IBA + IBA Foliar, there were no statistical differences with IBA Basal, regardless of a small increase, which caused the foliar treatment. Average of rooting is 27.2%, but it resulted in a wide range in a 6.0% to 54.6% and very large coefficient of variation, 42.4% by value. Rooting percentage variation comes from the effect of treatments applied. Preparations and concentrations have caused variation on the number of fallen leaves 159.7 average and 39.9% variation, caused by the toxic effect.

The variation on the blown buds, the number and length of roots has fluctuated moderately large, respectively from 12.6 to 15.4%. The important we result, has been bond between the resistant leaves and the percentage of indigenization. Connections between other indicators were not strong. Connections between foliar treatments – basal treatment was poor. Because in figure-4, seen dominance of averages of the basal treatments while on the other hand, we have inferior position of foliar treatments in this analysis. In this way, the effect of foliar treatments with any stimulant is not visible in each specific type of the olive Himara. As seen, treatments with different chemicals have been an impact in percentage of rooting they have also influenced their different concentrations as for rooting and the number of roots. The analysis results were statistically reliable because the F. factual value was greater than the theoretical F respectively for each indicator to analyze, Prob > F, <.0001*.

The data has testified to possibility of multiplication with green pieces apical for the Himara olive based on basal treatments with IBA. Meanwhile, when these basal treatments were accompanied with foliar treatments, especially with IBA, were stimulated the best results of their rooting. The awakening of buds during the process was unfavorable for the percentage of the rooting.

In general, is highlighted a slight positive effect of foliar treatments for the percentage of the rooting. Foliar treatments particularly contributed to the resistance of leaves, especially in the case of treatment with BAP and GA^3. Positive effects for the resistance of the fall leaves, was distinct in the case of foliar GA^3. Influence of cytokine as 6-benzylaminopurine (BAP) in combination with IBA basal, in the foliar method showed positive results for the preservation of leaves and buds differentiation, (Silva *et al.*, 2013; Siddiqui *et al.*, 2011); Naduvilpurakkal *et al.*, 2014). The same, *g*ibberellic acid promoting growth and elongation of cells, stimulates the cells for differentiation of roots. This acid is a very potent hormone whose natural occurrence in plants controls their development.

The existence of the leaves has been important for the start of the rooting and subsequently on the number of roots, *Dendrograme -2*. According sperman's, expressed graphically, figure-4, is seen and are statistically certified the strengths links between factors analyzed. Above all, they have confirmed the validity of mist propagation, in correlation with basal treatment in all these hypotheses.

Combining Basal / Foliar, has realized the improvement on the percentage of the rooting of this cultivar difficult for rooting. Especially, the number of leaves, buds and the type of preparation has an important role in this result. Showed that the resistance of the leaves until the end of the process, has a major influence on the rooting. The resistance 100% of the number of leaves has increased the percentage of the rooting and the number of roots. BAP and GA^3 Treatment, were greater numbers of leaves or 3.3 leaves / cutting and rooting higher percentage, but not compared with the treatment IBA / IBA. GA3 added incentive function of buds and leaves and indirectly to the process of rooting. The fall leaf was higher in the Control and NAA with 201 - 234 leaves / pieces, or about 50% and vas coincided with the smallest percentage of the rooting.

Naphthalene acetic acid (NAA) is the auxin family it has also been understood to prevent premature of leaf from sprig. Increased amounts of it can actually have negative effects however, and cause growth inhibition to the development of plant crops. Because, there are positive effects, it has been used on many different crops including apples, olives, oranges, potatoes, and various other hanging fruits. In order for it to obtain its desired effects it must be applied in concentrations ranging from 20-100 ug/mL. NAA present in the environment undergoes oxidation reactions with hydroxyl radicals and sulphate radicals (Siddiqui *et al.*, 2011; Silva *et al.*, 2013; Naduvilpurakkal *et al.*, 2014).

This experiment has certified a strong correlation between buds and leaves that have controlled the rooting and his character. The awakening and growth of buds had strong influence on the rooting and on the number of roots. The GA^3 treatment has the highest waking buds associated with the lower rooting. GA^3 has influenced in enlarge the volume and the number of cells of the leaves more than ANA and IBA foliar. This cytokine has added resistance of leaves more than others during the whole process and the same phenomenon is for BAP.

Data obtained have determined the variant that creates greater opportunity for the rooting. The basal treatments were efficient in the process of rooting. In this study is concluded, that a piece of green apical, with 4-6 buds is the best practice in correlation

with 4000 ppm IBA basal + foliar treatment 100-200 ppm.

The large number of leaves, to variant GA3 foliar, has not managed to modify results in favor of rooting percentage. In this way to this olive variety, GA3 should not apply. Nevertheless, this method it may be highly appreciated because it increases the resistance of leaves therefore deserves greater attention in the future.

Table.1 Statistical Data Analysis for the Main Treatments of IBAS, Rooting %, blown buds, nr. roots, L. of roots and F. Leafy, respective Dev stand, for the Himara Cultivar

Treatments	**Rooting (%)**	**Defoliation Leafy**	Nr. roots	**L. Roots**	blown buds
Bazal IBA 4000 ppm	52.6±1.52 ab	118.0±2.00 ef	4.60±0.26 a	4.23±0.15 a	61.6±4.72 **cd**
Control	6.3±0.57 d	201.0±12.1 a	1.93±0.20 d	5.63±0.60 a	37.6±3.05 **d**
IBA4000-BAP100ppm	48.6±1.15 bc	120.3±12.4 ef	3.13±0.51 abcd	4.76±0.35 a	94.0±6.00 **ab**
IBA4000-BAP200ppm	47.3±2.08 c	123.0±3.0 ef	2.03±0.87 d	5.50±1.04 a	110.6±11.6 **a**
IBA4000-BAP50ppm	47.0±2.64 c	108.0±5.29 f	3.10±0.43 bcd	4.66±0.29 a	80.3±7.09 **bc**
IBA4000-GA3-100ppm	47.3±1.10 c	129.6±5.50 de	3.13±0.51 abcd	4.76±0.35 a	94.0±6.12 **ab**
IBA4000-GA3-200ppm	46.6±2.00 c	126.3±6.50 ef	2.03±0.87 d	5.50±1.00 a	110.6±11.6 **a**
IBA4000-GA3-50ppm	45.6±1,42 c	114.6±7.37 ef	2.96±0.35 bcd	4.60±0.88 a	80.3±7.09 **bc**
IBA4000-IBA 100ppm	47.0±1.00 c	131.0±7.93 de	4.06±0.20 abc	5.66±0.32 a	57.3±19.0 **cd**
IBA4000-IBA 200ppm	54.6±1.12 a	117.6±3.05 ef	4.40±0.26 ab	4.53±0.15 a	52.3±7.02 **cd**
IBA4000-IBA 50ppm	46.0±1.04 c	148.3±7.76 cd	2.80±0.81 cd	4.96±0.85 a	51.0±8.71 **cd**
IBA4000-NAA 50ppm	46.6±1.32 c	168.6±7.63 bc	3.26±0.05 abcd	4.93±0.40 a	75.3±7.63 **bc**
IBA4000-NAA100ppm	46.0±1.89 c	183.6±6.02 ab	2.83±0.15 cd	5.33±0.32 a	68.6±9.50 **bc**
IBA4000-NAA200ppm	47.0±1.78 c	192.3±4.04 a	3.33±0.30 abcd	4.50±0.75 a	71.0±17.0 **bc**
Mean	44.9	141.6	3.11	4.97	74.6
Dev.Std	1.53	6.47	0.41	0.58	9.02
CAVY	3.4	4.5	12.9	1.20	12.1
Prob> F	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*
F Ratio	225.3	66.4	11.4	1.99	11.7

Levels not connected by same letter are significantly different

Photo: (from left to right: (1) nebulizing bank with the green pieces during rooting, (2))the green piece supplied with two pairs of leaves (3) callus tissue (4) root meristemes and (v) the completed rooting process.

Fig.2 Dendrograme onevay analysis of rooting by treatment for testing the variability analyzed all pairs Tukey-Kramer Lsd 3.91, F Ratio 606.19, Prob > F <.0001*

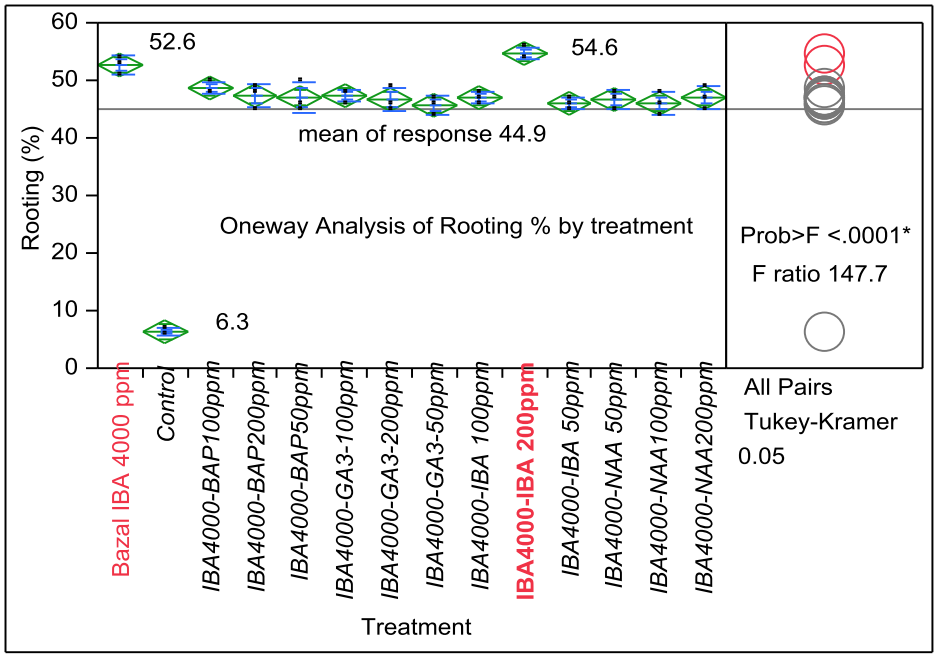


Fig.3 Dendrogram Onaway Analysis of Defoliation Leafy by Treatment for Testing the Variability Analyzed All Pairs Turkey-Kramer Lsd 3.67, F Ratio 606.19, Prob > F <.0001*

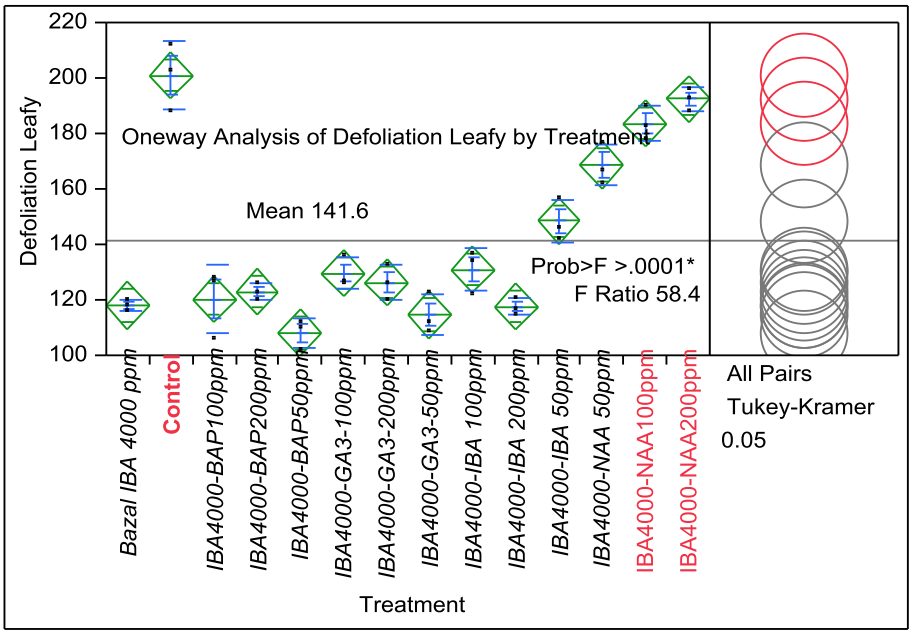
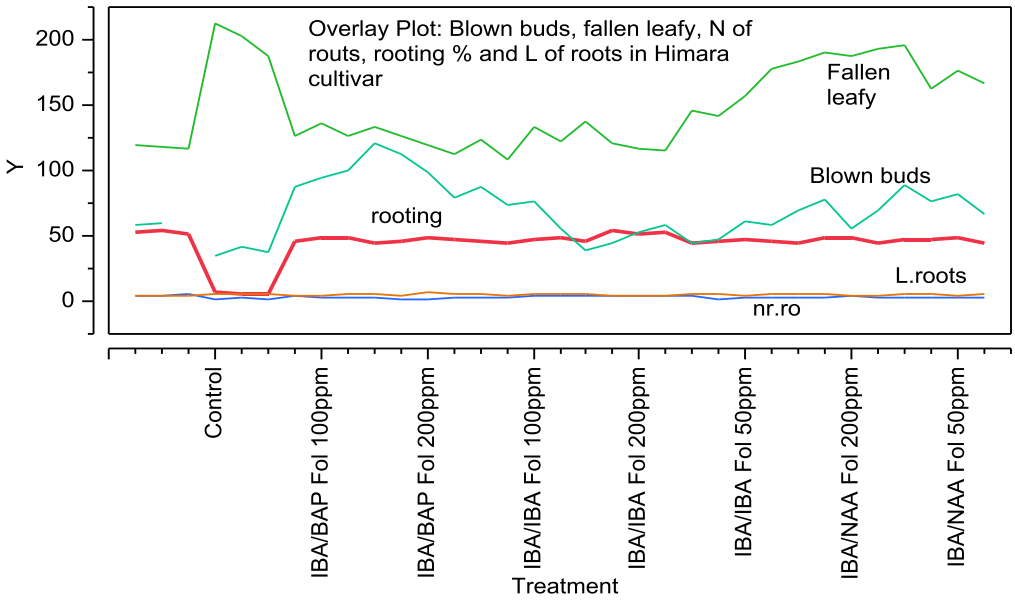


Fig.4 graphical presentation, for the blown buds, fallen leafy, Nr of routs, rooting % and L of roots, realized in Himara cultivar of olive in relation to the treatments applied.



Because, Cytokines in general stimulate the synthesis of RNA and as a consequence of protein synthesis, in the same time cell division (joint action with auxins), flower bud differentiation, interruption of lethargy of buds, movement of mineral resources, obstruction of tissue aging and in some olive cultivar it has helped rooting in reasonable percentage.

In conclusion, the basal treatment has favored better rooting process compared with the foliar treatment. The percentage of the rooting has wide amplitude and different treatments foliar and basal have stimulated in different ways this process. In general, foliar treatments have increased the rooting in inconsiderable quantities versus the Control. Four hormones have caused different reactions for defoliation whereas, for the biometrics index are small changes. During rhizogenous process, morphological changes have been accompanied with several relations among the factors and the cultivar, which should be taken into consideration.

Existence of leaves in the green cuttings, it was better when they are treated with GA_3 and BAP, but despite the importance of the presence of leaf for this method of multiplication, they have not stimulated the percentage of rooting more than others.

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